

A METHOD AND APPARATUS FOR MICROARRAYING OF BIOLOGICAL FLUIDS SAMPLES ON BIOLOGICAL SUBSTRATES

Field of the Invention

[001] The present invention generally relates to the field of depositing microarrays of biological fluid samples on biological substrates. In particular, the invention relates to a method and apparatus for improved throughput and quality of depositing biological fluid samples in a microarray on biological substrates.

Background of the Invention

[002] With increase research and development into genomics, demands on microarraying technology have also escalated significantly. Microarraying or the replication of thousands of biological fluid samples in small microarrays on biological substrates for gene expression verification and validation has long been in use in the fields of life sciences. The biological fluid samples are often mere spots measuring less than 100 microns across and 150 microns to 200 microns from each other. The quality of these spots as well as the speed and throughput of the production of the microarrays are now a growing concern.

[003] Conventional systems of microarraying involve the use of robotic systems depositing multiple spots of DNA (deoxyribonucleic acid) samples on a biological substrate of which an example is a chemically coated glass slide. The robotic systems typically use manipulators with end effectors having multiple spotting pins for dipping into a sample tray picking up biological fluid samples contained therein before depositing or spotting the biological fluid samples onto the biological substrates. Multiple spottings may be performed before the biological fluid samples on the spotting pins are depleted and the pins have to re-dip into the sample tray to pick up more biological fluid samples. Such

chemically coated glass slides containing the microscopic arrays of deposited spots comprising DNA samples are commonly referred to as DNA chips.

[004] Present systems by increasing number of spotting pins on the end effector have attempted to increase the production rate of the DNA chips. Sample trays having multiple compartments or wells allowing a variety of biological fluid samples to be deposited at each spotting have also been designed and utilized to increase production rates. Sample trays have developed into array trays having multiple wells of which the common ones are: 96 well and 384 well array plates.

[005] Studies have also shown that the throughput and quality of DNA chips are also affected by the need to wash the spotting pins properly at regular intervals with appropriate specified time duration. Spotting of DNA chips are usually conducted in sterile environments not unlike clean rooms. However, contamination still occurs and spotting pins need to be cleaned regularly especially before the start of spotting and in between spotting to maintain the cleanliness of the spotting pins which directly affects quality of the DNA chips.

[006] Studies have revealed that in a nominal batch of DNA chips, the rate of defective production is in the range of 0.5% to 10%. DNA chips are considered defective even if a single spot out of the desired several thousand spots of DNA material is missed and not deposited on the DNA chip. Insufficient deposition of DNA material on a spot may also render that spot useless and similarly the DNA chip may also be considered defective. Defective DNA chips are unusable and usually thrown away. Cleaning of the spotting pins in between spotting is thus very important and has a direct influence on the quality of DNA chips produced. Conventional microarray systems dedicate up to 25% of production time performing washing cycles. However, if too many washing cycles are implemented, then the actual production time of the DNA chips are also affected significantly.

[007] Detection of defective DNA chips is also an important concern as results of experiments and tests conducted can be severely impaired and compromised if a few crucial spots of DNA are not spotted properly on the biological substrates or if the amount of DNA deposited is insufficient. Presently, inspection of these DNA chips are carried out manually by experienced laboratory technicians, researchers and scientists using microscopes inspecting individual DNA chip one at a time. The inspection methodology deployed usually involves 100% visual checking with the aid of microscope. This method disadvantageously relies on the skill, experience and concentration of the laboratory technicians, researchers and scientists performing the inspection. Furthermore, by the time the inspection is carried out, it would not be possible to re-spot or re-deposit additional biological fluid samples on a missing spot once the biological substrate has been removed from the array table.

[008] It can thus be seen that present equipment for producing microarrayed biological slides or DNA chips in use today do still present several disadvantages in terms of throughput and quality control. Thus, there remains a need for a new and improved method and apparatus for microarraying or spotting biological fluid samples on biological substrates.

Summary of the Invention

[009] The present invention seeks to provide a method and apparatus for the depositing of microarrays of biological fluid samples on biological substrates.

[0010] Accordingly, in one aspect, the present invention provides an apparatus for depositing a plurality of spots of biological fluid samples on a plurality of biological substrates, the apparatus comprising: an array table for supporting the plurality of

biological substrates; at least two configurable spotting pin assemblies each having a plurality of spotting pins for depositing the plurality of spots of biological fluid samples on the plurality of biological substrates; the plurality of spotting pins capable of being selectively retracted and extended individually and independently; a first manipulator having a quick change device adapted for coupling to one of the at least two configurable spotting pin assemblies and for positioning the one of the at least two configurable spotting pin assemblies to deposit the plurality of spots of biological fluid samples on the plurality of biological substrates; a second manipulator adapted for coupling to an optical device for positioning the optical device for scanning and acquiring the image data of the plurality of spots of biological fluid samples on the plurality of biological substrates; a processor having memory means for receiving and processing image data from the optical device and for controlling the first manipulator, the second manipulator and the at least two configurable spotting pin assemblies; wherein the processor upon receiving the image data from the optical device processes the image data for detecting locations of desired spots of biological fluid samples which do not meet a predetermined specification on the biological substrates; and further wherein the processor further controls the first manipulator to position the one of the at least two configurable spotting pin assemblies to the locations of the desired spots of biological fluid samples which do not meet the predetermined specifications and selectively extend the desired spotting pin or a plurality of desired spotting pins independently to facilitate the deposition of the spots of biological fluid samples on these locations.

[0011] In another aspect, the present invention provides, a method for spotting a plurality of spots of biological fluid samples on a biological substrate using a first manipulator coupled to a first configurable spotting pin assembly having a plurality of spotting pins, each spotting pin capable of being selectively retracted and extended individually and independently, a second manipulator coupled to an optical device and a processor, the method comprising the steps: engaging the first configurable spotting pin assembly; dipping the plurality of spotting pins of the first configurable spotting pin assembly into

an array tray; positioning the first configurable spotting pin assembly in a desired location; spotting the biological substrate by lowering the plurality of spotting pins to contact the biological substrate depositing a plurality of spots of biological fluid samples on the biological substrate; and inspecting the plurality of spots of biological fluid samples on the biological substrate.

[0012] In a further aspect, the present invention provides, a method for spotting a plurality of spots of biological fluid samples in a microarray on a biological substrates using a first manipulator coupled to a first pre-cleaned configurable spotting pin assembly having a plurality of spotting pins, a second manipulator coupled to an optical device, a washing module having a second pre-cleaned configurable spotting pin assembly and a processor, the method comprising the steps: engaging the first pre-cleaned configurable spotting pin assembly; dipping the plurality of spotting pins of the configurable spotting pin assembly into an array tray; positioning the configurable spotting pin assembly in a desired location; spotting the biological substrate by lowering the plurality of spotting pins to contact the biological substrate depositing a plurality of spots of biological fluid samples on the biological substrate; performing a washing cycle on the first pre-cleaned configurable spotting pin assembly now soiled; engaging the second pre-cleaned configurable spotting pin assembly while the steps of performing a washing cycle are still being performed and repeating the steps of dipping to steps of spotting with the second pre-cleaned configurable spotting pin assembly.

[0013] In yet another aspect, the present invention provides, a method for spotting a plurality of spots of biological fluid samples on a biological substrate using a first manipulator coupled to a first configurable spotting module having means for spotting or depositing spots of biological fluid samples in microarrays on the biological substrate and having means for selectively re-spotting or re-depositing individual spots of biological fluid samples onto the biological substrate, a second manipulator coupled to an optical device and a processor, the method comprising the steps: engaging the first configurable spotting

module; loading the first configurable spotting module with the biological fluid samples; positioning the first configurable spotting module in a desired location; spotting the biological substrate using the first configurable spotting module; and inspecting the plurality of spots of biological fluid samples on the biological substrate.

[0014] In yet a further aspect, the present invention provides, a method for spotting a plurality of spots of biological fluid samples in a microarray on a biological substrate using a first manipulator coupled to a first pre-cleaned configurable spotting module having spotting means or depositing means for spotting or depositing spots of biological fluid samples in microarrays on the biological substrate and having spotting or depositing means for selectively re-spotting or re-depositing individual spots of biological fluid samples onto the biological substrate, a washing module having a second pre-cleaned configurable spotting module and a processor, the method comprising the steps: engaging the first pre-cleaned configurable spotting module; loading the configurable spotting module with the biological fluid sample; positioning the configurable spotting module in a desired location; spotting or depositing the biological substrate using the configurable spotting module; performing a washing cycle on the first pre-cleaned configurable spotting module now soiled; engaging the second pre-cleaned configurable spotting module while the steps of performing a washing cycle are still being performed and repeating the steps of dipping to steps of spotting with the second pre-cleaned configurable spotting module.

[0015] In a yet further aspect, the present invention provides, an apparatus for depositing a plurality of spots of biological fluid samples on a plurality of biological substrates, the apparatus comprising: an array table for supporting and securing the plurality of biological substrates; at least two configurable spotting modules each having spotting or depositing means for spotting or depositing the plurality of spots of biological fluid samples on the plurality of biological substrates; the at least two configurable spotting modules further having spotting or depositing means being capable of selectively re-spotting or re-depositing individual spots of biological fluid samples onto the biological substrates; a first

manipulator having a quick change device adapted for coupling to one of the at least two configurable spotting modules and for positioning the one of the at least two configurable spotting modules to deposit the plurality of spots of biological fluid samples on the plurality of biological substrates; a second manipulator adapted for coupling to an optical device for positioning the optical device for scanning and acquiring of image data of the plurality of spots of biological fluid samples on the plurality of biological substrates; a processor having memory means for receiving, processing and storing image data from the optical device and for controlling the first manipulator, the second manipulator and the at least two configurable spotting modules; wherein the processor upon receiving the image data from the optical device processes and stores the image data for detecting locations of desired spots of biological fluid samples which do not meet a predetermined specification on the biological substrates; and further wherein the processor further controls the first manipulator to position the one of the at least two configurable spotting modules to the locations of the desired spots of biological fluid samples which do not meet the predetermined specifications and selectively carry out re-spotting or re-depositing of the desired spots of biological fluid samples which do not meet the predetermined specifications.

Brief Description of the Drawings

[0016] A preferred and an alternative embodiment of the present invention will now be more fully described, with reference to the drawings of which:

[0017] FIG.1 illustrates an apparatus for the spotting of biological fluid samples on biological substrates in accordance with the present invention;

[0018] FIG.2A illustrates a configurable spotting pin assembly of FIG.1;

[0019] FIG.2B illustrates a second manipulator of FIG.1;

[0020] FIG.3A illustrates an array table of FIG.1;

[0021] FIG.3B illustrates a substrate well of FIG.3A;

[0022] FIG.3C illustrates a spring loaded ball plunger of FIG.3B;

[0023] FIG.4 illustrates a flowchart of the method for the spotting of biological fluid samples on biological substrates in accordance with the preferred embodiment;

[0024] FIG.5 illustrates a flowchart of the steps of performing the washing cycle of FIG.4;

[0025] FIG.6 illustrates a flowchart of the steps of inspecting the biological substrates of FIG.4;

[0026] FIG.7 illustrates a flowchart of the steps of analyzing image data of FIG.6.

[0027] FIG.8 illustrates a flowchart of the step re-spotting the biological substrates of FIG.4; and

[0028] FIG.9 illustrates a flowchart of the method for the spotting of biological fluid samples on biological substrates in accordance with the alternative embodiment.

Detailed description of the Drawings

[0029] A method and apparatus for microarraying biological fluid samples on biological substrates with a preferred embodiment and an alternative embodiment is described. It

shall be apparent to one skilled in the art, however that the invention may be practiced without such details. Some of the details may not be described at length so as not to obscure the invention.

[0030] Referring to FIG.1 an apparatus 10 for microarraying biological fluid samples on a plurality of biological substrates is illustrated. The apparatus 10 generally comprises a stand 24 for supporting a variety of components such as an array table 12, an array plate 22, a washing module, a first manipulator 16 for coupling to a configurable spotting pin assembly 14, a second manipulator 18 coupled to an optical device 20, a third manipulator 30 for coupling to and conveying a configurable spotting pin assembly 14 for washing in the washing module, and a processor for controlling the manipulators.

[0031] Referring to FIG.1 and FIG.2A, the configurable spotting pin assembly (hereafter referred to as CSPA) 14 has a plurality of spotting pins 40 for spotting or depositing spots of biological fluid samples in microarrays on the biological substrates. The plurality of spotting pins are each advantageously capable of being selectively retracted and extended individually and independently for re-spotting of missed spots at any point in time during the spotting process. The extending and the retracting individually and independently of each of the spotting pins 40 are pneumatically actuated via a plurality of vacuum nipples 42 each coupled to an individual spotting pin 40 respectively. The vacuum is created by means of a plurality of vacuum generating devices each capable of selectively activated and de-activated individually and independently. Alternatively, this vacuum source can be a vacuum pump coupled to a vacuum reservoir acting as central storage.

[0032] The first manipulator 16 is typically a 3-axis robotic arm and is able to manipulate and convey the CSPA 14 to access a full range of locations on and in proximity of the stand 24. The first manipulator 16 is adapted to couple to the CSPA 14 by means of a quick change device. This quick change device advantageously allows the first manipulator 16 to rapidly disengage an already coupled CSPA 14 that may be soiled and then to subsequently

engage another pre-cleaned CSPA 14. The first manipulator 16 may further have additional degrees of freedom incorporated to convert the 3-axis robotic arm to a multi-axis robotic arm.

[0033] The array plate 22 typically comprises multiple wells for containing a variety or a plurality of biological fluid samples for dipping and re-dipping of the multiple spotting pins of the CSPA 14. The array plate 22 is commercially available in several configurations such as a 384 well array plate.

[0034] The array table 12 is for securely supporting a plurality of biological substrates and allowing the first manipulator 16 to accurately and reliably perform the spotting of biological fluid samples on the securely supported biological substrates. The array table 12 comprises of a flat platform having multiple substrate wells 13, each substrate well 13 for securing a single biological substrate. Each substrate well 13 has at least two abutments 45 for placement and support of a biological substrate. Further, a spring loaded ball plunger 47 adds to the secure placement and support of the biological substrate. The spring loaded ball plunger 47 further comprises a ball bearing 50 and a compression spring 49. Placement of a biological substrate into a substrate well 13 involves seating at least two edges of the biological substrate onto the at least two abutments 45. At the same time, the biological substrate pushes against the ball bearing 50 of the spring loaded ball plunger 47 thus tensioning the compression spring 49 and this tensioned compression spring 49 acts to secure the biological substrate within the substrate well 13.

[0035] The array table 12 is typically situated in proximity from the array plate 22. This is to ensure that the first manipulator only needs to convey the CSPA 14 for a short distance from dipping the CSPA 14 into the array plate 22 and then spotting the biological fluid samples onto the biological substrates on the array table 12.

[0036] Referring to FIG.1 and FIG.2B, the second manipulator 18 is typically a 2-axis robotic arm and is able to manipulate and convey the coupled optical device 20 to access a full range of locations on the array table 12 where the biological substrates are supported. The array table 12 has a plurality of substrate wells 13 which advantageously allows visual and physical access of the biological substrates from below the array table 12. Thus the second manipulator 18 can be sited strategically underneath the array table 12 and advantageously access the full range of locations of the array table 12 particularly all the biological substrates. The optical device 20 is typically a CCD camera and may comprise more than a single camera. Two CCD cameras mounted in tandem can be used as the optical device 20 to simultaneously scan eight deposited biological spots on the biological substrate at any point in time instantaneously.

[0037] Referring to FIG.1, the washing module comprising a third manipulator 30, a holding station 34, an ultrasonic cleaner 35, a water bath 36 and a vacuum dryer 37, is for washing of a soiled CSPA 14 after performing a predetermined number of spottings on the biological substrates. The third manipulator 30 is typically a 2-axis robotic arm and is adapted to couple to the CSPA 14 via an end effector 32. The holding station 34 has at least two compartments, each for holding a single CSPA 14. During normal operation, there would be at least two CSPAs 14 in use by the apparatus 10. The first of the CSPAs 14 would be coupled to the first manipulator 16 for spotting biological fluid samples onto the biological substrates. The other CSPA 14 which if soiled would be undergoing cleaning in the washing module while the first manipulator 16 coupled to the first CSPA 14 is concurrently spotting the biological fluid samples onto the biological substrates. If the other CSPA 14 had already been washed, it would then be placed in one of the at least two compartments in the holding station 34 awaiting later use when the first CSPA 14 becomes soiled and requires washing.

[0038] While undergoing washing, the soiled CSPA 14 is conveyed from the holding station 34, to the ultrasonic cleaner 35, to the water bath 36 and then to the vacuum dryer 37 by

the third manipulator 30. When washing is completed, the third manipulator 30 then deposits the soiled CSPA 14 now cleaned back into one of the compartments of the holding station 34. The washing module advantageously allows the first manipulator to continue with spotting of the biological substrates with the pre-cleaned CSPA 14 while the third manipulator 30 of the washing module conveys the soiled CSPA 14 for cleaning. There is therefore zero time lost due to waiting for the soiled CSPA 14 to be washed.

[0039] Referring to FIG.4, the method 400 for microarraying biological fluid samples on biological substrates in accordance with the present invention starts with the step of engaging 410 the pre-cleaned CSPA 14 from the holding station 34 of the washing module. The CSPA 14 is then conveyed by the first manipulator 16 to the array plate 22. The multiple spotting pins 40 of the CSPA are then dipped 415 into the array plate 22. The CSPA 14 is then conveyed to the array table 12 and then positioned 420 in a desired location for the microarraying of the biological substrates.

[0040] When referencing locations on a flat plane as in the present case, two parameters of 2 reference axes are normally required. As such, the two parameters defining a location are each from a different axis and the intersection of the two axes is normally considered a base reference. In the present invention, the apparatus and method advantageously utilizes at least two adjacent edges of the biological substrates as the reference axes and the intersection of the at least two adjacent edges as the base reference for defining a location on the biological substrates.

[0041] Present microarraying systems have their reference axes and base references pre-programmed into the processors and controllers when the biological substrates are pre-arranged edge-to-edge and secured by magnetic strips unto a platform. This disadvantageously causes errors as the dimensional variances of the biological substrates may vary according to the quality control of different manufacturers. Such inherent dimensional errors will cause the spotting of the biological fluid samples to be spotted in

areas where the biological substrates are not present and may further result in spots of biological fluid samples meant for a particular biological substrate to be deposited on an adjacent biological substrate. The present invention thus advantageously obviates problems when biological substrates are inherently inconsistent in dimensions as the references are taken from the edges of individual biological substrates independent of the neighboring biological substrates.

[0042] Next, the step of spotting 425 the biological substrates is performed by lowering the CSPA 14 until the multiple spotting pins 40 come into contact with the biological substrates. This results in a minute spot of biological fluid sample being deposited by each of the multiple spotting pins 40 onto the biological substrate. Upon completion of the step of spotting 425 the biological substrates, the inspecting 430 of the biological substrates is then performed. If re-spotting of the biological substrates is required 435, the step of re-spotting 470 the biological substrates is then performed.

[0043] Re-spotting 470 maybe required when the inspecting 430 of the biological substrates determines that there are desired spots of biological fluid sample on the biological substrates that do not meet a predetermined specification. If re-spotting is not required 435, the process continues to determine whether all spotting 425 of biological substrates have been completed 440. Next, if the number of re-dippings 445 of the multiple pins of the CSPA 14 into the array table 12 has occurred a predetermined number of times N, then N is reset 460 to zero and the washing cycle 465 of the CSPA 14 engaged by the first manipulator 16 is performed.

[0044] The method 400 loops back and continues with the first manipulator 16 engaging 410 a pre-cleaned CSPA 14 and continues from there. If the re-dippings 415 of the CSPA 14 had not occurred N times 445, and if the number of spottings 425 by the engaged CSPA 14 had occurred a predetermined number of times n 450, then n is reset 455 to zero and the CSPA 14 is conveyed to the array tray 22 and re-dipped 415. If the number of spottings

425 by the engages CSPA 14 had not occurred n times 450 then the method 400 loops back and continues with the positioning 420 of the CSPA 14 for the next spotting 425. When all the spottings 425 and re-spottings 470 of all the biological substrates if any are completed 440, the method 400 ends.

[0045] However, in the event that one of the biological substrates is irrecoverable or damaged resulting in non-compliance to any predetermined specifications during the spotting processes, the processor will automatically activate a bypass routine which will instruct the first manipulator 16 to skip that particular defective biological substrate. This will prevent the spotting of subsequent biological fluids onto the defective biological substrate thereby eliminating further loss of production time and the wastage of the unspotted biological fluids.

[0046] Referring to FIG.5, the washing cycle 465 for the CSPA starts with the step of transferring 510 the CSPA 14 desired for washing to the holding station and depositing the CSPA 14 into one of the at least two compartments of the holding station 34. Next, the third manipulator 30 engages 515 the CSPA 14 desired for washing and conveys the CSPA 14 to the ultrasonic station 35 for cleaning 520. The third manipulator 30 then conveys the cleaned CSPA 14 to the water bath and immerses 525 the CSPA into the water bath 36. Next, the drying 530 of the CSPA 14 is performed in the vacuum dryer 37 after being conveyed to the vacuum dryer by the third manipulator 30. The third manipulator 30 then transfers 535 the now cleaned and dried CSPA 14 back to any one of the two compartments of the holding station 34.

[0047] Referring to FIG.6, the steps of inspecting 430 the biological substrates starts with the positioning 610 of the optical device by the second manipulator. The microarray of deposited spots of biological fluid samples on the biological substrate are then scanned 615 by the optical device 20 to acquire the image data of the spots of the deposited biological fluid samples. The image data is then sent 620 to the processor and the image data is

analyzed 625. The optical device 20 is typically a CCD camera and may comprise more than a single camera. Two CCD cameras mounted in tandem can be used as the optical device 20 to simultaneously scan eight deposited biological fluid samples on the biological substrate at any point in time instantaneously thus accelerating the scanning rate and acquisition of the image data of the spots of biological fluid samples on the biological substrate thereby reducing the image data acquisition time by 50% as compared to using a single CCD camera.

[0048] Referring to FIG.7, the steps of analyzing the image data 625 by the processor starts with the step of defining 710 the region of interest. In the present invention, the optical device 20 scans the spots of biological fluid samples on the biological substrates. The region of interest would primarily be the image of the deposited spot of biological fluid sample. Next, differentiating 715 the deposited spot of biological fluid sample from its immediate background. This would involve known image processing techniques especially histogram calculation to determine the grey scale levels involved, Gaussian filtering to smoothen the histogram and certain known algorithms to distinguish the deposited spot of biological fluid samples from its background. The step of determining 720 the characteristics of the deposited spot of biological fluid sample is then performed.

[0049] The spot characteristics being: area, compactness, maximum diameter and circularity. The spot characteristic of area refers to the actual area occupied by the spot of biological fluid sample on the biological substrate. Using the maximum diameter of the spot, the apparent area of the spot is computed. By dividing the actual area of the spot of biological fluid sample by this apparent area of the spot, an index of compactness is then derived. This index of compactness is also referred to as the index of irregularity as the index compares the actual area of the spot of biological fluid sample with respect to that of the apparent area had the entire area been covered by the biological fluid. The characteristic of circularity is simply an index that indicates the degree of conformity the spot of biological fluid sample has as compared to an actual perfect circle.

[0050] The spot characteristics are then compared 730 with a comprehensive set of predetermined specification that determine the requirements of the desired spot characteristics. If the spot characteristics fail 735 any of the predetermined specifications, a software marker (which comprises the location of the spot, the biological substrate where the spot is on and the spot characteristics) for that failed spot requiring re-spotting 470 is sent 750 to the processor for re-spotting 470 and the software marker recorded is then recorded 740. If the spot characteristics do not fail 735 the specifications, then the software marker is simply recorded 740.

[0051] Referring to FIG.8, the re-spotting 470 of the biological substrates starts with the step of the processor receiving 810 the software markers for the spots that require re-spotting. Next selectively extending 815 the required spotting pin or a plurality of spotting pins for re-spotting in accordance with the software marker. The extended plurality of spotting pins are then dipped 820 into the desired wells of the array plate 22. Next the CSPA 14 are positioned 825 in a desired location for re-spotting of at least one desired spot which does not meet the predetermined specifications. The CSPA 14 is then lowered by the first manipulator 16 onto the biological substrate and the biological fluid sample re-deposited or re-spotted 830. The respective extended spotting pin or a plurality of spotting pins 40 are then retracted 835. If there are more re-spotting required 840, the spotting pins 40 are selectively and individually extended again, else the re-spotting 470 of the biological substrates ends 845.

[0052] For allowing easy access to control and maintain the apparatus 10, the apparatus 10 can further be provided with a Graphical User Interface (GUI) system. Such a GUI system would comprise some displaying means and input means. Alternatively, the input means could be incorporated into the displaying means an example of which is a touch screen Liquid Crystal Display (LCD). The displaying means would provide data such as the operational status of the apparatus 10, recorded data of scanned images of the spots of

biological fluid samples, software markers of spots of biological fluid samples that failed the predetermined specifications and status of the spotting 425 and re-spotting 470 processes.

[0053] In the alternative embodiment, the apparatus 10 is substantially similar, but the spotting technology for spotting or depositing biological fluid samples on the biological substrates is not limited to that of multiple spotting pins as used in the CSPA 14. Consequently, a Configurable Spotting Module (CSM) can be used to replace the CSPA 14. The CSM would comprise spotting or depositing means for spotting or depositing spots of biological fluid samples in microarrays on the biological substrates. An example of such an alternative means is to use piezoelectric crystals to eject droplets of the fluid samples onto the biological substrates, this is sometimes referred to as the "Ink Jet printing" method. Furthermore, the CSM would also comprise means for selectively re-spotting or re-depositing individual spots of biological fluid samples onto the biological substrates.

[0054] Referring to FIG.9, the second embodiment of a method 900 for microarraying biological fluid samples on biological substrates in accordance with the present invention starts with the step of engaging 910 a pre-cleaned Configurable Spotting Module (CSM). The CSM is then loaded 915 with the spotting material which is the biological fluid samples. The CSM is then conveyed to the array table 12 and then positioned 920 in a desired location for the microarraying of the biological substrates.

[0055] Next, the step of spotting 925 the biological substrates is performed by lowering the CSM until the CSM is in range of the biological substrates. The step of spotting 925 the biological substrates is then carried out with the CSM. Following which, the inspecting 930 of the biological substrates is then performed. If re-spotting or depositing of the biological substrates is required 935, the step of re-spotting or re-depositing 970 the biological substrates is then performed.

[0056] Re-spotting or re-depositing 970 maybe required when the inspecting 930 of the biological substrates determines that there are desired spots of biological fluid sample on the biological substrates that do not meet a predetermined specification. If re-spotting or re-depositing is not required 935, the process continues to determine whether all spotting or depositing 925 of biological substrates have been completed 940. Next, if the number of re-loadings 995 of the CSM has occurred a predetermined number of times N, then N is reset 960 to zero and the washing cycle 965 of the CSM engaged by the first manipulator 16 is performed.

[0057] The method 900 loops back and continues with the first manipulator 16 engaging 910 a pre-cleaned CSM and continues from there. If the re-loadings 915 of the CSM had not occurred N times 995, and if the number of spottings 925 by the engaged CSM had occurred a predetermined number of times n 950, then n is reset 955 to zero and the CSM is then re-loaded 915. If the number of spottings or depositings 925 by the engages CSM had not occurred n times 950 then the method 900 loops back and continues with the positioning 920 of the CSM for the next spotting or depositing 925. When all the spottings or depositing 925 and re-spottings or re-depositing 970 of all the biological substrates if any are completed 940, the method 900 ends.

[0058] The details of performing the washing cycle 965 can be substantially similar to that of the washing cycle 465 in the preferred embodiment as illustrated in FIG.5. However, depending on the spotting or depositing means employed in the CSM, the steps in the washing cycle may well differ. An example of an alternative washing cycle using "Ink Jet printing" method would involve clearing of nozzles of the CSM using a neutral medium or using compressed air.

[0059] Furthermore, inspecting of the biological substrates 930 is also substantially similar to the inspecting 430 of the biological substrates in the preferred embodiment as illustrated in FIG.6. The re-spotting or re-depositing 970 step of the alternative embodiment is also

substantially similar to the re-spotting 470 of the preferred embodiment as illustrated in FIG.8. However, the CSM replaces the CSPA 14 and the means for selectively re-spotting individual spots on the biological substrates also differ.

[0060] It will be appreciated that the embodiments described and illustrated do not limit the scope of the present invention and various modifications and improvements can be made by a person skilled in the art without departing from the scope of the present invention.

Claims

1. An apparatus for depositing a plurality of spots of biological fluid samples on a plurality of biological substrates, said apparatus comprising:

an array table for supporting and securing said plurality of biological substrates;

at least two configurable spotting pin assemblies each having a plurality of spotting pins for depositing said plurality of spots of biological fluid samples on said plurality of biological substrates;

said plurality of spotting pins each capable of being selectively retracted and extended;

a first manipulator having a quick change device adapted for coupling to one of said at least two configurable spotting pin assemblies and for positioning said one of said at least two configurable spotting pin assemblies to deposit said plurality of spots of biological fluid samples on said plurality of biological substrates;

a second manipulator adapted for coupling to an optical device for positioning said optical device for scanning and acquiring of image data of said plurality of spots of biological fluid samples on said plurality of biological substrates;

a processor having memory means for receiving, processing and storing image data from said optical device and for controlling said first manipulator, said second manipulator and said at least two configurable spotting pin assemblies;

wherein said processor upon receiving said image data from said optical device processes and stores said image data for detecting locations of desired spots of biological fluid samples which do not meet a predetermined specification on said biological substrates; and

further wherein said processor further controls said first manipulator to position said one of said at least two configurable spotting pin assemblies to said locations of said desired spots of biological fluid samples which do not meet said predetermined

specifications and selectively extend a plurality of desired spotting pins individually and independently to facilitate the deposition of said spots of biological fluid samples on said locations.

2. The apparatus in accordance with claim 1, further comprising a washing module for performing a washing cycle on said one of said at least two configurable spotting pin assemblies, said washing module comprising; a holding station for holding said at least two configurable spotting pin assemblies; an ultrasonic cleaner; a water bath and a vacuum dryer; wherein said washing cycle is performed concurrently and independently with said depositing of said plurality of spots of biological fluid samples on said plurality of biological substrates
3. The apparatus in accordance with claim 2, further comprising a third manipulator adapted for conveying said one of said at least two configurable spotting pin assemblies between said holding station, said ultrasonic cleaner, said water bath and said vacuum dryer for performing said washing cycle.
4. The apparatus in accordance with claim 3, wherein said third manipulator further comprises an end effector adapted for coupling to said one of said at least two configurable spotting pin assemblies
5. The apparatus in accordance with claim 1, further comprising an array plate adapted for holding and securing said biological fluid samples and for dipping of said plurality of spotting pins before said depositing of said plurality of spots of biological fluid samples on said plurality of biological substrates.
6. The apparatus in accordance with claim 1, further comprising a displaying means coupled to said processor for Graphical User Interface (GUI).

7. The apparatus in accordance with claim 1, wherein said optical device further comprises at least one CCD camera.
8. The apparatus in accordance with claim 1, wherein said array table further comprises a plurality of substrate wells.
9. The apparatus in accordance with claim 8, wherein said each of said plurality of substrate wells further comprises at least two abutments and a spring ball loaded ball plunger.
10. A method for spotting a plurality of spots of biological fluid samples on a biological substrate using a first manipulator coupled to a first configurable spotting pin assembly having a plurality of spotting pins, each spotting pin capable of being selectively retracted and extended independently, a second manipulator coupled to an optical device and a processor, said method comprising the steps:
 - a. engaging said first configurable spotting pin assembly;
 - b. dipping said plurality of spotting pins of said first configurable spotting pin assembly into an array plate;
 - c. positioning said first configurable spotting pin assembly in a desired location;
 - d. spotting said biological substrate by lowering said plurality of spotting pins to contact said biological substrate depositing a plurality of spots of biological fluid samples on said biological substrate; and
 - e. inspecting said plurality of spots of biological fluid samples on said biological substrate.
 - f. re-spotting on locations where desired spots of biological fluid samples on said biological substrate that do not meet a predetermined specification using said first configurable spotting pin assembly.

11. The method in accordance to claim 10 wherein said step e. further comprises:
 - i. positioning said optical device to said location of said plurality of spots of biological fluid samples;
 - ii. scanning and acquiring image data of said spots of biological fluid samples on said biological substrate;
 - iii. sending said image data to said processor; and
 - iv. analyzing said image data.
12. The method in accordance to claim 11 wherein said step iv. further comprises:
 - i. defining region of interest;
 - ii. differentiating said spots of biological fluid samples from background of said spots of biological fluid samples;
 - iii. determining spot characteristics: area, compactness, maximum diameter, and circularity
 - iv. comparing spot characteristics with a predetermined set of specifications;
 - v. sending a software marker of said spot which fails said predetermined set of specifications to said processor; and
 - vi. recording said software marker.
13. The method in accordance to claim 12 wherein said software marker comprises: location of said spot, said biological substrate where said spot is on and said spot characteristics.
14. The method in accordance to claim 10 wherein step f further comprises:
 - i. receiving said software marker for said spot requiring e-spotting;
 - ii. extending selectively independently said at least one spotting pin;
 - iii. dipping said at least one extended spotting pin into array plate;
 - iv. positioning said configurable spotting pin assembly to said location; and

- v. re-spotting said biological substrate by lowering said at least one extended spotting pin to contact said biological substrate at said location;
15. The method in accordance to claim 10 further comprising the step:
- g. further providing a second configurable spotting pin assembly and a washing module comprising: a third manipulator having an end effector adapted for coupling to said configurable spotting pin assembly, a holding station, a ultrasonic cleaner, a water bath and a vacuum dryer.
16. The method in accordance to claim 15 further comprising the step:
- h. performing a washing cycle on said first configurable spotting pin assembly now soiled;
 - i. engaging said second configurable spotting pin assembly while step h. is still being performed and
 - j. repeating steps b to d with said second configurable spotting pin assembly.
17. The method in accordance to claim 16 wherein said step h. further comprises:
- i. transferring said first configurable spotting pin assembly to said holding station by said first manipulator;
 - ii. conveying said first configurable spotting pin assembly to said ultrasonic cleaner;
 - iii. cleaning said first configurable spotting pin assembly;
 - iv. immersing said first configurable spotting pin assembly into said water bath;
 - v. drying said first configurable spotting pin assembly in said vacuum dryer; and
 - vi. transferring said first configurable spotting pin assembly to said holding station.

18. A method for spotting a plurality of spots of biological fluid samples in a microarray on a biological substrates using a first manipulator coupled to a first pre-cleaned configurable spotting pin assembly having a plurality of spotting pins, a second manipulator coupled to an optical device, a washing module having a second pre-cleaned configurable spotting pin assembly and a processor, said method comprising the steps:

- a. engaging said first pre-cleaned configurable spotting pin assembly;
- b. dipping said plurality of spotting pins of said configurable spotting pin assembly into an array plate;
- c. positioning said configurable spotting pin assembly in a desired location;
- d. spotting said biological substrate by lowering said plurality of spotting pins to contact said biological substrate leaving a plurality of spots of biological fluid samples on said biological substrate;
- e. performing a washing cycle on said first pre-cleaned configurable spotting pin assembly now soiled;
- f. engaging said second pre-cleaned configurable spotting pin assembly while step e. is still being performed and
- g. repeating steps b to d with said second pre-cleaned configurable spotting pin assembly.

19. The method in accordance to claim 18 wherein said washing module further comprises: a third manipulator having an end effector adapted for coupling to said configurable spotting pin assembly, a holding station, a ultrasonic cleaner, a water bath and a vacuum dryer.

20. The method in accordance to claim 19 wherein said step e. further comprises:

- i. transferring said first pre-cleaned configurable spotting pin assembly to said holding station by said first manipulator;
- ii. conveying said first pre-cleaned configurable spotting pin assembly to said ultrasonic cleaner;

- iii. cleaning said first pre-cleaned configurable spotting pin assembly;
- iv. immersing said first pre-cleaned configurable spotting pin assembly into said water bath;
- v. drying said first pre-cleaned configurable spotting pin assembly in said vacuum dryer; and
- vi. transferring said first pre-cleaned configurable spotting pin assembly to said holding station.

21. A method for spotting a plurality of spots of biological fluid samples on a biological substrate using a first manipulator coupled to a first configurable spotting module having means for spotting or depositing spots of biological fluid samples in microarrays on said biological substrate and having means for selectively re-spotting or re-depositing individual spots of biological fluid samples onto said biological substrate, a second manipulator coupled to an optical device and a processor, said method comprising the steps:

- a. engaging said first configurable spotting module;
- b. loading said first configurable spotting module with said biological fluid samples;
- c. positioning said first configurable spotting module in a desired location;
- d. spotting or depositing said biological substrate using said first configurable spotting module; and
- e. inspecting said plurality of spots of biological fluid samples on said biological substrate;
- f. re-spotting or re-depositing on locations where desired spots of biological fluid samples on said biological substrate that do not meet a predetermined specification using said first configurable spotting module.

22. The method in accordance to claim 21 further comprising the step:
- g. further providing a second configurable spotting module and a washing module comprising: a third manipulator having an end effector adapted for coupling to said configurable spotting module, a holding station, a ultrasonic cleaner, a water bath and a vacuum dryer.
23. The method in accordance to claim 22 further comprising the step:
- h. performing a washing cycle on said first configurable spotting module now soiled;
 - i. engaging said second configurable spotting module while step h. is still being performed and
 - j. repeating steps b to d with said second configurable spotting module.
24. A method for spotting a plurality of spots of biological fluid samples in a microarray on a biological substrates using a first manipulator coupled to a first pre-cleaned configurable spotting module having means for spotting or depositing spots of biological fluid samples in microarrays on said biological substrate and having means for selectively re-spotting or re-depositing individual spots of biological fluid samples onto said biological substrate, a washing module having a second pre-cleaned configurable spotting pin assembly and a processor, said method comprising the steps:
- a. engaging said first pre-cleaned configurable spotting module;
 - b. loading said first configurable spotting module with said biological fluid samples;
 - c. positioning said configurable spotting module in a desired location;
 - d. spotting or depositing said biological substrate using said first configurable spotting module;
 - e. performing a washing cycle on said first pre-cleaned configurable spotting module now soiled;

- f. engaging said second pre-cleaned configurable spotting module while step e. is still being performed and
- g. repeating steps b to d with said second pre-cleaned configurable spotting module.

25. The method in accordance to claim 24 wherein said washing module further comprises: a third manipulator having an end effector adapted for coupling to said configurable spotting module, a holding station, a ultrasonic cleaner, a water bath and a vacuum dryer.

26. The method in accordance to claim 25 wherein said step e. further comprises:

- i. transferring said first pre-cleaned configurable spotting module to said holding station by said first manipulator;
- ii. conveying said first pre-cleaned configurable spotting module to said ultrasonic cleaner;
- iii. cleaning said first pre-cleaned configurable spotting module;
- iv. immersing said first pre-cleaned configurable spotting module into said water bath;
- v. drying said first pre-cleaned configurable spotting module in said vacuum dryer; and
- vi. transferring said first pre-cleaned configurable spotting module to said holding station.

27. An apparatus for depositing a plurality of spots of biological fluid samples on a plurality of biological substrates, said apparatus comprising:

- an array table for supporting and securing said plurality of biological substrates;
- at least two configurable spotting modules each having spotting or depositing means for spotting or depositing said plurality of spots of biological fluid samples on said plurality of biological substrates;

said at least two configurable spotting modules further having spotting or depositing means being capable of selectively re-spotting or re-depositing individual spots of biological fluid samples onto said biological substrates;

a first manipulator having a quick change device adapted for coupling to one of said at least two configurable spotting modules and for positioning said one of said at least two configurable spotting modules to deposit said plurality of spots of biological fluid samples on said plurality of biological substrates;

a second manipulator adapted for coupling to an optical device for positioning said optical device for scanning and acquiring of image data of said plurality of spots of biological fluid samples on said plurality of biological substrates;

a processor having memory means for receiving, processing and storing image data from said optical device and for controlling said first manipulator, said second manipulator and said at least two configurable spotting modules;

wherein said processor upon receiving said image data from said optical device processes and stores said image data for detecting locations of desired spots of biological fluid samples which do not meet a predetermined specification on said biological substrates; and

further wherein said processor further controls said first manipulator to position said one of said at least two configurable spotting modules to said locations of said desired spots of biological fluid samples which do not meet said predetermined specifications and selectively carry out re-spotting or re-depositing of said desired spots of biological fluid samples which do not meet said predetermined specifications.