(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 December 2002 (27.12.2002)

PCT

(10) International Publication Number
WO 02/102514 A1

(51) International Patent Classification[7]: B01L 3/02, G01N 35/10

(21) International Application Number: PCT/CA02/00922

(22) International Filing Date: 19 June 2002 (19.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/298,911 19 June 2001 (19.06.2001) US

(71) Applicant: **B.C. CANCER AGENCY** [CA/CA]; 600 West 10th Avenue, Technology Development Office, Vancouver, British Columbia V5Z 4E6 (CA).

(72) Inventors: **MACAULAY, Calum, E.**; 338 East 37th Avenue, Vancouver, British Columbia V5W 1E7 (CA). **KORBELIK, Jogoda**; 3944 Dunbar Street, Vancouver, British Columbia V6S 2E4 (CA). **CARDENO, Mark**; 8593 149A Street, Surrey, British Columbia V3S 7K7 (CA).

(74) Agents: **CLARK, Neil, S.** et al.; Fetherstonhaugh & Co., Box 11560, Vancouver Centre, Suite 2200, 650 West Georgia Street, Vancouver, British Columbia V6B 4N8 (CA).

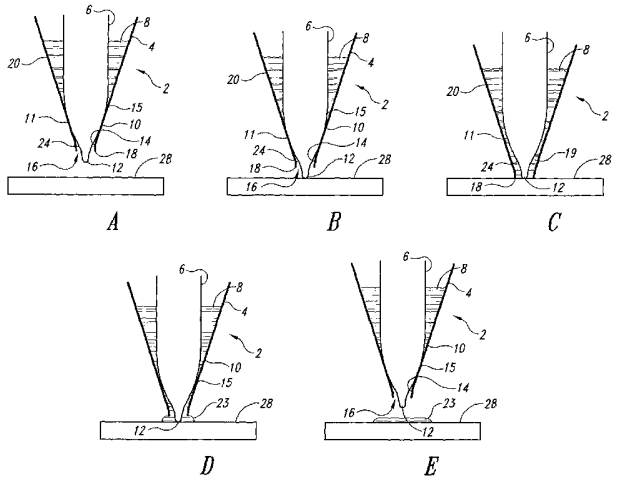
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with amended claims*

[Continued on next page]

(54) Title: MICROVOLUME LIQUID DISPENSER SUITABLE FOR MICROARRAYS AND METHODS RELATED THERETO



(57) Abstract: Microvolume liquid dispensers that provide simple and inexpensive approaches to making cytology microarrays. In some embodiments, the dispensers comprise tips that comprise an outer sleeve, typically shaped like a funnel, that holds a reciprocating needle or pin. The tip of the pin slightly extends beyond the distal opening of the outer sleeve in one position, and is retracted in another position. When the pin is in the distal position the pin contacts the inner surfaces of the sleeve and blocks cytology liquid from flowing through the opening of the sleeve. Thus the pin and sleeve cooperate to form a reservoir behind the blockage. When the pin is pushed up into the sleeve, for example by touching the tip to a glass slide, a passage is formed between the outer surface of the pin and the inner surface of the sleeve. The liquid in the reservoir then flows through the passage and onto the slide. Removing the tip from the substrate moves the pin back to its original position, re-forming the reservoir and leaving a predetermined microvolume amount of the liquid on the slide.

WO 02/102514 A1

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

MICROVOLUME LIQUID DISPENSER SUITABLE FOR MICROARRAYS AND METHODS RELATED THERETO

FIELD

[0001] The field of the present application is micropipettes and microarrays.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] The present application claims priority from United States provisional patent application No. 60/298,911, filed June 19, 2001.

BACKGROUND

[0003] A "microarray" is a device that is used in biotechnology and other science research. A microarray can be made by putting a large number of tiny samples on a microscope slide (usually made of glass, nylon, plastic, metal, etc.). In a "cytology microarray," the samples are typically individual cells or groups of cells (or disrupted tissue) in a solution such as water and alcohol. In a "tissue microarray," the samples are typically whole tissue (as opposed to the substantially free-floating cells in a cytology microarray). In order to examine the samples in microarray closely, the microarrays are typically stained with special dyes, and/or probed with DNA, proteins or antibodies (or other probes). The microarray are then examined under a microscope or in a specialized kind of computerized microscope called an image cytometer. This can determine the makeup or identity of the cells or tissues under review. This can be helpful for a variety of medical purposes, such as identifying or diagnosing diseases.

[0004] Tissue microarrays are sometimes advantageous because they keep the cells in their original tissue structure, and thus keep them in their original relationship with each other. However, tissue microarrays can be difficult to create and to assay because they can suffer from problems, known as "artifacts." For example, when the cells are cut into thin sections, individual cells may be cut in half and thus important information can be lost. When the tissue is cut in thick sections it can be difficult to see the cells, and determine where one cell ends and another begins, because the cells overlap. Further, the tissue sections for one microarray are never precisely the same as the tissue sections for the next microarray because the microarrays are cut from different layers of the tissue. As a loose analogy, this is similar to a loaf of sliced

bread. Each slice is a little bit different from the previous slice, and sometimes, in just one slice, the bread changes from middle pieces to an end piece, or even to nothing at all (once the loaf is finished). The same kind of thing happens with the individual cells in the tissue microarrays; the cells in one slice are not the same as the cells in the next slice. Tissue microarrays are also typically expensive to create.

[0005] Cytology microarrays, where the cells have been separated from each other and suspended in a suitable liquid, can be advantageous because they can be less expensive to make, and typically the cells can be put down on the slides in a "monolayer," which means in a single layer so that there is little overlap of one cell and the next. However, making such cytology microarrays can also be expensive and difficult, for example because of inconsistent dispensing of the micro-volumes of liquid used for the cytology microarrays.

[0006] Accordingly, there is gone unmet a need for inexpensive and simple methods and devices for making cytology microarrays. The present systems and methods provide these and other advantages.

SUMMARY

[0007] The microvolume liquid dispensers disclosed herein provide simple and inexpensive approaches to making cytology microarrays. Briefly, the tips comprise an outer sleeve, typically shaped like a funnel, that holds a needle or pin. The pin moves back and forth inside the sleeve, or reciprocates. The tip of the pin slightly extends beyond the distal opening of the outer sleeve in one position, and is retracted in another position. When the pin is in the extended, or distal, position the shoulders of the pin contact the inner surfaces of the sleeve and block the cytology liquid from flowing through the opening. Thus the pin and sleeve cooperate to form a reservoir behind the blockage. When the pin is pushed up into the sleeve by touching the tip to a glass slide or other substrate, a passage is formed between the outer surface of the pin and the inner surface of the sleeve. The liquid in the reservoir then flows through the passage and onto the slide. Removing the tip from the substrate moves the pin back to its original position, re-forming the reservoir and leaving a precise droplet of liquid - a predetermined microvolume amount of the liquid - on the slide.

[0008] The size and shape of the pin and sleeve can be cooperatively configured in any desired shape so that a precise amount of liquid flows from the reservoir when the tip is contacted with the substrate. Although a wide variety of additional attachments, such as springs or other biasing members, automated motion detectors, etc., can be provided and added, it is an advantage of the present tips that they do not need such attachments; they can be nothing more than routine plastic micropipette tips and simple metal needles (any other desired material can be used for either the sleeve or the pin), and the device can, if desired, be operated solely via manual operation and the effects of gravity.

[0009] In one aspect, the present disclosure provides a microvolume liquid dispenser comprising a body, an outer sleeve extending from the body, and a reciprocating pin located within the outer sleeve. The outer sleeve comprises a distal opening and the pin reciprocates relative to the sleeve between a distal position wherein a distal tip of the pin extends beyond the distal opening and a proximal position. The outer sleeve and the reciprocating pin can be configured to cooperatively form a reservoir when the pin can be in the distal position and configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid from the reservoir when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position.

[00010] In some embodiments, wherein the dispenser can be a hand-held dispenser and the body comprises a handle, or the dispenser can be stationary and the body can be attached to a frame sized to fit on a substantially flat surface. (Unless expressly stated otherwise or clear from the context, all embodiments, aspects, features, etc., can be mixed and matched, combined and permuted in any desired manner.) The sleeve and pin can be configured to cooperatively dispense a volume per cycle that is suitable for a cytology microarray, and the passage can be sized to substantially avoid clogging by cells. The sleeve and pin can be configured such that the predetermined microvolume amount can be from about 0.05 µl to 0.5 µl per cycle, or otherwise as desired. The dispenser can further comprise a biasing element operably connected to at least one of the body and the outer sleeve and configured to urge the pin toward the distal position.

[00011] In another aspect, the present disclosure provides a microvolume liquid dispenser tip comprising an outer sleeve and a reciprocating pin located within the outer sleeve, configured to cooperatively interact as discussed above. The inner surface of the sleeve, and the sleeve itself, can be substantially frustoconical and the outer surface of the pin can be correspondingly substantially frustoconical. The substantially frustoconical shape of the pin can comprise a concave curve near the distal tip. The distal opening of the sleeve can have a diameter from about 0.5 mm to 1.5 mm. The tip can be one of an array of the microvolume liquid dispenser tips, which array can be configured and sized to make a cytology microarray.

[00012] In a further aspect, the present disclosure provides a cytology microarray maker comprising a frame operably connected to a body holding an array of microvolume liquid dispenser tips, at least two stages sized to support cytology microarrays, at least one upright member operably attached to the body to move the body and the array of tips substantially normal to the stages between at least an extended position wherein the tips contact a cytology microarray substrate located on the stage and a retracted position wherein the tips do not contact the cytology microarray substrate, and at least one axial member disposed along the frame and operably connected to the upright members to provide a track along which the upright members, the body and the array of tips can be movable along the track between the first and the second stage.

[00013] The microvolume liquid dispenser tips can be configured as discussed elsewhere herein or can be other configurations, and the maker can further comprise at least a third stage. The maker can be stationary and the frame can be sized to fit on a substantially flat surface or other surface as desired. The at least one axial member can comprise two rails extending along the frame, either separately from or as a part of the frame. The upright members can comprise two substantially planar elements slidably connected to the two rails and situated on either side of the stages, the substantially planar elements comprising corresponding elongated axial channels configured to slidably receive projections extending from the body. At least one of the frame and the upright members can be operably connected to body biasing element urging the body away from the stages.

[00014] The stages can be substantially planar stands and can further comprise at least x-axis and y-axis adjustment mechanisms configured to adjust positions of the stages relative to at least one of the frame and each other. The body can comprise a plurality of floating channels each sized to releasably hold one tip. The maker (as with other devices and systems herein) can be substantially automated or substantially manually operated.

[00015] The present disclosure also provides methods of dispensing a microvolume of liquid. The methods can comprise, a) providing a microvolume liquid dispenser tip as discussed herein; b) transiently contacting the distal tip and distal opening with a substrate thereby causing the pin to cycle; and, c) during the cycle, dispensing the liquid to the substrate. The sleeve and pin can be configured to cooperatively dispense a volume per cycle that is suitable for a cytology microarray. The tip can be one of an array of the tips, and the methods can comprise substantially simultaneously transiently contacting the array of tips with a cytology microarray platform, thereby causing the pin to cycle, and thereby forming the cytology microarray on the platform.

[00016] The methods can also comprise, before providing the tip containing the liquid, loading the liquid into the tip by placing the tip into a source of the liquid and suctioning up the liquid using capillary action. The tip can also be loaded by loading the liquid into the tip through a proximal opening located at a proximal area of the tip, or otherwise as desired.

[00017] The present disclosure further provides methods of making a cytology microarray comprising: a) providing a cytology microarray maker as discussed herein; b) loading the array of tips with liquid cytological specimens by transiently moving the array of tips into the liquid cytological specimens and suctioning up the liquid cytological specimens using capillary action; c) moving the array of tips along the axial member to the second stage; and, d) making the cytology array by transiently contacting the array of tips with the cytology microarray substrate. If desired, the microvolume liquid dispenser tips can comprise an outer sleeve and a reciprocating pin as discussed herein. The frame can further comprise a third stage, and the methods can comprise moving the array of tips along the axial member to the third

stage; then making a second cytology array. The second cytology array can be made without reloading the tips.

[00018] The methods can comprise sliding the upright members along the two rails between the cytology microarray template and substrate, and then pushing the array downwardly (for example by pushing down on the array itself or on the body) to contact the cytology microarray template and substrate, respectively. The methods can also comprise adjusting the stages on at least one of an x-axis and a y-axis. Where the body comprises a plurality of floating channels each sized to releasably hold one tip, the methods can comprise placing the tips in the body to create the array of tips and removing the tips from the body after making the cytology array. The methods can also comprise removing the cytology array template and the cytology array from the stages then placing new cytology array substrates on the stages and making additional cytology arrays. The additional cytology arrays can be made without reloading the tips.

[00019] The present disclosure still further provides tip means for microvolume liquid dispensing comprising: a) an outer sleeve means for holding the liquid, b) a reciprocating pin means located within the outer sleeve for cooperatively dispensing, through a passage formed between a side of the outer sleeve means and the pin means, a predetermined microvolume amount of liquid when the pin moves in a cycle from a distal position to a proximal position then returns to a distal position. A means for making cytology microarrays can comprise: a) a frame means for holding a body means, b) the body means for holding an array of tips means for dispensing a microvolume of liquid, c) at least two stage means for supporting cytology microarrays, d) at least two upright member means operably attached to the body for moving the body means substantially normal to the stage means, and e) at least one axial member means disposed along the frame and operably connected to the upright members for moving the upright member means between the two stage means.

[00020] A methods of dispensing a microvolume of liquid can comprise the steps of: a) a step of providing a microvolume liquid dispenser tip means, as discussed herein, containing the liquid; b) transiently contacting the distal tip and distal opening with a substrate thereby causing the pin to cycle; and, c) during the cycle, dispensing the liquid onto the substrate. The sleeve means and pin means can be configured for

cooperatively dispensing a volume per cycle that can be suitable for a cytology microarray, and the methods comprise the step of dispensing a spot of cell-containing liquid sized for the cytology microarray.

[00021] These and other aspects, features and embodiments are set forth within this application, including the following Detailed Description and attached drawings. In addition, various references are set forth herein, including in the Cross-Reference To Related Applications, that discuss in more detail certain systems, apparatus, methods and other information; all such references are incorporated herein by reference in their entirety and for all their teachings and disclosures, regardless of where the references may appear in this application.

BRIEF DESCRIPTION OF THE DRAWINGS

[00022] Figure 1 depicts schematically a microvolume liquid dispenser tip transiently contacting a cytology microarray substrate and dispensing a desired, predetermined microvolume amount of liquid.

[00023] Figure 2 depicts schematically a microvolume liquid dispenser tip configured to dispense a smaller spot of liquid than the tip in Figure 1.

[00024] Figure 3 depicts schematically a microvolume liquid dispenser tip configured to dispense a larger spot of liquid than the tip in Figure 1.

[00025] Figure 4 depicts a hand-held micropipette comprising a microvolume liquid dispenser tip as discussed herein.

[00026] Figure 5 depicts a schematically an elevated perspective view of various elements of a cytology microarray maker as discussed herein.

[00027] Figure 6 depicts a front-side view of a cytology microarray maker.

[00028] Figure 7 depicts a schematically an exploded, elevated perspective view of a tabletop suitable for use with the cytology microarray maker of Figure 5.

[00029] Figure 8 depicts a hand spotted cytology microarray with large spots, made using a funnel without a reciprocating pin.

[00030] Figure 9 depicts a hand spotted cytology microarray with large spots, made using a funnel without a reciprocating pin.

[00031] Figure 10 depicts a hand spotted cytology microarray with medium spots, made using a microvolume dispensing tip as discussed herein.

[00032] Figure 11 depicts a hand spotted cytology microarray with small spots, made using a microvolume dispensing tip as discussed herein.

[00033] Figure 12 depicts photomicrographs at different magnifications of a single spot from the cytology microarray of Figure 8.

[00034] Figure 13 depicts photomicrographs at different magnifications of a single spot from the cytology microarray of Figure 11.

[00035] Figure 14 depicts photomicrographs at different magnifications of a single spot from a cytology microarray with small spots made using a microvolume dispensing tip as discussed herein.

[00036] Figure 15 depicts screen shots collected by an automated image cytometer for spots created using a funnel without a reciprocating pin.

[00037] Figure 16 depicts screen shots of images collected by an automated image cytometer for spots made using a microvolume dispensing tip as discussed herein.

[00038] Figures 17a and 17b provide graphs demonstrating spot sized in comparison to spot makers comprising a funnel only or comprising a funnel and needle and at different concentrations of cell concentration.

[00039] Figure 18 depicts graphs indicating the effect of different alcohol and cellular concentration on spot size and liquid flow through the tips.

DETAILED DESCRIPTION

[00040] High throughput genomic screening methodologies generate very large amounts of genetic, gene expression, and protein content information, and can be mined to determine possible markers (e.g., DNA sequence, mRNA, protein and antibodies to same) for a wide variety of clinical conditions (e.g., disease state, environmental induced damage, infection, or genetic susceptibility markers). Many of these markers can be evaluated, tested, verified and utilized on cellular material such as tissue sections, cytological preparations or extracted cellular components. It is generally accepted that many more markers will be suggested than will eventually be found to be clinically useful. Additionally, these markers are likely to be costly to manufacture and market. Thus, strategies that assist effective testing, verification and utilization of these markers would be of benefit. For these and other reasons, tissue microarrays are made from wax blocks that have tens to thousands of cylindrical

tissue samples from random (cylinders adjacent to each other can be arbitrarily determined) arrangements of sources are constructed and used for these purposes. However, tissue microarrays have a number of drawbacks.

[00041] Cytology microarray provide a less labor-intensive, more uniform representation, and use less tissue from a sample. These arrays of spotted (deposited) cytological material may have from one to several thousands of sample cells per spot. The cells deposited may be unfixed, fixed, pre-processed disaggregated cells from solid tissue samples, etc. Each spot of cells may be from different sources, or may be from the same source, or some of each. The spots may be spatially distinct or over lapping. The spatial extent of each spot will be determined by the fluid containing the cells, the surface they are deposited onto and the environment in which they are deposited (humidity, temperature, vapor pressure of the atmosphere, etc.).

[00042] The systems and methods discussed herein provide simple and easy ways to make such cytological microarrays.

[00043] Turning to the Figures, Figures 1-3 each schematically depict one cycle of a microvolume liquid dispenser tip. In Figure 1, for example, the microvolume liquid dispenser tip 2 has an outer sleeve 4 and a reciprocating pin 6. Reciprocating pin 6 is located within the outer sleeve, and may or may not be physically attached to outer sleeve 4. Outer sleeve 4 comprises a distal opening 16 and an inner surface 14. As depicted, outer sleeve 4 is substantially frustoconical, ending in distal opening 16; other shapes are possible as desired. Reciprocating pin 6 comprises an outer surface 10, a shoulder 11 and a distal tip 12. As depicted, the shoulder 11 and distal tip 12 provide a substantially frustoconical form to reciprocating pin 6. As can be seen in the Figure, in this embodiment shoulder 11 further provides for a concave curve 24 near distal tip 12.

[00044] When reciprocating pin 6 is in a distal position with respect to outer sleeve 4, the outer surface 10 of reciprocating pin 6 contacts an inner surface 14 of outer sleeve 4 to substantially form a seal 15 at the point of contact. Because of the seal 15, any liquid maintained proximal to the seal 15 forms a reservoir 8. When reciprocating pin 6 is moved proximally relative to outer sleeve 4, a passage 19 is created between reciprocating pin 6 and a side 18 of distal opening 16 of outer sleeve 4. Accordingly,

liquid, which in Figure 1 is a cytological fluid 20, can flow through passage 19 to reach cytology platform 28 which, as embodied in Figure 1, is a substantially planar substrate. If desired, other forms of substrate, such as substantially spherical or otherwise curved substrates as may be found in the bottom of certain wells of desirable microarray substrates, such as 96-well plates, are also suitable for use. Passage 19 can be sized to substantially avoid clogging by the cells when the liquid being dispensed is a cytological fluid.

[00045] If desired, the reciprocation of reciprocating pin 6 can be caused by various assorted attachments to either the outer sleeve or the pin, for example a biasing element 37 as depicted in Figure 4, but it is a feature and an advantage of the present systems and methods that no additional elements are necessary to provide the precisely dispensed quantities of cytological fluids or other chemical solutions (such as chemical solution 22 dispensed onto substrate 30 in Figure 2). Thus, it is inexpensive and easy to move reciprocating pin 6 in a cycle from the distal position to the proximal position and then back to the distal position simply by contacting the dispenser tip 2 with the desired substrate. Similarly, it is possible to load the tip with a liquid merely by placing the tip into the source of the liquid and suctioning up the liquid using capillary action. Conversely, if desired, the liquid may be loaded into the tip through a proximal opening located at a proximal area of the dispensing tip, 2 for example at the top of dispensing tip 2 where it abuts body 36 in Figure 4.

[00046] The result of moving the reciprocating pin through a cycle is the dispensing, and typically deposit, of a spot of the desired fluid onto the receiving surface such as the cytology platform 28 or substrate 30 depicted in Figures 1-3. Thus, a spot 23 is formed on the receiving surface. The spot can be of medium size, as depicted in Figure 1, of small size as depicted in Figure 2, or of a large size as depicted in Figure 3. Generally, the spots comprise from about 0.05 µl to about .5 µl with a typical spot being about 0.1 µl. The spots be either larger or smaller if desired. Typically, depending upon the desired format, the solution comprising the cells (or other chemical solution if not a cytological application), the distal opening diameter will typically be from about 0.5 mm to about 1.5 mm, for example about 0.83 mm to 1 mm.

[00047] As already noted, the distal tip 12 of reciprocating pin 6 extends beyond the distal opening 16 of outer sleeve 4. Such extension can be effected by a single point

of reciprocating pin 6, or reciprocating pin 6 can be shaped to provide a plurality of points or otherwise configured to extend beyond distal opening 16. Typically reciprocation pin 6 and distal tip 12 are unitary, but if desired they can be operably connected to provide the same functions (indeed, for example where the tip is designed to be used with a deep well plate such as certain 96-well plates, the distal tip may be configured to contact the side of the well as opposed to the bottom of the well yet still releasing the fluid at the desired point, for example substantially when the dispensing tip 2 contacts the bottom of the well (or other desired location).

[00048] The spot size can be controlled by a variety of factors in addition to the size of the reciprocating pin 6 in the outer sleeve 4. For example, as depicted in Figure 18, spot size can be affected by alcohol to water concentration, the concentration of cells, or other factors as desired. In view of the present application, a skilled person will be able to control the spot size quite precisely.

[00049] Figure 4 depicts a hand-held embodiment of the microvolume liquid dispenser discussed herein. In particular, hand-held micropipette 32 has a handle 34 and a body 36. As depicted, micropipette 32 additionally comprises a plunger 38 that is useful for typical operation of the micropipette but which is not necessary for the present systems for dispensing microvolumes of liquid.

[00050] Figure 5 depicts a cytology microarray maker 40 having a frame 42. As depicted, frame 42 is sized to be stationary and fit on a substantially flat surface although it can be configured or sized to fit any desired surface. Maker 42 has a first stage 44 second stage 46 and third stage 54, each of which are capable of supporting or holding a cytology array template, cytology array substrate or other desired platform or surface. A cytology microarray template is a cytology microarray that comprises a plurality of liquid cytological specimens or other suitable samples (such as control samples or reference samples). This template can provide samples to the microvolume dispensing tips by transiently contacting the tips into the sample, i.e., the source of the liquid, and then suctioning up the liquid, for example by using capillary action, an active vacuum or otherwise as desired. It is an advantage of the present embodiment that enough liquid from the template can be loaded into the tips at one time to make a plurality of cytological microarrays without reloading. Accordingly, a plurality of different stains, probes or other investigative material can be used with

different cytological microarrays but without significant variation in the samples in the microarrays, both in the volume of a given sample in the microarray and in the location of the samples in one microarray to another.

[00051] Cytology microarray maker 40 further comprises a body 36 that holds an array 26 of tips (see Figure 6) between upright members 48. Frame 42 further comprises at least one axial member 50, which in the embodiment depicted comprises two rails 52 extending along frame 42. In Figure 5, rails 52 are attached to the frame via rail attachments 56. Conversely, the rails 52 or other axial member 50 can be integrally formed in frame 42, for example being formed by the provision of axial slots along frame 42. Rails 52, or other axial member, provide a track disposed along the frame such that the array of tips, the body, the upright members 48, etc., are movable along the track between the various stages.

[00052] As depicted in Figure 5, two sets of removable slide cards 47 are shown, one each above the first stage and second stage 46. Upright members 48, which as depicted are substantially planar elements 49 can be moved along frame 42 by pushing or pulling them along rails 52. If desired, the positioning of the upright members 48 can be facilitated by the provision of retaining elements indicating when the upright members are in the proper location, for example by the provision of spring-loaded ball and indent centering and lock mechanisms, or any other desired positioning mechanism. The maker 40 in Figure 5 also has a frame support 58 sized for a substantially planar surface.

[00053] Figure 6 depicts a cytology microarray maker 40 comprising a body 36 holding an array of tips 26 and a cytology microarray template 60. Upright members 48 comprise substantially planar elements 49, which in turn comprise elongated axial channels 62. Substantially planar elements 49, are slidably connected to the rails 52 shown in Figure 5, and are situated on either side of the stages. Elongated axial channels 62 provide locations configured to slidably receive projections 64 extending from body 36. Figure 6 also depicts a floating channel 66 in dotted line for one of the tips 2; similar floating channels are provided for each of the microvolume dispenser tips in array 26 but not depicted. The floating channels are each sized to releasably hold one tip. If desired, two or more of the channels can be interlocked, provided that adequate spacing between the tips is maintained when moving the array 26 from one

stage to another. Also depicted are body biasing elements 68 which urge the body 35 away from the various stages. This facilitates both loading the tips and making the microarrays because one need merely push down on the body to load the tips/dispense from the tips; the tips then automatically reciprocate away from the given cytology element upon release of the pressure. As depicted, the various embodiments are used in an orientation where gravity is below the tips and assists in maintaining the tips in place in the body and in maintaining the fluid in the reservoirs. It is possible to provide other orientations for the various elements if desired. In addition, the body 36 moves in an orientation that is substantially normal to the various cytology templates/substrates. As used herein, substantially normal includes angles other than 90 degrees if desired by the user.

[00054] Figure 7 depicts a tabletop 43 suitable for use with, and comprising a part of, frame 42. In tabletop 43 as depicted, a variable Y adjustment device 70 and a variable X adjustment device at 74 are provided. Movement of these devices in the desired direction enhances the ability to precisely place templates and substrates under the body and array of tips. Also depicted are a plurality of cytological microarray substrates 76, in this case glass slides.

[00055] Figures 8-11 provides photographs of a variety of arrays made using various dispensing tips. In each of Figures 8-11, each of the spots provide a cytological specimen and has been stained with H & E. In Figure 8 and 9, the spots were made without using the reciprocating needle 6 discussed elsewhere and, as can be see, the spots are diffuse and large. In contrast, in Figures 10 and 11, medium and small spots were created using outer sleeves or funnels and reciprocating pins. Small pins and a high cell concentration solution were used to make medium spots in Figure 10, and large pins and a high cell concentration solution were used to make small spots in Figure 11.

[00056] Figures 12 and 13 provide photomicrographs at various magnifications (4x, 10x and 20x) of a single spot from the cytological microarrays depicted in figures 8 and 11 respectively. As can be seen, the spots in Figure 13 are smaller, as can also be seen in Figure 11, and the cells are not as clustered and there is reduced overlapping. Thus the cells are better capable of analysis using certain analysis methods such as certain image cytometry analyses. Figure 14 also depicts a series of

micrographs of magnification 4x, 10x and 20x of a single spot from a hand spotted cytology microarray that was stained with H & E wherein the microvolume dispensing tip had a large reciprocating pin and a low cell concentration solution.

[00057] Figures 15 and 16 depict screen shots of cells images collected by an automated image cytometer. The Figures demonstrating the distribution of the images collected from the spots both with and without the cytological microvolume dispensing tip discussed herein. In Figure 15, the spots were created using a funnel only, with out a reciprocating pin, whereas in Figure 16 the spots were created using both the outer sleeve and the reciprocating pin (which was a small needle in this case). In each figure, low cell concentration solutions were used for the spots in the graphs on the left, high cell concentration solutions were used for the spots in the graphs on the right. As can be seen, the spots in Figure 15 are significantly larger and the spots in Figure 16 are better suited for some analyses than are the spots in Figure 15.

[00058] Figures 17a and 17b provide graphs depicting the distribution of cell images collected by an automated image cytometer wherein the spots were created using an outer sleeve only (on the left in each graph) and an outer sleeve with a small reciprocating pin (on the right in each graph). Two different cell concentrations were used for each pair in each graph, with low concentrations on the left and high concentrations on the right). The low cell concentrations, and the funnels without reciprocating pins created larger spots, with more cells imaged per spot. For the high cell concentration dispensed through a funnel with a reciprocating pin, the cell density was too high and it appears that the number of overlapping cell clusters artificially reduced the number of cells counted by the automated image cytometer. It appears that the cell concentration changes the viscosity of the solution and that the high concentration solution exhibits the characteristics of a viscous or slow spreading or rapidly evaporating solution.

[00059] In Figure 18, different alcohol concentrations were used. As can be seen, increasing the alcohol concentration increased the spot size.

[00060] Turning to some additional discussion of various aspects, the amount of fluid deposited depends in part upon the shape of the outer sleeve and the shape of the reciprocating pin. The size to which the fluid spreads to create the spot depends in part

on the suspension fluid, the type of planar surface, and the environment in which the process takes place. For example, low humidity, moderate temperature and a hydrophilic surface will cause the formation of a smaller spot than will high humidity, low temperature and a hydrophilic surface. Additionally, the suspension fluid may comprise rapidly drying fluids such as alcohol. The rate of spread of the fluid affects creation of a cellular monolayer. Too slow with the spreading and too fast with the evaporation with a high cell density will lead to many clumped, overlapping cells. Too fast with the spreading and too slow with evaporation will lead to larger than desired spots.

[00061] In addition to creating arrays of distinct spots on a planar surface, the same techniques and approach can be used to very rapidly turn a cell suspension into a spatially localized monolayer-type preparation for traditional cytological applications, as well as for automated quantitative cytological applications. This can be done by creating an area of spots that just touch or slightly overlap. These spots would be placed in an interleaved fashion such that new spots are either deposited on a virgin surface or adjacent to completely dry spots so as to create the optimal monolayer without causing all the deposited cells to bunch up along the edge or into clumps. The cytological preparation is typically disaggregated so as to not plug or clump up the outer sleeves or outer sleeve reciprocating pin combinations.

[00062] The outer sleeve or reciprocating pin outer sleeve combinations may also be used to disaggregate cytological samples by utilizing the shear forces involved in flowing the sample multiple times backwards and/or forwards through the outer sleeve or outer sleeve reciprocating pin combination. It is possible to create different controllable shear forces by varying the position of the reciprocating pin within the outer sleeve and by designing the shape of the reciprocating pin-outer sleeve contact areas appropriately.

[00063] The methodologies and systems herein can typically be implemented in parallel such that many (2 to 32 or more) spots could be deposited in parallel.

[00064] Applications for cytology microarrays in addition to those discussed elsewhere herein include 1) Use with multiple FISH probes where one probe is applied to a cytology microarray comprising samples from multiple subjects; 2) Use with multiple messenger RNA probes for expression analysis from multiple subjects; 3) Use with disease markers across multiple subjects or samples to reduce cost and/or increase

throughput; 4) Use with an automated cytometry device to allow ploidy data to be rapidly collected from many samples/subjects disposed on a single slide, which can assist in reducing slide-to-slide staining variations.

[00065] The ability to make many equivalent cytology microarrays confers other possibilities. For example, given 200 tumor samples which need to be examined for about 1000 genetic changes or about 1000 expression changes, one can disaggregate the samples, create 200 cell suspensions, deposit 200 spots (one per sample) on each of 1000 slides (one spot from each sample for each slide, 100 cells per spot for a total cell count of about 100,000 cells) and then mark each slide with either a specific FISH probe (1 or multicolor per slide) or a specific mRNA marker for expression analysis. Thus, instead of running 600 DNA tissue microarrays, each of which typically uses about 1 million cells costs more per slide than cytology microarrays, one can run 1000 cytology microarrays for less cost, in some cases possibly about 10% the cost.

[00066] The data produced by each of the tissue microarray and the cytology microarray would be the about same except with the cytology microarray DNA data one would have FISH spot counts which can detect single deletions very reliably, as well as be able to differentiate the contamination cells (stromal, connective tissue, blood, vessel wall, etc.) from the tumor cells, which could reduce the need for tissue microdissection. The differentiation could be on the basis of morphological features or various counter stains. For the expression data, the result could be intensity expression for individual cells in a spot, the average expression, and the variance of expression. Given that mRNA marker and DNA FISH probe staining processes do not interact significantly it would be possible to perform both tests on the same samples.

[00067] Thus, in one aspect, one can match the FISH probe to the expression marker, or otherwise match gene and protein expression assays, and do both gene and gene expression at the same time. Also at a later date as more specific protein markers become available, one could measure all three of gene, gene expression and gene product on the same cells at the same time.

[00068] The present systems and methods are also useful for combining cytology microarrays (or for that matter, tissue microarrays) with complex liquid handling. For example, it would be possible to take multiple specimens from a single sample the deposit (or block) many spots of cells (or tissue cores) on a slide or slides and then

deposit fixed (typically very small) amounts (usually no more than a single drop) of different marker solutions on different tissue or cell spots on the slide. This allows the different markers to bind the cell components (DNA, mRNA, etc.). The cell markers are then typically washed off the slide. To reduce the risk of cross contamination of marker solutions to adjacent spots, the small amounts of marker solution could be removed using a blotter (wicking material) in soft contact with the slide to wick away most of the marker solution then wash the slide. In all of these applications, a flexible automated cytometer (transmission and fluorescence mode) would be extremely valuable to automate the interpretation of the slides.

[00069] To reduce the spot to spot contamination (of either fluids and cells) it can be beneficial to use a slide with a removable or non-removable mask that creates shallow or deep wells, then depositing one spot into each well. The mask would contain the cell spot as well as any added solutions. For an example of a masked slide with removable mask see US patent No. 5,784,193.

[00070] Turning to some additional discussion of the methods herein, in some aspects the methods comprising dispensing a microvolume of liquid. Such methods can comprise a) providing a microvolume liquid dispenser tip as discussed herein, b) transiently contacting the distal tip and distal opening with a substrate thereby causing the pin to cycle, for example by briefly touching the tip and the substrate; and, c) during the cycle, dispensing the liquid to the substrate.

[00071] The sleeve and pin can be configured to cooperatively dispense a volume per cycle suitable for a cytology microarray, and the passage can be sized to substantially avoid clogging by the cells. The microvolume liquid dispenser tip can be one of an array of tips, the tips and array configured and sized to make a cytology microarray. The method can comprise substantially simultaneously transiently contacting the array of tips with a cytology microarray platform, thereby causing the pin to cycle, and thereby forming the cytology microarray on the platform.

[00072] The methods can also make cytology microarrays. Such methods can comprise providing a frame holding a body holding an array of microvolume liquid dispenser tips, at least first and second stages sized to support cytology microarrays, upright members operably attached to the body to move the body and tips substantially normal to the stages between at least an extended position wherein the tips contact a

cytology microarray substrate located on the stage and a retracted position wherein the tips do not contact the cytology microarray substrate, and at least one axial member disposed along the frame and to move the upright members between the stages. The first stage holds a cytology microarray template comprising an array of liquid cytological specimens and the second stage holds a cytology microarray substrate. The tips in the array are then loaded with the liquid cytological specimens by transiently moving the array of tips into the liquid cytological specimens and suctioning up the liquid cytological specimens using capillary action. Next, the array of tips is moved to the second stage, where the cytology array is made by transiently contacting the array of tips with the cytology microarray substrate.

[00073] The frame can further comprises a third stage holding a cytology microarray substrate and a second cytology array can be made by moving the array to the third stage then transiently contacting the array of tips with the second cytology microarray substrate. In some embodiments, this can be done without reloading the tips. Additionally, the substrates and the template(s) can be removed or covered, then additional substrates can be provided and additional cytology arrays created. The method can further comprise adjusting the stages on at least one of an x-axis and a y-axis relative to at least one of the frame and each other. The methods can also comprise placing the tips in the body to create the array of tips and removing the tips from the body after making the cytology array. As with the devices herein, the methods can be either substantially manual or automated. If automated, the devices can be operably connected to a controller, which is a device that is capable of controlling various elements of the apparatus and methods discussed herein. For example, the controller can control the location and movement of the body, the loading of and dispensing from the tips, and the collection of images form a microarray. Typically, a controller is a computer or other device comprising a central processing unit (CPU) or other logic-implementation device, for example a stand alone computer such as a desk top or laptop computer, a computer with peripherals, a local or internet network, etc. Controllers are well known and selection of a desirable controller for a particular aspect or feature is within the scope of a skilled person in view of the present disclosure.

[00074] All terms used herein, including those specifically discussed below in this section, are used in accordance with their ordinary meanings unless the context or

definition clearly indicates otherwise. Also unless indicated otherwise, except within the claims, the use of "or" includes "and" and vice-versa. Non-limiting terms are not to be construed as limiting unless expressly stated, or the context clearly indicates, otherwise (for example, "including," "having," and "comprising" typically indicate "including without limitation"). Singular forms, including in the claims, such as "a," "an," and "the" include the plural (for example, "a" means "at least one") unless expressly stated, or the context clearly indicates, otherwise.

[00075] The scope of the present disclosure includes both means plus function and step plus function concepts. However, the terms set forth in this application are not to be interpreted in the claims as indicating a "means plus function" relationship unless the word "means" is specifically recited in a claim, and are to be interpreted in the claims as indicating a "means plus function" relationship where the word "means" is specifically recited in a claim. Similarly, the terms set forth in this application are not to be interpreted in method or process claims as indicating a "step plus function" relationship unless the word "step" is specifically recited in the claims, and are to be interpreted in the claims as indicating a "step plus function" relationship where the word "step" is specifically recited in a claim.

[00076] From the foregoing, it will be appreciated that, although specific embodiments have been discussed herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the present disclosure. Accordingly, the disclosure includes such modifications as well as all permutations and combinations of the subject matter set forth herein and is not limited except as by the appended claims.

What is claimed is:

1. A microvolume liquid dispenser comprising a body, an outer sleeve extending from the body, and a reciprocating pin located within the outer sleeve, wherein the outer sleeve comprises a distal opening and the pin reciprocates relative to the sleeve between a distal position wherein a distal tip of the pin extends beyond the distal opening and a proximal position, wherein the outer sleeve and the reciprocating pin are configured to cooperatively form a reservoir when the pin is in the distal position and configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid from the reservoir when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position.

2. The microvolume liquid dispenser of claim 1 wherein the dispenser is a hand-held dispenser and the body comprises a handle.

3. The microvolume liquid dispenser of claim 1 or 2 wherein the dispenser is stationary and the body is attached to a frame sized to fit on a substantially flat surface.

4. The microvolume liquid dispenser of any one of claims 1 to 3 wherein the sleeve and pin are configured to cooperatively dispense a volume per cycle that is suitable for a cytology microarray.

5. The microvolume liquid dispenser of any one of claims 1 to 4 wherein the passage is sized to substantially avoid clogging by cells when the liquid is a cytological fluid.

6. The microvolume liquid dispenser of any one of claims 1 to 5 wherein the sleeve and pin are configured such that the predetermined microvolume amount is from about 0.05 μl to 0.5 μl per cycle.

7. The microvolume liquid dispenser of any one of claims 1 to 6 wherein the dispenser further comprises a biasing element operably connected to at least one of the body and the outer sleeve and configured to urge the pin toward the distal position.

8. A microvolume liquid dispenser tip comprising an outer sleeve and a reciprocating pin located within the outer sleeve, the outer sleeve comprising a distal opening and the pin reciprocating relative to the sleeve between at least a distal position wherein a distal tip of the pin extends slightly beyond the distal opening and a proximal position, wherein an inner surface of the sleeve and an outer surface of the pin are configured to cooperatively form a reservoir when the pin is in the distal position and wherein the sleeve and pin are configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid from the reservoir when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position.

9. The microvolume liquid dispenser tip of claim 8 wherein the inner surface of the sleeve is substantially frustoconical and the outer surface of the pin is correspondingly substantially frustoconical.

10. The microvolume liquid dispenser tip of claim 9 or 10 wherein the substantially frustoconical shape of the pin comprises a concave curve near the distal tip.

11. The microvolume liquid dispenser tip of any one of claims 8 to 10 wherein the passage is sized to substantially avoid clogging by cells when the liquid is a cytological fluid.

12. The microvolume liquid dispenser tip of any one of claims 8 to 11 wherein the sleeve and pin are configured to cooperatively dispense about 0.1 μl per cycle.

13. The microvolume liquid dispenser tip of any one of claims 8 to 12 wherein the distal opening has a diameter from about 0.5 mm to 1.5 mm.

14. The microvolume liquid dispenser tip of any one of claims 8 to 13 wherein the tip is one of an array of the microvolume liquid dispenser tips, the array configured and sized to make a cytology microarray.

15. A cytology microarray maker comprising

a frame, the frame operably connected to

a body holding an array of microvolume liquid dispenser tips,

at least two stages sized to support cytology microarrays,

upright members operably attached to the body to move the body and the array of tips substantially normal to the stages between at least an extended position wherein the tips contact a cytology microarray substrate located on the stage and a retracted position wherein the tips do not contact the cytology microarray substrate, and

at least one axial member disposed along the frame and operably connected to the upright members to provide a track along which the upright members, the body and the array of tips is movable along the track between the first and the second stage.

16. The cytology microarray maker of claim 15 wherein the microvolume liquid dispenser tips comprise an outer sleeve and a reciprocating pin located within the outer sleeve, the outer sleeve comprising a distal opening and the pin reciprocating relative to the sleeve between at least a distal position wherein a distal tip of the pin extends slightly beyond the distal opening and a proximal position, wherein an inner surface of the sleeve and an outer surface of the pin are configured to cooperatively form a reservoir when the pin is in the distal position and wherein the sleeve and pin are configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid from the reservoir when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position.

17. The cytology microarray maker of claim 15 or 16 wherein the maker further comprises a third stage sized to support a cytology microarray and the at least one axial member is configured to move the array of tips between the first, second, and third stages.

18. The cytology microarray maker of any one of claims 15 to 17 wherein the maker is stationary and the frame is sized to fit on a substantially flat surface.

19. The cytology microarray maker of any one of claims 15 to 18 wherein the at least one axial member comprises two rails extending along the frame.

20. The cytology microarray maker of claim 19 wherein the two rails form a part of the frame.

21. The cytology microarray maker of claim 19 wherein the upright members comprise two substantially planar elements that are slidably connected to the two rails and situated on either side of the stages, the substantially planar elements comprising

corresponding elongated axial channels configured to slidably receive projections extending from the body, and at least one of the frame and the upright members comprises an operably connected body biasing element urging the body away from the stages.

22. The cytology microarray maker of any one of claims 15 to 21 wherein the stages are substantially planar stands and further comprise at least x-axis and y-axis adjustment mechanisms configured to adjust positions of the stages relative to at least one of the frame and each other.

23. The cytology microarray maker of any one of claims 15 to 22 wherein the body comprises a plurality of floating channels each sized to releasably hold one tip.

24. The cytology microarray maker of any one of claims 15 to 23 wherein the maker is substantially automated.

25. The cytology microarray maker of any one of claims 15 to 23 wherein the maker is substantially manually operated.

26. A method of dispensing a microvolume of liquid comprising:

a) providing a microvolume liquid dispenser tip containing the liquid, the tip comprising an outer sleeve and a reciprocating pin located within the outer sleeve, wherein the outer sleeve comprises a distal opening and the pin reciprocates relative to the sleeve between a distal position wherein a distal tip of the pin extends beyond the distal opening and a proximal position, wherein the outer sleeve and the reciprocating pin are configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position;

b) transiently contacting the distal tip and distal opening with a substrate thereby causing the pin to cycle; and,

c) during the cycle, dispensing the liquid to the substrate.

27. The method of claim 26 wherein the sleeve and pin are configured to cooperatively dispense a volume per cycle that is suitable for a cytology microarray, the substrate is a cytology platform, and the method comprises dispensing a spot of cell-containing liquid on the platform sized for the cytology microarray.

28. The method of claim 26 or 27 wherein the passage is sized to substantially avoid clogging by the cells.

29. The method of any one of claims 26 to 28 wherein the sleeve and pin are configured to cooperatively dispense about 0.1 μl per cycle.

30. The method of any one of claims 26 to 29 wherein an internal surface of the sleeve is substantially frustoconical and an outer surface of the pin is correspondingly substantially frustoconical.

31. The method of any one of claims 26 to 30 wherein the microvolume liquid dispenser tip is one of an array of the microvolume liquid dispenser tips, the tips and array configured and sized to make a cytology microarray, and the method further comprises substantially simultaneously transiently contacting the array of tips with a cytology microarray platform, thereby causing the pin to cycle, and thereby forming the cytology microarray on the platform.

32. The method of any one of claims 26 to 31 wherein the method further comprises, before providing the tip containing the liquid, loading the liquid into the tip by placing the tip into a source of the liquid and suctioning up the liquid using capillary action.

33. The method of any one of claims 26 to 32 wherein the method further comprises, before providing the tip containing the liquid, loading the liquid into the tip through a proximal opening located at a proximal area of the tip.

34. A method of making a cytology microarray comprising:

a) providing a frame holding a body holding an array of microvolume liquid dispenser tips, at least first and second stages sized to support cytology microarrays, upright members operably attached to the body to move the body and the array of tips substantially normal to the stages between at least an extended position wherein the tips contact a cytology microarray substrate located on the stage and a retracted position wherein the tips do not contact the cytology microarray substrate, and at least one axial member disposed along the frame and operably connected to the upright members to move the upright members, the body and the array of tips between the first and the second stage, wherein the first stage holds a cytology microarray template comprising an array of liquid cytological specimens and the second stage holds a cytology microarray substrate;

b) loading the array of tips with the liquid cytological specimens by transiently moving the array of tips into the liquid cytological specimens and suctioning up the liquid cytological specimens using capillary action;

c) moving the array of tips along the axial member to the second stage; and

d) making the cytology array by transiently contacting the array of tips with the cytology microarray substrate.

35. The method of claim 34 wherein the microvolume liquid dispenser tips comprise an outer sleeve and a reciprocating pin located within the outer sleeve, the outer sleeve comprising a distal opening and the pin reciprocating relative to the sleeve between at least a distal position wherein a distal tip of the pin extends slightly beyond the distal opening and a proximal position, wherein an inner surface of the sleeve and an outer surface of the pin are configured to cooperatively form a reservoir

configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid from the reservoir when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position, and wherein the transient contacting causes the pin to cycle.

36. The method of claim 34 or 35 wherein the frame further comprises a third stage holding a cytology microarray substrate and the at least one axial member is configured to move the array of tips between the first, second and third stages, and the method further comprises moving the array of tips along the axial member to the third stage; and making a second cytology array by transiently contacting the array of tips with the second cytology microarray substrate.

37. The method of claim 36 wherein the second cytology array is made without reloading the tips.

38. The method of any one of claims 34 to 37 wherein the at least one axial member comprises two rails extending along the frame, the upright members comprise two substantially planar elements slidably connected to the two rails and situated on either side of the stages, the substantially planar elements comprising corresponding elongated axial channels configured to slidably receive projections extending from the body, and the method comprises sliding the upright members along the two rails between the cytology microarray template and substrate, and then pushing the array downwardly to contact the cytology microarray template and substrate, respectively.

39. The method of any one of claims 34 to 38 wherein the method further comprises adjusting the stages on at least one of an x-axis and a y-axis relative to at least one of the frame and each other.

40. The method of any one of claims 34 to 39 wherein the body comprises a plurality of floating channels each sized to releasably hold one tip, and the method further comprises placing the tips in the body to create the array of tips and removing the tips from the body after making the cytology array.

41. The method of any one of claims 34 to 40 wherein the method further comprises removing the cytology array template and the cytology array from the stages then placing new cytology array substrates on the stages and making additional cytology arrays.

42. The method of claim 41 wherein the additional cytology arrays are made without reloading the tips.

43. The method of any one of claims 34 to 42 wherein the method is substantially automated.

44. The method of any one of claims 34 to 42 wherein the method is substantially manual.

45. A tip means for microvolume liquid dispensing comprising:

a) an outer sleeve means for holding the liquid,

b) a reciprocating pin means located within the outer sleeve for cooperatively dispensing, through a passage formed between a side of the outer sleeve means and the pin means, a predetermined microvolume amount of liquid when the pin moves in a cycle from a distal position to a proximal position then returns to a distal position.

46. A means for making cytology microarrays comprising:

a) a frame means for holding a body means,

b) the body means for holding an array of tips means for dispensing a microvolume of liquid,

c) at least two stage means for supporting cytology microarrays,

d) at least two upright member means operably attached to the body for moving the body means substantially normal to the stage means, and

e) at least one axial member means disposed along the frame and operably connected to the upright members for moving the upright member means between the two stage means.

47. A method of dispensing a microvolume of liquid comprising the steps of:

a) a step of providing a microvolume liquid dispenser tip means containing the liquid, the tip comprising an outer sleeve and a reciprocating pin located within the outer sleeve, wherein the outer sleeve comprises a distal opening and the pin reciprocates relative to the sleeve between a distal position wherein a distal tip of the pin extends beyond the distal opening and a proximal position, wherein the outer sleeve and the reciprocating pin are configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position;

b) transiently contacting the distal tip and distal opening with a substrate thereby causing the pin to cycle; and,

c) during the cycle, dispensing the liquid onto the substrate.

48. The method of claim 47 wherein the sleeve means and pin means are configured for cooperatively dispensing a volume per cycle that is suitable for a cytology microarray, and the method comprises the step of dispensing a spot of cell-containing liquid sized for the cytology microarray.

[Received by the International Bureau on 20 November 2002 (20.11.02): original claims 11 to 48 replaced by amended claims 11 to 52 (9 pages)]

11. The microvolume liquid dispenser tip of any one of claims 8 to 10 wherein the passage is sized to substantially avoid clogging by cells when the liquid is a cytological fluid.

12. The microvolume liquid dispenser tip of any one of claims 8 to 11 wherein the sleeve and pin are configured to cooperatively dispense about 0.1 µl per cycle.

13. The microvolume liquid dispenser tip of any one of claims 8 to 12 wherein the distal opening has a diameter from about 0.5 mm to 1.5 mm.

14. The microvolume liquid dispenser tip of any one of claims 8 to 13 wherein the tip is one of an array of the microvolume liquid dispenser tips, the array configured and sized to make a cytology microarray.

15. The microvolume liquid dispenser of any one of claims 1 to 14 wherein the reciprocating pin comprises a shoulder and an inner surface of outer sleeve is substantially frustoconical, wherein the shoulder contacts the inner surface to form the reservoir.

16. The microvolume liquid dispenser of any one of claims 1 to 6, 8 to 14 and 15 wherein the reciprocating pin and the outer sleeve are attached to no additional elements that cause reciprocation of the reciprocating pin relative to the outer sleeve.

17. The microvolume liquid dispenser of any one of claims 1 to 6, 8 to 14 and 15 wherein the dispenser does not comprise any biasing element operably connected to any of the pin and the outer sleeve and configured to urge the pin toward the distal position.

18. The microvolume liquid dispenser of any one of claims 1 to 6, 8 to 14 and 15 wherein the reciprocating pin and the outer sleeve are configured such that the reciprocating pin is reciprocated relative to the outer sleeve by transiently contacting the reciprocating pin with a desired substrate to push the reciprocating pin up into the outer sleeve, and wherein the predetermined microvolume amount of liquid is dispensed simultaneously with the transient contact.

19. A cytology microarray maker comprising
a frame, the frame operably connected to
a body holding an array of microvolume liquid dispenser tips,
at least two stages sized to support cytology microarrays,
upright members operably attached to the body to move the body and the array of tips substantially normal to the stages between at least an extended position wherein the tips contact a cytology microarray substrate located on the stage and a retracted position wherein the tips do not contact the cytology microarray substrate, and
at least one axial member disposed along the frame and operably connected to the upright members to provide a track along which the upright members, the body and the array of tips is movable along the track between the first and the second stage.

20. The cytology microarray maker of claim 19 wherein the microvolume liquid dispenser tips comprise an outer sleeve and a reciprocating pin located within the outer sleeve, the outer sleeve comprising a distal opening and the pin reciprocating relative to the sleeve between at least a distal position wherein a distal tip of the pin extends slightly beyond the distal opening and a proximal position, wherein an inner surface of the sleeve and an outer surface of the pin are configured to cooperatively form a reservoir when the pin is in the distal position and wherein the sleeve and pin are configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid

from the reservoir when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position.

21. The cytology microarray maker of claim 19 or 20 wherein the maker further comprises a third stage sized to support a cytology microarray and the at least one axial member is configured to move the array of tips between the first, second, and third stages.

22. The cytology microarray maker of any one of claims 19 to 21 wherein the maker is stationary and the frame is sized to fit on a substantially flat surface.

23. The cytology microarray maker of any one of claims 19 to 22 wherein the at least one axial member comprises two rails extending along the frame.

24. The cytology microarray maker of claim 23 wherein the two rails form a part of the frame.

25. The cytology microarray maker of claim 23 wherein the upright members comprise two substantially planar elements that are slidably connected to the two rails and situated on either side of the stages, the substantially planar elements comprising corresponding elongated axial channels configured to slidably receive projections extending from the body, and at least one of the frame and the upright members comprises an operably connected body biasing element urging the body away from the stages.

26. The cytology microarray maker of any one of claims 19 to 25 wherein the stages are substantially planar stands and further comprise at least x-axis and y-axis adjustment mechanisms configured to adjust positions of the stages relative to at least one of the frame and each other.

27. The cytology microarray maker of any one of claims 19 to 26 wherein the body comprises a plurality of floating channels each sized to releasably hold one tip.

28. The cytology microarray maker of any one of claims 19 to 27 wherein the maker is substantially automated.

29. The cytology microarray maker of any one of claims 19 to 27 wherein the maker is substantially manually operated.

30. A method of dispensing a microvolume of liquid comprising:

a) providing a microvolume liquid dispenser tip containing the liquid, the tip comprising an outer sleeve and a reciprocating pin located within the outer sleeve, wherein the outer sleeve comprises a distal opening and the pin reciprocates relative to the sleeve between a distal position wherein a distal tip of the pin extends beyond the distal opening and a proximal position, wherein the outer sleeve and the reciprocating pin are configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position;

b) transiently contacting the distal tip and distal opening with a substrate thereby causing the pin to cycle; and,

c) during the cycle, dispensing the liquid to the substrate.

31. The method of claim 30 wherein the sleeve and pin are configured to cooperatively dispense a volume per cycle that is suitable for a cytology microarray, the substrate is a cytology platform, and the method comprises dispensing a spot of cell-containing liquid on the platform sized for the cytology microarray.

32. The method of claim 30 or 31 wherein the passage is sized to substantially avoid clogging by the cells.

33. The method of any one of claims 30 to 32 wherein the sleeve and pin are configured to cooperatively dispense about 0.1 µl per cycle.

34. The method of any one of claims 30 to 33 wherein an internal surface of the sleeve is substantially frustoconical and an outer surface of the pin is correspondingly substantially frustoconical.

35. The method of any one of claims 30 to 34 wherein the microvolume liquid dispenser tip is one of an array of the microvolume liquid dispenser tips, the tips and array configured and sized to make a cytology microarray, and the method further comprises substantially simultaneously transiently contacting the array of tips with a cytology microarray platform, thereby causing the pin to cycle, and thereby forming the cytology microarray on the platform.

36. The method of any one of claims 30 to 35 wherein the method further comprises, before providing the tip containing the liquid, loading the liquid into the tip by placing the tip into a source of the liquid and suctioning up the liquid using capillary action.

37. The method of any one of claims 30 to 36 wherein the method further comprises, before providing the tip containing the liquid, loading the liquid into the tip through a proximal opening located at a proximal area of the tip.

38. A method of making a cytology microarray comprising:

a) providing a frame holding a body holding an array of microvolume liquid dispenser tips, at least first and second stages sized to support cytology microarrays, upright members operably attached to the body to move the body and the array of tips substantially normal to the stages between at least

an extended position wherein the tips contact a cytology microarray substrate located on the stage and a retracted position wherein the tips do not contact the cytology microarray substrate, and at least one axial member disposed along the frame and operably connected to the upright members to move the upright members, the body and the array of tips between the first and the second stage, wherein the first stage holds a cytology microarray template comprising an array of liquid cytological specimens and the second stage holds a cytology microarray substrate;

b) loading the array of tips with the liquid cytological specimens by transiently moving the array of tips into the liquid cytological specimens and suctioning up the liquid cytological specimens using capillary action;

c) moving the array of tips along the axial member to the second stage; and

d) making the cytology array by transiently contacting the array of tips with the cytology microarray substrate.

39. The method of claim 38 wherein the microvolume liquid dispenser tips comprise an outer sleeve and a reciprocating pin located within the outer sleeve, the outer sleeve comprising a distal opening and the pin reciprocating relative to the sleeve between at least a distal position wherein a distal tip of the pin extends slightly beyond the distal opening and a proximal position, wherein an inner surface of the sleeve and an outer surface of the pin are configured to cooperatively form a reservoir when the pin is in the distal position and wherein the sleeve and pin are configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid from the reservoir when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position, and wherein the transient contacting causes the pin to cycle.

40. The method of claim 38 or 39 wherein the frame further comprises a third stage holding a cytology microarray substrate and the at least one axial

member is configured to move the array of tips between the first, second and third stages, and the method further comprises moving the array of tips along the axial member to the third stage; and making a second cytology array by transiently contacting the array of tips with the second cytology microarray substrate.

41. The method of claim 40 wherein the second cytology array is made without reloading the tips.

42. The method of any one of claims 38 to 41 wherein the at least one axial member comprises two rails extending along the frame, the upright members comprise two substantially planar elements slidably connected to the two rails and situated on either side of the stages, the substantially planar elements comprising corresponding elongated axial channels configured to slidably receive projections extending from the body, and the method comprises sliding the upright members along the two rails between the cytology microarray template and substrate, and then pushing the array downwardly to contact the cytology microarray template and substrate, respectively.

43. The method of any one of claims 38 to 42 wherein the method further comprises adjusting the stages on at least one of an x-axis and a y-axis relative to at least one of the frame and each other.

44. The method of any one of claims 38 to 43 wherein the body comprises a plurality of floating channels each sized to releasably hold one tip, and the method further comprises placing the tips in the body to create the array of tips and removing the tips from the body after making the cytology array.

45. The method of any one of claims 38 to 44 wherein the method further comprises removing the cytology array template and the cytology array from

the stages then placing new cytology array substrates on the stages and making additional cytology arrays.

46. The method of claim 45 wherein the additional cytology arrays are made without reloading the tips.

47. The method of any one of claims 38 to 46 wherein the method is substantially automated.

48. The method of any one of claims 38 to 46 wherein the method is substantially manual.

49. A tip means for microvolume liquid dispensing comprising:

a) an outer sleeve means for holding the liquid,

b) a reciprocating pin means located within the outer sleeve for cooperatively dispensing, through a passage formed between a side of the outer sleeve means and the pin means, a predetermined microvolume amount of liquid when the pin moves in a cycle from a distal position to a proximal position then returns to a distal position.

50. A means for making cytology microarrays comprising:

a) a frame means for holding a body means,

b) the body means for holding an array of tips means for dispensing a microvolume of liquid,

c) at least two stage means for supporting cytology microarrays,

d) at least two upright member means operably attached to the body for moving the body means substantially normal to the stage means, and

e) at least one axial member means disposed along the frame and operably connected to the upright members for moving the upright member means between the two stage means.

51. A method of dispensing a microvolume of liquid comprising the steps of:

a) a step of providing a microvolume liquid dispenser tip means containing the liquid, the tip comprising an outer sleeve and a reciprocating pin located within the outer sleeve, wherein the outer sleeve comprises a distal opening and the pin reciprocates relative to the sleeve between a distal position wherein a distal tip of the pin extends beyond the distal opening and a proximal position, wherein the outer sleeve and the reciprocating pin are configured to cooperatively dispense, through a passage formed between a side of the distal opening and the pin, a predetermined microvolume amount of liquid when the pin moves in a cycle from the distal position to the proximal position then returns to the distal position;

b) transiently contacting the distal tip and distal opening with a substrate thereby causing the pin to cycle; and,

c) during the cycle, dispensing the liquid onto the substrate.

52. The method of claim 51 wherein the sleeve means and pin means are configured for cooperatively dispensing a volume per cycle that is suitable for a cytology microarray, and the method comprises the step of dispensing a spot of cell-containing liquid sized for the cytology microarray.

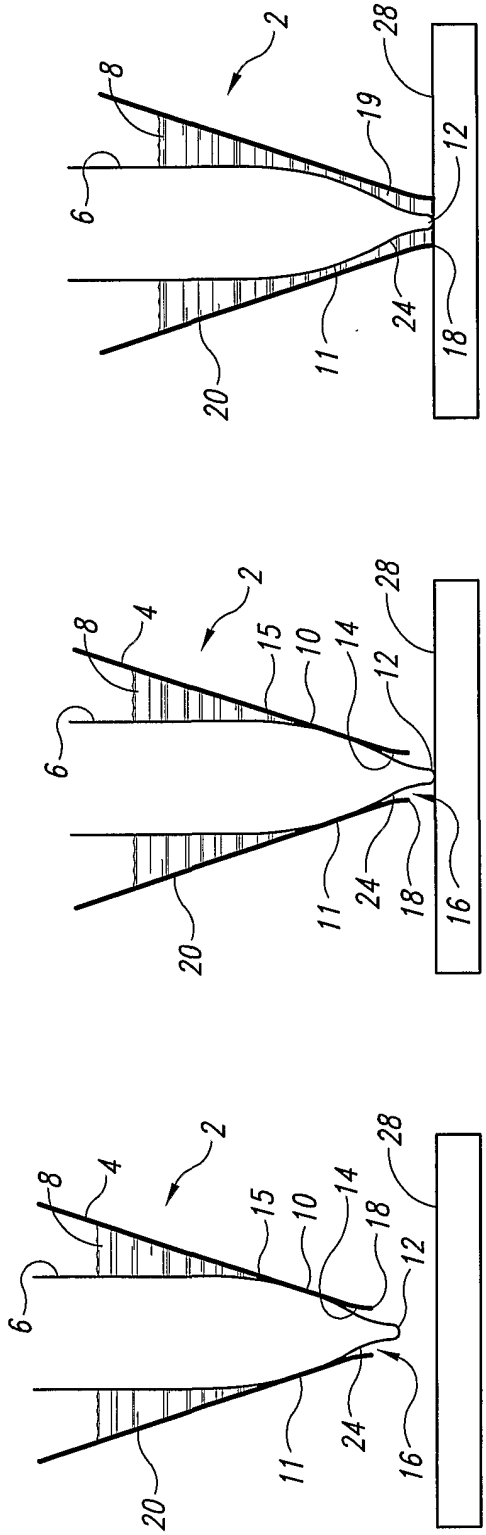


Fig. 1A

Fig. 1B

Fig. 1C

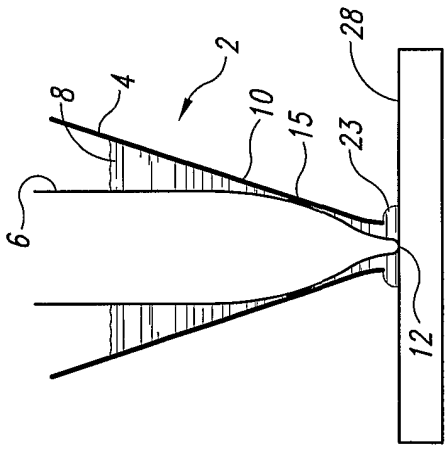


Fig. 1D

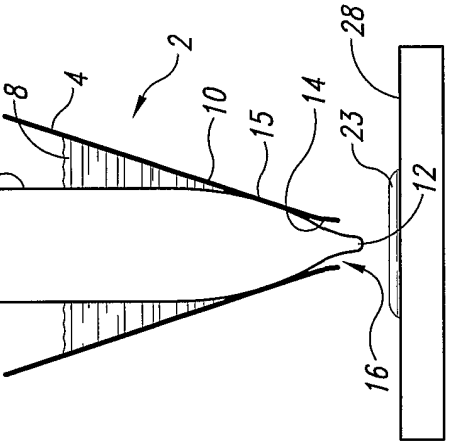


Fig. 1E

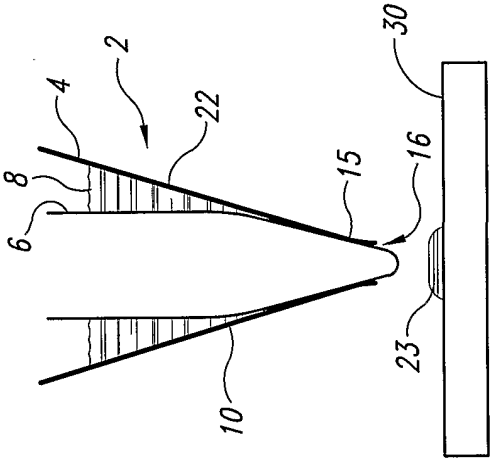


Fig. 2C

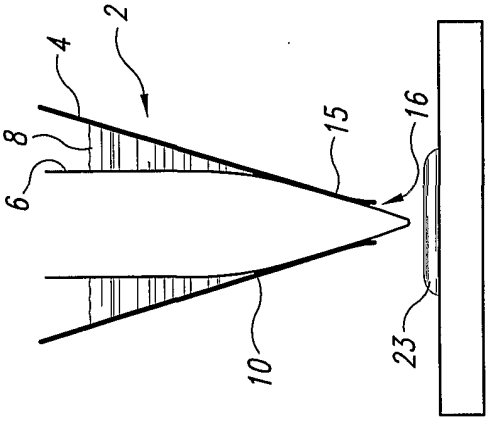


Fig. 3C

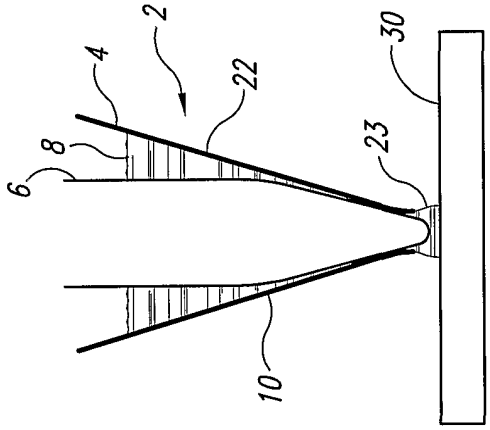


Fig. 2B

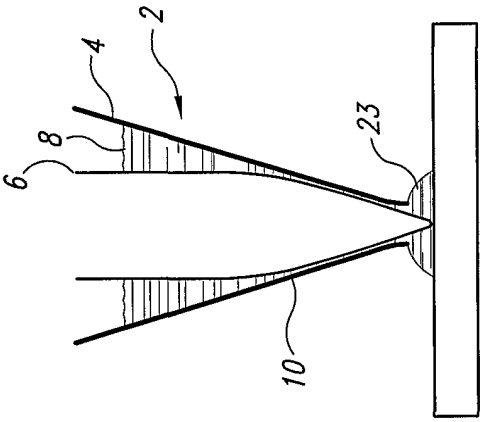


Fig. 3B

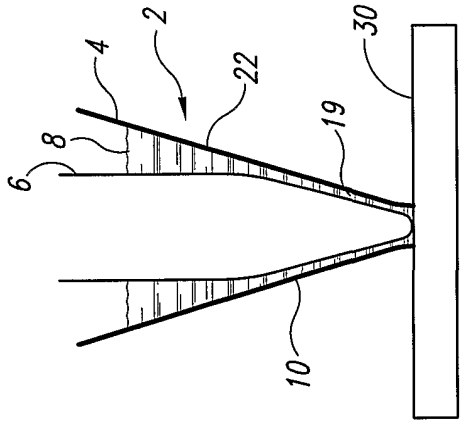


Fig. 2A

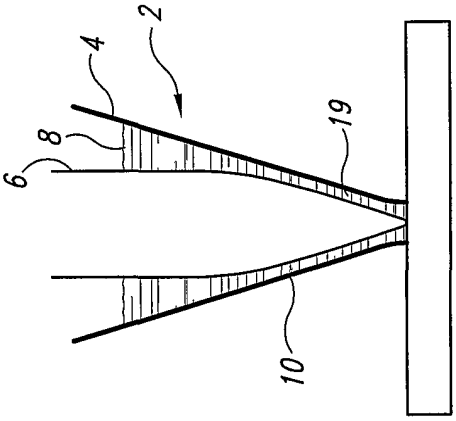


Fig. 3A

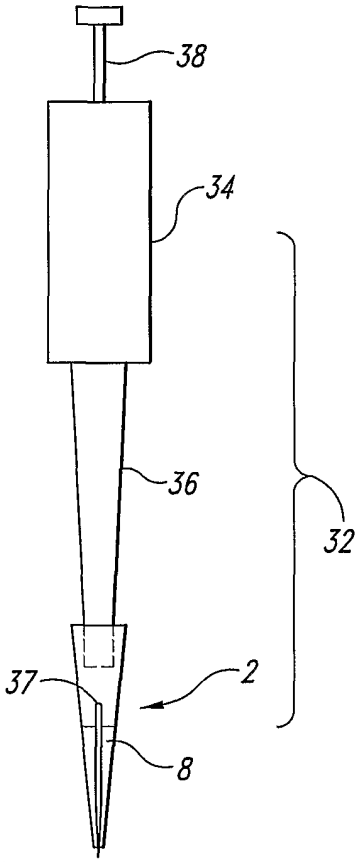


Fig. 4

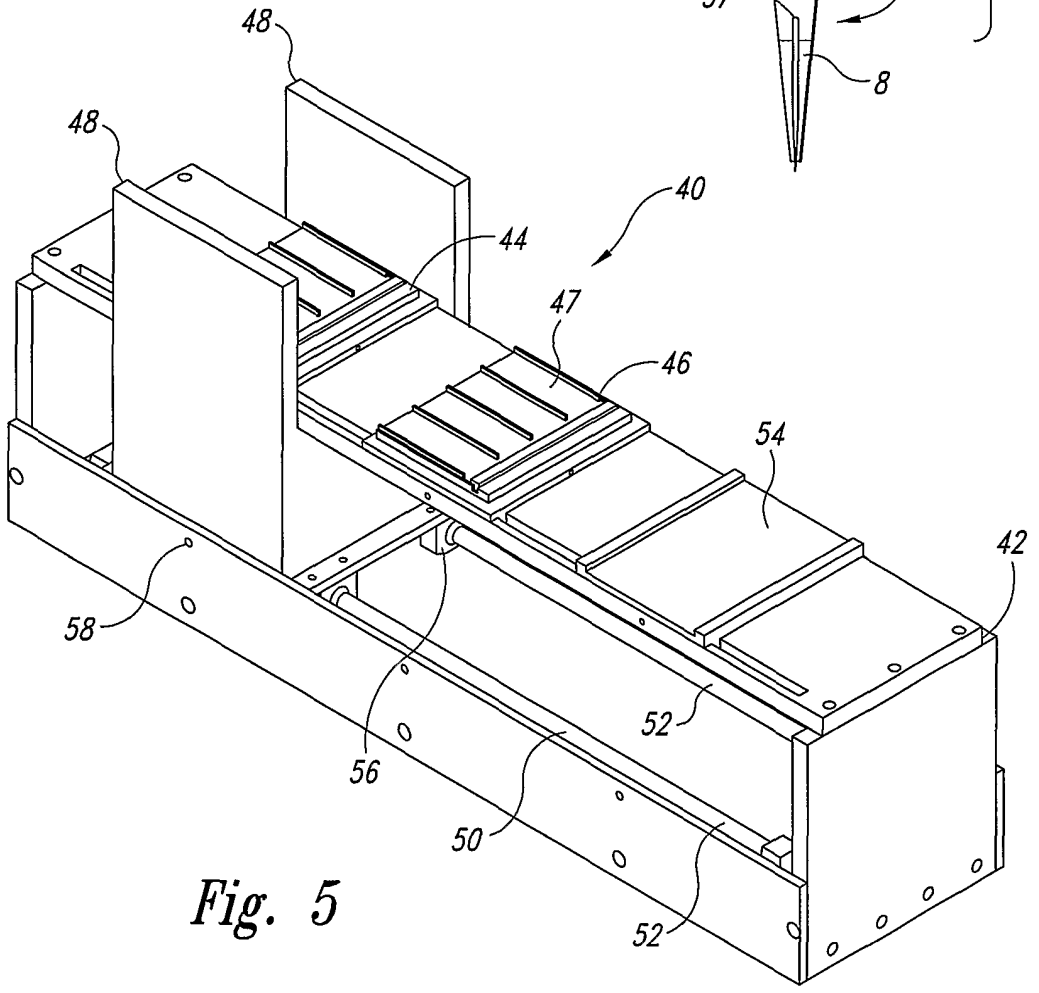


Fig. 5

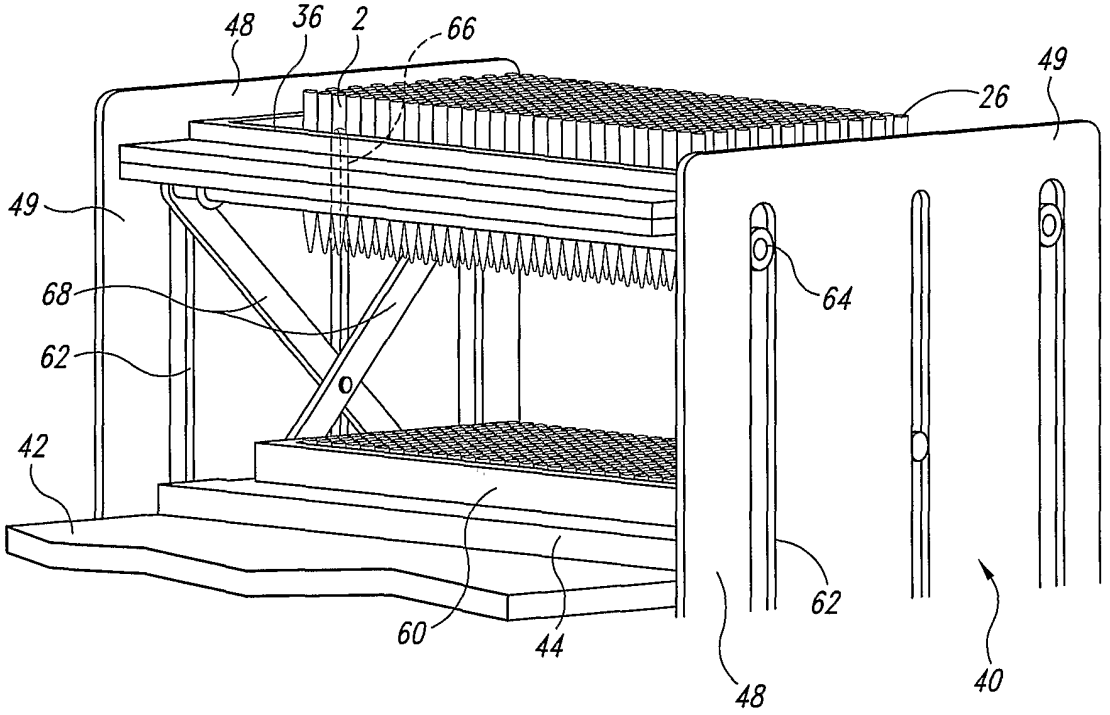

48
36
2
66
49
26
49
68
62
42
64
62
60
44
48
40

Fig. 6

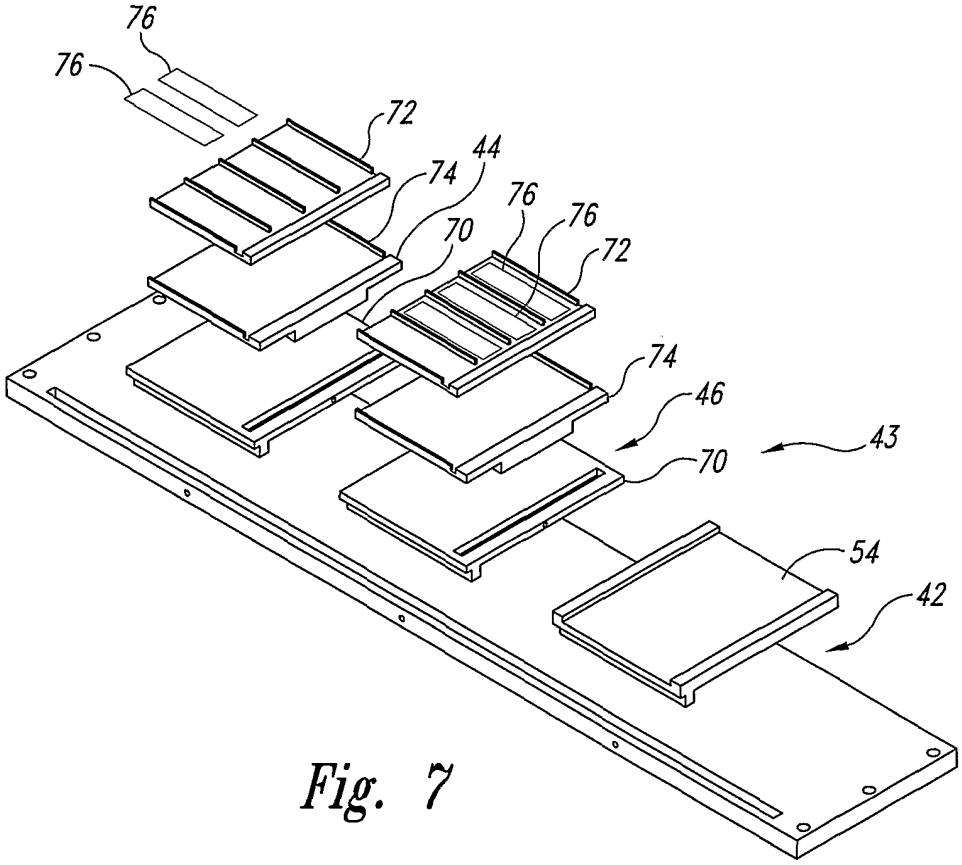

76
76
72
44
74
76
76
70
72
74
46
43
70
54
42

Fig. 7

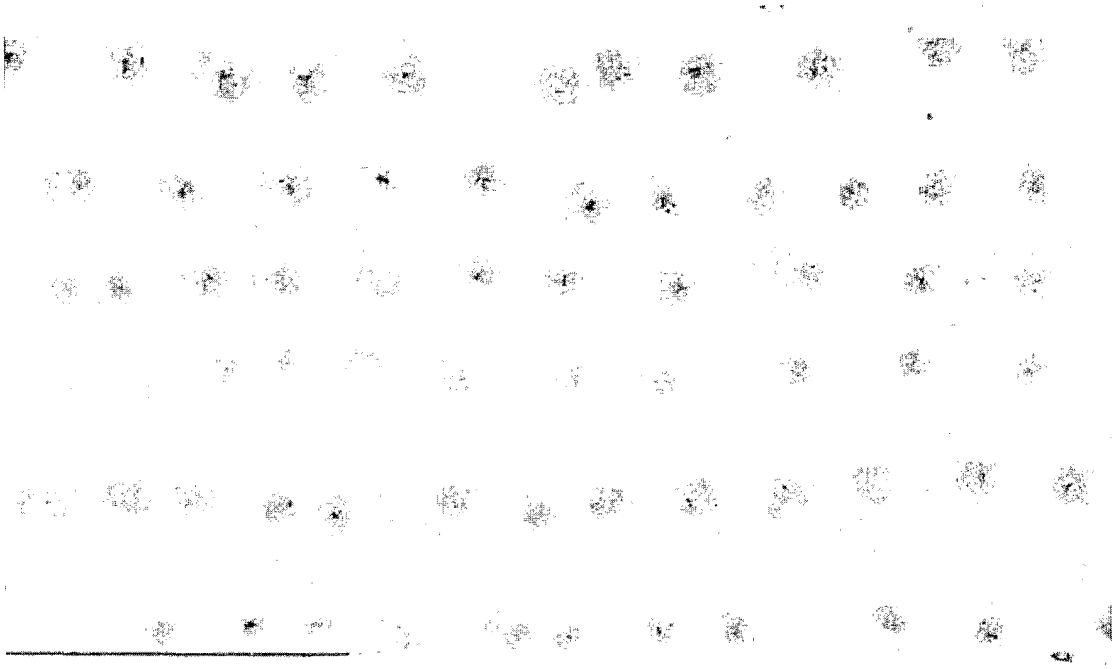


Fig. 8
(Prior Art)

Fig. 9
(Prior Art)

Fig. 10

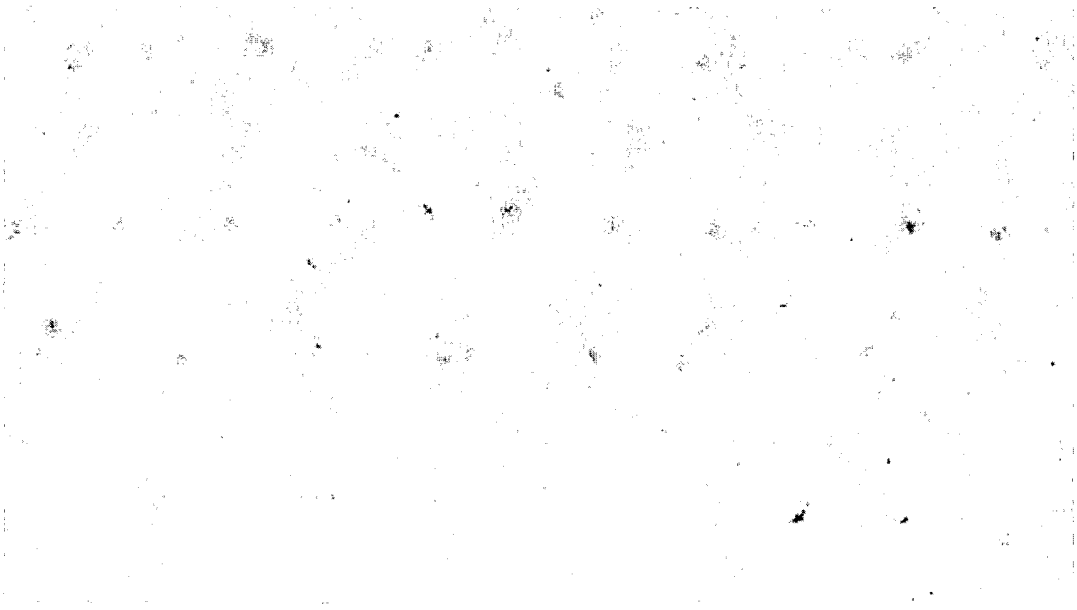


Fig. 11

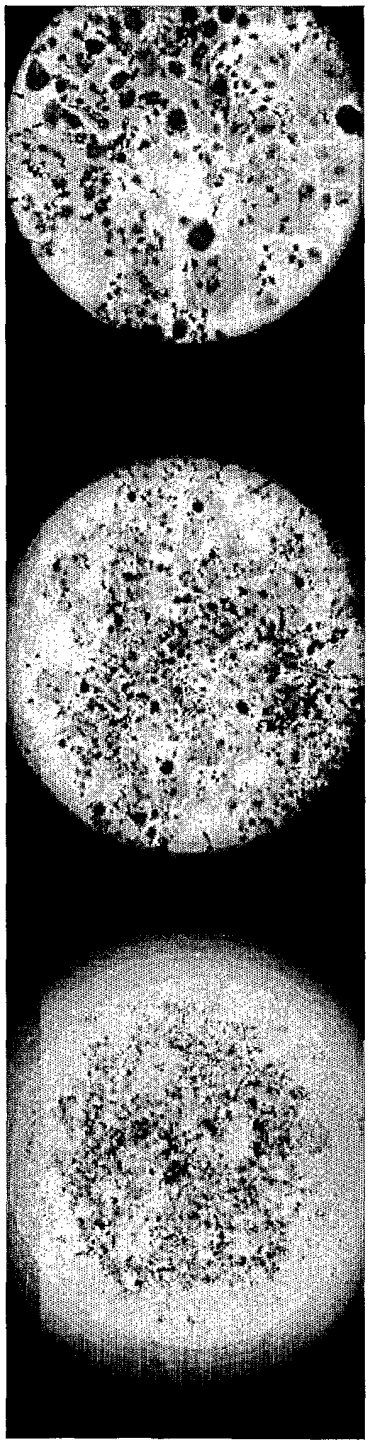


Fig. 12

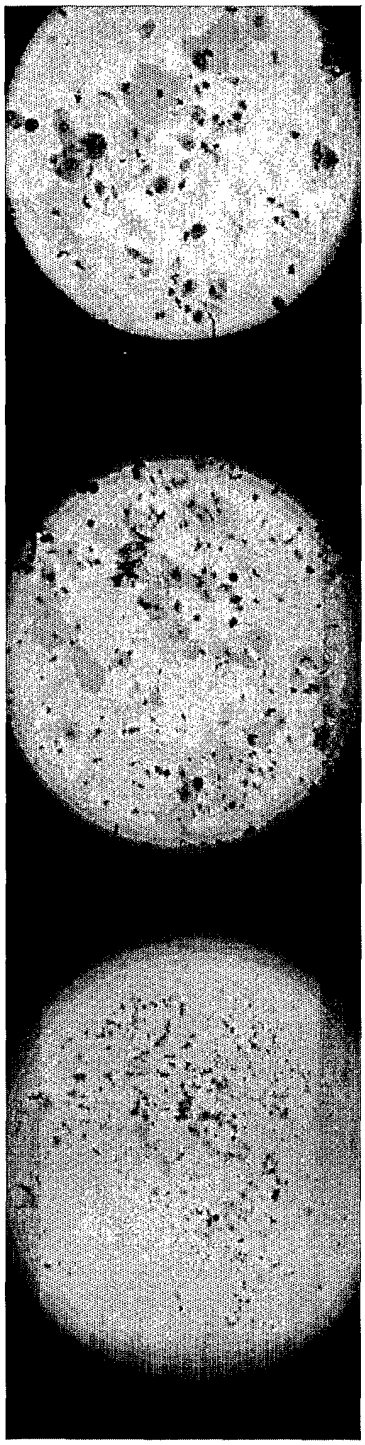


Fig. 13

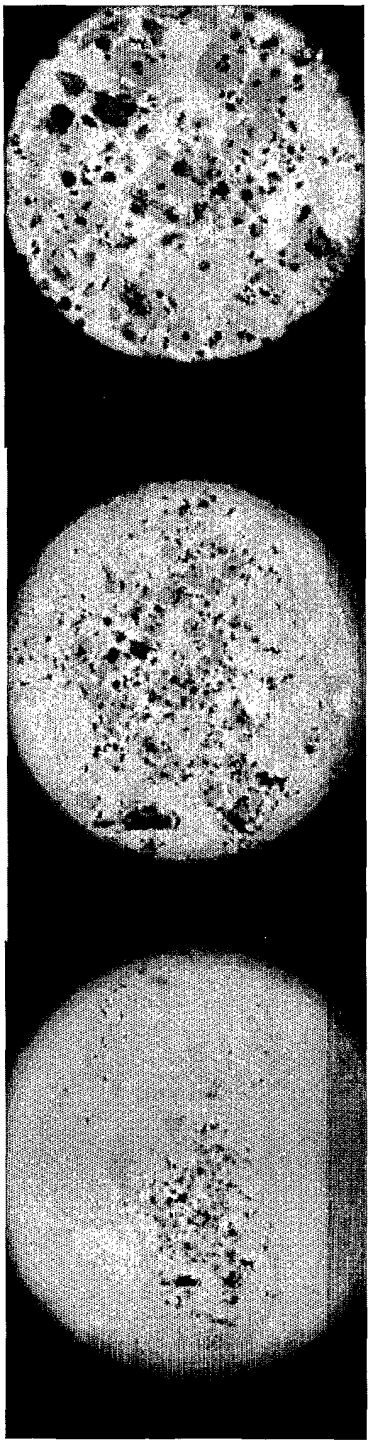


Fig. 14

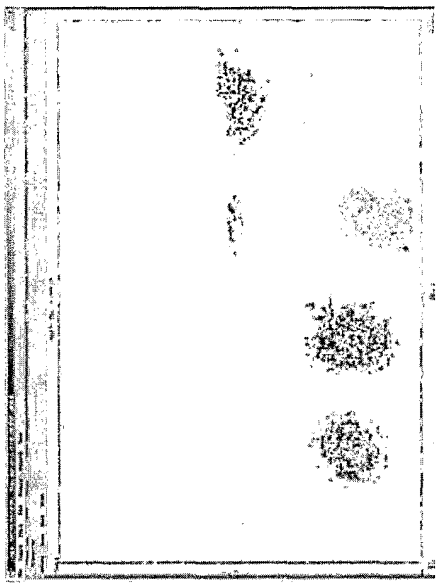


Fig. 15B

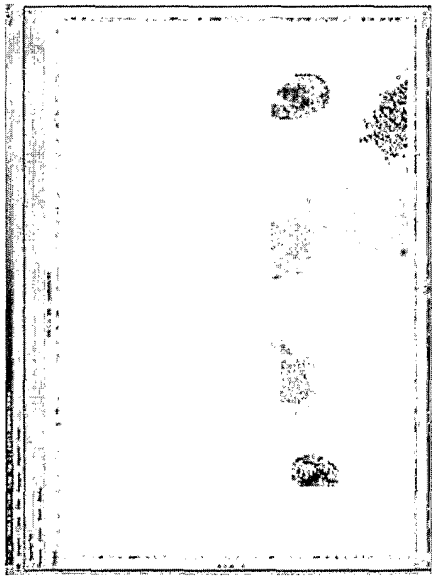


Fig. 15A

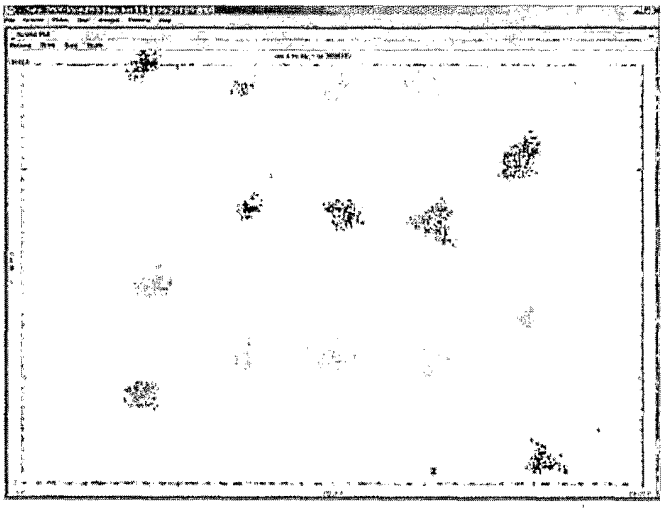


Fig. 16A

Fig. 16B

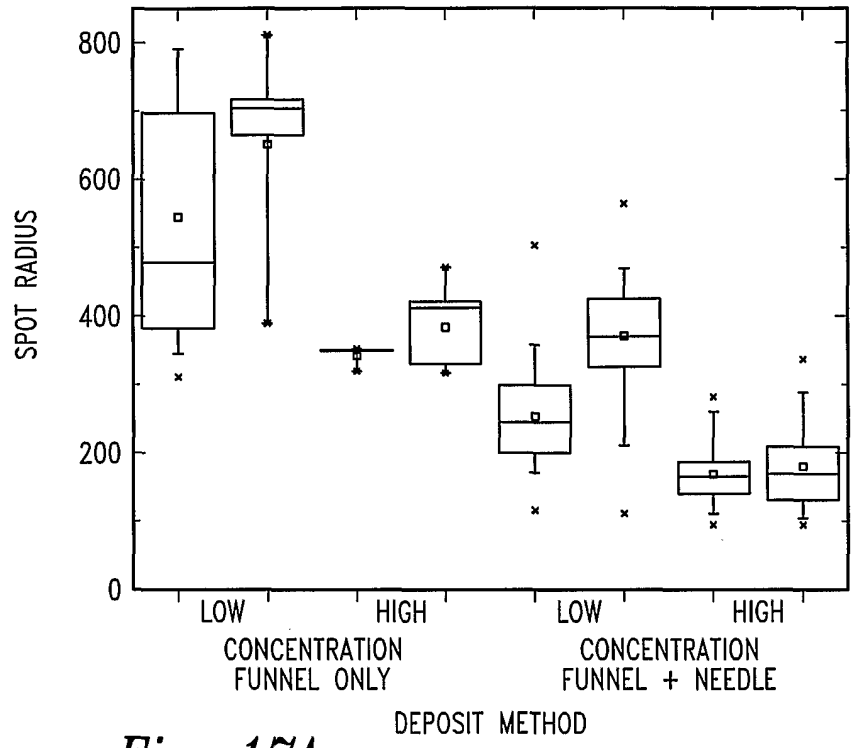


Fig. 17A

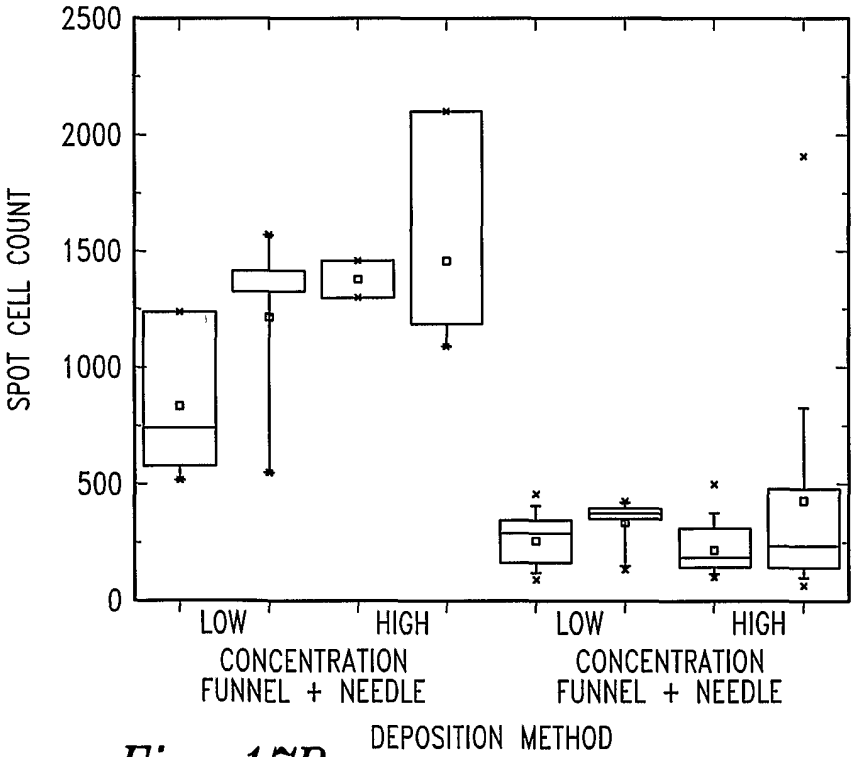


Fig. 17B

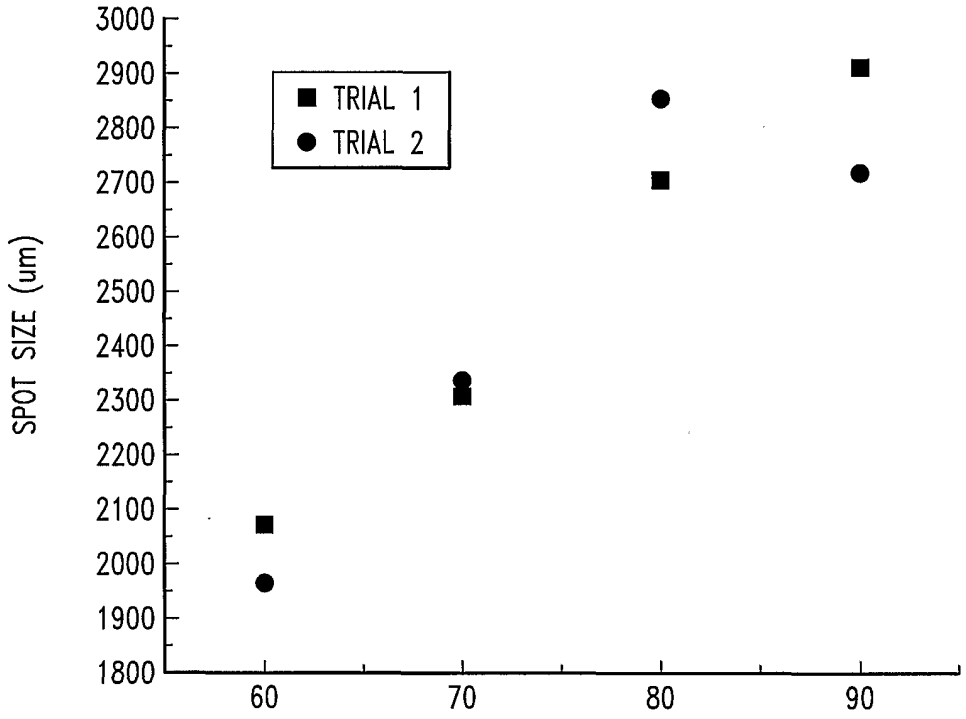

SPOT SIZE (um)
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
60
70
80
90
TRIAL 1
TRIAL 2

Fig. 18

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 02/00922

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/02 G01N35/10

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 7 B01L G01N B41F H04N

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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 075 869 A (THK CO LTD) 14 February 2001 (2001-02-14)	1,3-9, 11-14, 26-33, 45,47,48
Y	column 3, line 21 -column 3, line 22 column 7, line 13 -column 16, line 33; figures 1-7,10	16-24, 35-44
Y	WO 00 54883 A (PERKIN ELMER CORP) 21 September 2000 (2000-09-21) page 21, line 7 -page 26, line 21; figure 8	16-24, 35-44
A	US 5 540 889 A (CHRISTOPHER ANTHONY J ET AL) 30 July 1996 (1996-07-30) abstract; figures column 1, line 45 -column 3, line 2	1-48

-/--

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

[X] Patent family members are listed in 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 document but published on or after the international filing date

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 September 2002	20/09/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Smith-Hewitt, L

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 02/00922

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 133 918 A (SIMMS DOUGLAS P ET AL) 9 January 1979 (1979-01-09) column 1, line 62 -column 3, line 40; figures	1,6-8, 26,45,47
X	US 3 329 964 A (ULDIS KLAVSONS ET AL) 4 July 1967 (1967-07-04) column 3, line 32 -column 7, line 15; figures	1,6-8, 26,45,47
X	US 4 023 716 A (SHAPIRO JUSTIN JOEL) 17 May 1977 (1977-05-17) column 2, line 17 -column 4, line 28; figures	2,25,44
X	EP 1 070 540 A (COSMOTEC CO LTD) 24 January 2001 (2001-01-24) column 4, line 17 -column 7, line 21; figures 1-3	15,34,46
A	US 5 021 217 A (OSHIKUBO YUJU) 4 June 1991 (1991-06-04) column 2, line 58 -column 7, line 45; figures	2,25,44
A	WO 99 44062 A (SAUTER GUIDO ;US HEALTH (US); KONONEN JUHA (US); KALLIONIEMI OLLI) 2 September 1999 (1999-09-02) the whole document	15-24, 34-44

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. Claims: 1-14,16-25,26-33,35-44,45,47-48

   A microvolume dispenser comprising a body, an outer sleeve and a reciprocating pin and method of use thereof.

2. Claims: 15,17-25,34,36-44,46

   A cytology microarray maker comprising a frame, a body holding an array of dispenser tips, two stages for cytology microarrays, upright members attached to the body and an axial member disposed along the frame, and a method of use thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 02/00922

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 02/00922

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1075869 A	14-02-2001	JP 2001046062 A	20-02-2001
		JP 2001050959 A	23-02-2001
		EP 1075869 A1	14-02-2001
WO 0054883 A	21-09-2000	US 6296702 B1	02-10-2001
		AU 3745400 A	04-10-2000
		EP 1156880 A1	28-11-2001
		WO 0054883 A1	21-09-2000
		US 2001009136 A1	26-07-2001
		US 2001016359 A1	23-08-2001
		US 2001010206 A1	02-08-2001
US 5540889 A	30-07-1996	AT 191370 T	15-04-2000
		CA 2190184 A1	23-11-1995
		DE 69516134 D1	11-05-2000
		DE 69516134 T2	11-01-2001
		EP 0758923 A1	26-02-1997
		WO 9531284 A1	23-11-1995
US 4133918 A	09-01-1979	NONE	
US 3329964 A	04-07-1967	GB 1095098 A	13-12-1967
US 4023716 A	17-05-1977	DE 2648037 A1	03-11-1977
EP 1070540 A	24-01-2001	JP 2001033463 A	09-02-2001
		EP 1070540 A2	24-01-2001
US 5021217 A	04-06-1991	JP 2169043 A	29-06-1990
		JP 2701900 B2	21-01-1998
WO 9944062 A	02-09-1999	AU 2875799 A	15-09-1999
		AU 2973599 A	15-09-1999
		CA 2318789 A1	02-09-1999
		CA 2318984 A1	02-09-1999
		EP 1068528 A2	17-01-2001
		EP 1066517 A1	10-01-2001
		JP 2002505431 T	19-02-2002
		JP 2002505432 T	19-02-2002
		WO 9944062 A1	02-09-1999
		WO 9944063 A2	02-09-1999
		EP 1131470 A1	12-09-2001
		WO 0024940 A1	04-05-2000