Title: A MICROSPOTTING PIN FOR THE PRODUCTION OF MICROARRAYS

Abstract: The present invention relates to a high precision microspotting pin for utilisation in an apparatus for preparing microarrays, comprising a pin member with a dispensing tip at a first end thereof with an end wall surface, a reservoir defined by at least one reservoir wall, a first channel defined by at least one channel wall and extending from the reservoir to the dispensing tip providing fluid communication between the reservoir and the dispensing tip, and wherein the reservoir is a closed reservoir.
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.
A MICROSPOTTING PIN FOR THE PRODUCTION OF MICROARRAYS

FIELD OF THE INVENTION

The present invention relates to a high precision microspotting pin for utilisation in an apparatus for preparing microarrays.

BACKGROUND OF THE INVENTION

Microarray technology is emerging as one of the principal and fundamental investigational tools for a wide variety of biological phenomena. Microarrays are formed by depositing very small amounts of a liquid, e.g. with nucleic acid fragments, onto a substrate in an array.

It is well known in the art to deposit a small amount of a liquid utilising a microspotting pin or by applying inkjet technology. Microspotting pins are competitive in terms of speed, quality and cost, and utilisation of high precision metal microspotting pins is widespread. However, the metal microspotting pins are individually machined at a significant cost. Moreover, the metal pins are susceptible to bending damage.

Therefore, in order to increase the usefulness of microspotting pin technology and make it even more attractive, microspotting pins with improved performance are needed.

In WO 02/063272, a microspotting pin made of silicon is disclosed providing a microspotting pin at a lower cost than the cost of metal microspotting pins.

SUMMARY OF THE INVENTION

Known non-metal microspotting pins suffer from the disadvantages that the sample to be spotted is subjected to significant evaporation. The volume of sample in the spotting pins may be less than 1 μl, and even moderate evaporation of the solvent of the sample will significantly affect the concentration of e.g. bio molecules present in the sample. Furthermore, known microspotting pins typically require a pre-spotting process to obtain a uniform spot size. The pre-spotting process is both time consuming and consumes sample. Moreover, known microspotting pins have turned out to be fragile requiring careful and troublesome handling. Still further, bio molecules of the sample to be spotted may adsorb or bind to the surface of the microspotting pin leading to variation of the concentration of biomolecules in spots produced by the microspotting pin. Further, known microspotting pins only deliver a limited number of spots.

It is an object of the present invention to provide a microspotting pin that overcomes the above-mentioned disadvantages.
It is a further object of the present invention to provide a microspotting pin that produces highly uniform spots, i.e. uniform with relation to the size of the individual spots and/or uniform with relation to the amount of a bio molecules deposited in the spots.

It is a further object of the present invention to provide a pin that minimizes pin to pin variation and has very low variation from the first to the last spot.

It is a still further object of the present invention to provide a microspotting pin that is insensitive to changes in temperature and/or in humidity of the surroundings during its use.

According to the present invention the above-mentioned and other objects are fulfilled by a microspotting pin for dispensing a volume of a liquid on a substrate, comprising a pin member with a dispensing tip at a first end thereof with an end wall surface, a reservoir defined by at least one reservoir wall, a first channel defined by at least one channel wall and extending from the reservoir to the dispensing tip providing fluid communication between the reservoir and the dispensing tip.

During preparation of a microarray on a substrate, the sample is contacted with the microspotting pin whereby the first channel and the reservoir are filled with sample, e.g. by capillary attraction, aspiration or a combination thereof, and a liquid film is formed on the dispensing tip. Then, the substrate is contacted with the liquid film of the dispensing tip followed by breaking the contact between the substrate and the liquid film thereby forming a spot of sample on the substrate.

The substrate may be glass (e.g. standard microscope slides) or polymer (e.g. PMMA) or Silicon or organic matter (e.g. a cell, a slice of tissue) activated by different surface coatings (silanes, silylated, epoxy treated, covered with polyacrylamide, agarose, cellulose). The surface may be flat and may be the surface of a microscope slide, or the substrate may reside within a lab-on-a-chip. The substrate may be hydrophilic and hydrophobic as appropriate for the actual assay.

The sample may be in liquid form and may comprise particulate material, such as a cell, a microbead, etc. The sample may be hydrophilic and it may comprise water, or the sample may be hydrophobic and it may comprise organic solvents. Typical buffers include (e.g. SSC (sodium chloride and citric acid, trisodium salt), phosphate buffer, DMF (dimethylformamide), DMSO (dimethyl sulfoxide), polyvinylpyrrolidone, TRIS (amino-2-(hydroxymethyl)-1,3-propanediol) are all diluted in water. Additives may be added, such as detergents for lowering of the surface tension of the liquid, e.g. Tween
20, SDS (Sodium Dodecyl Sulfate). BSA (Bovine Serum Albumine) and Betaine may be added to stabilise the dissolved sample. The sample may comprise a biological component selected from the group consisting of proteins, polypeptides, amino acids, DNA molecules, oligonucleotides, RNA molecules, beads, cells, antibodies, and combinations thereof.

In a preferred embodiment of the invention, the pin member includes a pin mounting head disposed at a second end thereof for engagement with an aperture in a holder. The pin mounting head may prevent the microspotting pin from falling through the holder and/or may be part of a surface arrangement used to avoid rotation of the microspotting pin in the aperture of the pin holder.

In a preferred embodiment of the present invention, the reservoir is a substantially closed reservoir.

Enclosing sample contained in the pin member significantly reduces evaporation of the enclosed sample as compared to the sample being exposed to air.

The reservoir is substantially closed when the surface area of sample contained in the pin member exposed to air is less than or equal to 200000 \( \mu \text{m}^2 \), such as less than or equal to 100000 \( \mu \text{m}^2 \), 80000 \( \mu \text{m}^2 \), 60000 \( \mu \text{m}^2 \), 40000 \( \mu \text{m}^2 \), 20000 \( \mu \text{m}^2 \), 10000 \( \mu \text{m}^2 \), 5000 \( \mu \text{m}^2 \), 1000 \( \mu \text{m}^2 \), 500 \( \mu \text{m}^2 \) or 100 \( \mu \text{m}^2 \), such as less than or equal to 10 \( \mu \text{m}^2 \). For example, the surface area of the sample that is in contact with air, when the reservoir and the first channel of the microspotting pin have been filled with the sample, is within the range 1 \( \mu \text{m}^2 \) - 100000 \( \mu \text{m}^2 \), such as 1 \( \mu \text{m}^2 \) - 100 \( \mu \text{m}^2 \), 100 \( \mu \text{m}^2 \) - 1000 \( \mu \text{m}^2 \), 1000 \( \mu \text{m}^2 \) - 10000 \( \mu \text{m}^2 \), or 10000 \( \mu \text{m}^2 \) - 100000 \( \mu \text{m}^2 \).

Further, the reservoir is substantially closed when the ratio between the area of sample exposed to air and the volume of sample contained in the pin member is less than or equal to 500 \( \text{m}^{-1} \), such as less than or equal to 200 \( \text{m}^{-1} \), 150 \( \text{m}^{-1} \), 100 \( \text{m}^{-1} \), 75 \( \text{m}^{-1} \), 50 \( \text{m}^{-1} \), 25 \( \text{m}^{-1} \), 15 \( \text{m}^{-1} \), 10 \( \text{m}^{-1} \), 5 \( \text{m}^{-1} \), 3 \( \text{m}^{-1} \), 2 \( \text{m}^{-1} \), 1 \( \text{m}^{-1} \), 0.5 \( \text{m}^{-1} \), 0.1 \( \text{m}^{-1} \), or 0.01 \( \text{m}^{-1} \), such as less than or equal to 0.001 \( \text{m}^{-1} \).

In a preferred embodiment of the present invention, the ratio between the area of sample exposed to air and the volume of sample contained in the pin member is less than or equal to 100 \( \text{m}^{-1} \).

In another embodiment of the present invention, the ratio between the area of sample exposed to air and the volume of sample contained in the pin member is less than or equal to 100 \( \text{m}^{-1} \), and the surface area of the sample that is exposed to air is less than or equal to 40000 \( \mu \text{m}^2 \).
Further, the reservoir and first channel are substantially closed when the sample in the reservoir and the first channel, respectively, is in contact with the walls of the reservoir and the first channel, respectively, the dispensing tip and possible gas trapped inside the reservoir and/or the first channel.

Still further, the reservoir and the first channel are substantially closed when the contents of the reservoir and the first channel, respectively, is confined by walls on all sides except for an opening connecting the reservoir with the first channel and optionally an opening connecting the reservoir to a vent, e.g. a second channel, as further described below.

The pin member may further comprise a vent in fluid communication with the reservoir. The vent allows equalization of the pressure within the reservoir with the pressure of the surroundings of the micropointing pin. The vent may be a second channel in fluid communication with the reservoir and with the surroundings. Preferably, the vent includes a liquid stop which prevents passage of sample. The liquid stop may be e.g. a hydrophobic porous material, a hydrophobic or hydrophilic surface of the second channel, a geometry of the second channel or an opening in fluid communication with the reservoir that terminates capillary flow from the reservoir.

The micropointing pin according to the invention may be composed of any material or combination of materials suitable for microfabrication including but not limited to semiconductor materials such as silicon (Si), silicon carbide (SiC), silicon nitride (Si₃N₄), insulator materials such as silicon dioxide (SiO₂), polymers, ceramics, non-ferric alloys. Any suitable microfabrication method or combination of methods may be used for making the micropointing pin depending upon the material or materials selected for the pin, the desired dimensional precision of the pin, and/or the desired manufacturing yield. Suitable microfabrication methods include but are not limited to chemical and physical microfabrication, photolithography, photoresist methods, microelectromechanical methods, e-beam lithography, and x-ray lithography. Precision machining techniques including but not limited to EDM, laser cutting, hot embossing, and injection moulding may be used to supplement the microfabrication methods.

Preferably, the pin member is manufactured from a material selected from the group consisting of semiconductors, organic polymers, ceramics, non-ferric alloys, ferric alloys, and glass. The glass may be a borosilicate glass, such as Pyrex ®.

In one preferred embodiment, the pin member is made of crystalline silicon.
In another preferred embodiment, the pin member is made of crystalline silicon and glass.

Generally, the glass of a microspotting pin may be a borosilicate glass or other suitable glass types. An example could be a PYREX® glass which has the following composition 80.6% SiO₂, 13.0% B₂O₃, 4.0% Na₂O and 2.3% Al₂O₃.

The dispensing tip may comprise crystalline silicon and/or it may comprise an organic polymer. The at least one first channel wall may comprise crystalline silicon. The at least one reservoir wall may comprise crystalline silicon.

Advantages of microfabricated microspotting pins, especially those made from silicon, over machined metal pins include 10-100 fold higher dimensional tolerances, less than 10% of the weight (lighter pressure gives more uniform spots and makes the pin last longer because of less mechanical wear), far less plastic deformation of the tip, the ability to chemically modify the SiO₂ surface of the microspotting pins to control wetting and liquid uptake/release, higher microspotting pin density in array, higher spot density in microarray, more precise volumetric uptake into microspotting pin, lower surface friction (ease of sliding movement in holder), resistance of tip to bending damage and the ability to fabricate complex features not obtainable by traditional machine shop fabrication. The combination of increased tip hardness and lower microspotting pin weight results in far less wear on the tip. The decreased tip wear will result in reduced wear of the dispensing tip, which may be inspected visually. The thin shafts on the Si microspotting pins, combined with the far greater elastic deformation of Si versus steel (suffers plastic deformation), suggest that the Si microspotting pins will not suffer from bending damage. The deposited drop size from the Si microspotting pin is uniform than those from the steel pins not only because of the higher tolerances, greater uniformity of machined dimensions from pin-to-pin and slower tip wear but the precision of the volumetric uptake of liquid into the microspotting pin should be higher as well.

Multiple wafers can be processed simultaneously to further cut the cost of production of the microspotting pins. The microspotting pins last longer and pin to pin variation is low. This makes a higher number of pins feasible leading to higher printing speeds, which again leads to a lower cost per spotted spot.

The microspotting pin may further comprise an adapter for receiving and holding the pin member.

The adapter facilitates handling of the pin member. For example, the operator may handle the head with his or her hands thereby avoiding the use of tweezers.
Further, the combination of the pin member and the adapter is more robust, since the adapter protects the shaft of the pin member.

The adapter may comprise an adapter head for handling the microspotting pin.

Preferably, the outer shape of the adapter fits the holes of a holder for the microspotting pins. If, e.g., the cross-sectional shape of the pin member is rectangular, quadratic, pentagonal or hexagonal, and the microspotting pin is mounted in a substantially circular hole in the holder, the microspotting pin without an adapter would typically move horizontally in the hole thereby changing the distance between spots and may even produce overlapping spots. Provision of a microspotting pin with an adapter having a substantially circular outer circumference eliminates this problem.

The cross section of the adapter may be various shapes as appropriate for mounting of the microspotting pin in the holder, for example substantially circular, such as circular, substantially rectangular, such as rectangular, substantially square, such as square, substantially triangular, such as triangular, etc.

The adapter may comprise a surface arrangement that prevents rotation of the microspotting pin in the aperture of a pin holder. For example, the adapter head may have a substantially cylindrical shape with a planar side fitting a similar hole in the holder so that the microspotting pin mounted in that hole cannot rotate. Alternatively, the holder may have a protrusion adjacent the hole for receiving and holding the microspotting pin, the adapter head abutting the protrusion when mounted in the hole whereby rotation of the pin is eliminated.

The adapter may comprises an adapter aperture for receiving a pin member and a fixing means for fixing the adapter to the pin member or the pin holder. The fixing means could be a glue for fixing the adapter to the pin holder hole. Also, the fixing means could be a protrusion of the adapter, said protrusion fitting into an aperture of a pin holder. In a preferred embodiment, the protrusion fitting into an aperture of a pin holder may furthermore comprise an adapter aperture capable of receiving, and preferably retaining, a pin member. For example, the adapter may be forced or glued into the aperture of the pin holder. In one embodiment, the adapter aperture is designed so that the pin member is able to move smoothly up and down through the adapter aperture.

In a preferred embodiment of the present invention, the adapter is non-removably fixed to the microspotting pin. The adapter may e.g. be non-removably fixed to the shaft.
and/or the pin mounting head and/or the dispensing tip. For example, the adapter may be non-removably fixed to the shaft of the microspotting pin.

The microspotting pin may be cleaned in the apparatus. The microspotting pin is dipped in water for some seconds and then dried in a vacuum station where each microspotting pin is lowered into a circular hole connected to a vacuum pump. The microspotting pin may not fill the circular hole completely so that an inefficient vacuum is achieved and therefore there may be liquid left both on the inner and outer surfaces of the microspotting pin. Provision of a microspotting pin with an adapter having a circular outer circumference eliminates this problem since the adapter preferably fits the hole in the vacuum stage and thereby make the vacuum much more efficient.

The adapter may be produced from a material selected from the group consisting of metals, semiconductors, polymers, ceramics, non-ferric alloys, ferric alloys and glass.

The adapter may comprise a slot for receiving a microspotting pin, the cross sectional circumference of the slot being less than or equal to 10% larger than the cross sectional circumference of the shaft of the microspotting pin, such as less than or equal to 8%, 6%, 4%, 2%, 1%, or 0.5% such as less than or equal to 0.1% larger than the cross sectional circumference of the shaft of the microspotting pin.

The at least one reservoir wall and/or the at least one first channel wall may comprise a protection layer that reduces or inhibits adsorption or non-specific binding of biomolecules to the walls.

The protection layer may comprise a material selected from the group consisting of a silane, a macromolecule, an inorganic film, an organic polymer, a semiconductor, a metal. Furthermore the protection layer may comprise combinations of these materials such as e.g. an inorganic film and silane, a metal and a silane, or a silane and a macromolecule.

The protection layer may be covalently or non-covalently attached to the at least one channel wall and/or the at least one reservoir wall.

In a preferred embodiment of the present invention, the protection layer comprises a silane. The silanizing agent for preparing the silane protection layer may for example be selected from group consisting of dimethylchlorosilane (DMCS), dimethyldichlorosilane (DMDCS), hexamethyldisilazane (HMDS), and trimethylchlorosilane (TMCS), poly(ethylene glycol) silane and combinations thereof. Hydrophilic silanes may be preferred for the protection layer and uncharged, hydrophilic silanes such as poly(ethylene glycol) silanes are even more preferred.
Hydrophilic surfaces can be converted to hydrophobic surfaces through the use of certain organic compounds. These organic compounds contain a ‘hydrophobic tail’ and a ‘hydrophilic head’ and are known as surfactants or surface active agents.

The hydrophobic tail typically consists of a hydrocarbon chain containing e.g. 12 carbon atoms [ex. \( \text{C}_{12}\text{H}_{25} \)]. The hydrophilic head may be non-polar (OH) or polar, (SO\(_2\)\(\text{COO}^-\)). These compounds absorb at the solid-liquid interface. Some silane based organic compounds can react with surface hydroxyl groups to make the surface hydrophobic. Reaction of octadecyltrichlorosilane [\( \text{C}_{18}\text{H}_{37}\text{SiCl}_3 \)] dissolved in an organic solvent with surface hydroxyl groups in the presence of controlled amounts of water produce a highly hydrophobic surface e.g. having a water contact angle of \( \sim 120^\circ \). The reaction may produce a self-assembled monolayer (SAM). The different silane products are applied as specified by the manufacturer. The hydrophobicity may be measured by applying a drop of liquid to the surface and measuring the contact angle.

For example, the silane protection layer may be prepared using of gauge needle and syringe filled with silanizing agent to run a small amount of silanizing agent through e.g. the first channel and/or reservoir of the microspoting pin, allowing excess silane to drip into a tube. The microspoting pins are rinsed 6 times in MilliQ-water and the water is blown out of the channels and reservoirs by nitrogen gas after each rinse. The microspoting pins are hereafter rinsed 6 x in 5% dextrose (Travenol water) adjusted to Ph 2.5 with 5M HCl. The liquid is blown out of the pin using pressurized nitrogen gas, between washing steps. Microspoting pins are put in the oven at 100 degrees till they are completely dry.

The protection layer may comprise a silicone. The siliconizing agent used for preparing the silicone may be selected from the group consisting of AquasilTM or SurfasilTM (Pierce, Rockford, Ill.), or SigmacoteTM (Sigma Chemical Co., St. Louis, Mo.).

The protection layer may comprise a macromolecule. The macromolecule may be selected from the group consisting of an amino acid polymer, or polymers such as polyvinylpyrrolidone, polyadenylic acid, or polymaleimide or compositions such as maleimide, poly-L-alanine, poly-L-aspartic acid, polyglycine, poly-L-phenylalanine, or poly-L-tryptophan.

The protection layer may comprise an inorganic film such as a metal oxide, a semiconductor oxide, a metal nitride or a semiconductor oxide. For example, the inorganic film may comprise silicon dioxide, silicon nitride, tantalum oxide, silicon carbide, silicides or combinations thereof.
In a preferred embodiment of the present invention, the inorganic film comprises silicon dioxide.

The protection layer may comprise a metal or semiconductor material. The metal or semiconductor material may e.g. be selected from the group consisting of aluminium, gold, silver, platinum, titanium, chromium, tungsten, palladium, silicon such as crystalline silicon or polycrystalline silicon and combinations thereof.

Also, the protection layer may comprise a polymer such as polyvinylchloride (PVC), polytetrafluoroethylene (PTFE), fluorinated ethylene propylene (FEP), polyhexafluoropropylene or paralyene-polymer (e.g. poly xylene).

The protection layer may have an average thickness in the range 0.5 μm–50 μm, such as 1 μm to 40 μm, 5-30 μm or 10-20 μm.

Alternatively, the protection layer may have an average thickness in the range 5 Å – 2 μm, such as 100 Å - 1 μm or 500 Å - 0.5 μm. The average thickness of the protection layer may be measured by standard techniques known to the person skilled in the art.

For example ellipsometry may be used.

In a microspotting pin, the thickness of the protection layer within the reservoir and/or the first channel may have a relative standard deviation of less than or equal to 30% such as less than or equal to 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% such as less than or equal to 0.5%.

The protection layer may cover at least at least 5% of the at least one wall of the first channel and/or the at least one wall of the reservoir, such as at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97.5%, or 99% such as 100%. Preferably, the protection layer covers all walls of the reservoir and the first channel.

An inorganic film may e.g. be prepared when two silicon wafer are used in the production of the microspotting pin. After the reservoir and the first channel have been micromachined in the silicon wafers but before joining the cover wafer and the pin wafer the silicon wafers are thermally oxidised in an O₂ or H₂O gas atmosphere e.g. at temperatures ranging from 900-1200 °C. Then the wafers may be contacted in a piranha solution (H₂SO₄: H₂O₂ / 4:1) for e.g. 30 minutes in order to make the surface hydrophilic prior to bonding.

Alternative an oxidised silicon nitride thin film can be formed on the surfaces. This also results in a hydrophilic surface. A non-oxidised nitride will be hydrophobic.
In the case of anodic bonded pins it is also possible to deposit a metal in the channels and reservoir prior bonding by standard silicon micro technology.

In a preferred embodiment of the present invention, the microspotting pin has an outer surface that cannot be wetted by the sample to be spotted. The outer surface of the microspotting pin that cannot be wetted by the sample may comprise the surface of the dispensing tip. In a preferred embodiment, the outer surface that cannot be wetted by the sample does not include the surface of the dispensing tip.

The outer surface that cannot be wetted by the sample may comprise a hydrophobic layer. For example the hydrophobic layer may be a material selected from a polymer, a metal or semiconductor nitride and a silane.

The polymer may be selected from the group consisting of polyvinylchloride (PVC), polytetrafluoroethylene (PTFE), fluorinated ethylene propylene (FEP), polyhexafluoropropylene and paralyene-polymers such as polyxylene.

In a preferred embodiment of the present invention, the silane is a silane comprising a hydrophobic side group. The side group may e.g. be C3, C6, C8, C12 or C18.

The hydrophobic layer may have an average thickness in the range 0.5 μm – 50 μm, such as 1 μm - 40 μm, 5 - 30 μm or 10 - 20 μm.

Alternatively, the hydrophobic layer may have an average thickness in the range 5 Å – 2 μm, such as 100 Å - 1 μm or 500 Å - 0.5 μm.

The average thickness of the hydrophobic layer may be measured by standard techniques known to the person skilled in the art. For example by spectroscopic ellipsometry using a Variable Angle Spectroscopic Ellipsometre (VASE®) produced by J.A.Woollam Co.

The average thickness of the hydrophobic layer may have a relative standard deviation of less than or equal to 30% such as less than or equal to 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% such as less than or equal to 0.5%. In a preferred embodiment of the present invention, the average thickness of the hydrophobic layer has a relative standard deviation of less than or equal to 5%.

The hydrophobic layer may cover at least 5% of the outer surface, such at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97.5%, or 99% such as 100%.

Preferably, the hydrophobic layer covers at least 95% of the outer surface.

The hydrophobic layer may be applied to the microspotting pin, e.g. to the shaft and/or to the dispensing tip, without being applied to the dispensing tip by first dipping the
microspotting pin in hydrophilic sample such as water in order to fill the first channel, optionally also the reservoir. Then the outer surface of the microspotting pin may be spray coated with for instance Teflon® spray, or, the surface can be coated with Teflon® in a STS ASE system. The first channel and the reservoir of the microspotting pin and optionally also the dispensing tip is protected against the hydrophobic coating by the hydrophilic sample.

SiO₂ on the outside of the microspotting pin can be removed by dipping in a Buffered Hydrofluoric Acid (BHF) solution while the first channel, reservoir and venting channel is filled with water. The time it takes for the BHF to diffuse into the water filled channel is much longer than the typical time it takes to etch the oxide on the outside. This will give a hydrophobic surface on the outside for at least 4 hours and a hydrophilic surface inside the pin.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings, the like reference characters have been used to identify like elements.

Figs. 1a-1d illustrate an exemplary embodiment of a microspotting pin having a closed reservoir and a partially closed first channel,

Figs. 2a-2b illustrate the details of the dispensing tip of the exemplary embodiment shown in Figs. 1a-1d,

Figs. 3a-b illustrate cross sectional views of a fusion bonded microspotting pin,

Figs. 4a, 4b illustrate cross sectional views of an anodic bonded microspotting pin,

Figs. 5a-5c illustrate three exemplary embodiments of the dispensing tip,

Figs. 6a-6e illustrate five exemplary embodiments of the end wall surface of the dispensing tip,

Figs. 7a-7f illustrate exemplary process steps for the preparation of the microspotting pins of the present invention,

Figs. 8a-8b illustrate the problem of loose fit between the pin holder and the microspotting pin and Figs. 8c-e illustrate the effect of using a microspotting pin comprising an adapter,

Figs. 9a-9d illustrate an exemplary embodiment of a microspotting pin having a head and an adapter with a recess for receiving the head,

Figs. 10a-10c illustrate an exemplary embodiment of a microspotting pin including an adapter, the adapter comprising two parts that are glued together,
Figs. 11a-11c illustrate an exemplary embodiment of a microspotting pin including an adapter, where the adapter comprises a tube that can receive the microspotting pin,

Figs. 12a-12c illustrate an exemplary embodiment of the microspotting pin where the head of the microspotting pin comprises the adapter,

Figs. 13a-13c illustrate an exemplary embodiment of the microspotting pin where the adapter is glued onto the head of the microspotting pin,

Fig. 14a-14c schematically illustrates mounting of microspotting pins in a holder,

Fig. 15 shows arrays of spots produced by a microspotting pin according to the present invention and a prior art microspotting pin, and

Fig. 16 shows plots of spot sizes of the arrays shown in Fig. 15.

It should be understood that the drawings are solely for the purpose of illustrating the concepts of the invention and are not intended as a level of the limits of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Figs. 1a – 1d illustrate a pin member 20 according to the present invention. Fig. 1 is a rear view, Fig. 1b is a cross-section along the length of the pin member 20, and Fig. 1c is a front view of the inventive pin member 20.

The pin member 20 has a dispensing tip 24 at a first end thereof with an end wall surface 34, a reservoir 28 defined by at least one reservoir wall 29, a first channel 30 defined by at least one channel wall 31 and extending from the reservoir 28 to the dispensing tip 24 providing fluid communication between the reservoir 28 and the dispensing tip 24. The pin member 20 further includes a shaft 22, and a pin mounting head 26 located at the end opposite the dispensing tip 24. In the illustrated embodiment, the pin mounting head 26 has a rectangular shape. The pin mounting head 26 may prevent the microspotting pin from falling through a mounting aperture in a holder and/or may be part of a surface arrangement used to avoid rotation of the microspotting pin in the aperture of the pin holder.

The length $L_p$ of the illustrated pin member 20 ranges from 0.5 cm to 5 cm, e.g. 2 cm or 3 cm. The length $L_s$ of the shaft 22 ranges from 0.5 cm to 5 cm, e.g. 2 cm or 3 cm, and the width $W_s$ of the shaft 22 ranges from 100 $\mu$m to 2 mm. The height $L_h$, the width $W_h$, and the depth of the pin mounting head ranges from 100 $\mu$m to 1 cm, from 100 $\mu$m to 4.5 mm, and 200 $\mu$m to 2 mm, respectively. Due to the nature of the wet etching of
crystalline silicon a <111> plane a4 forms a part of an outer surface of the illustrated pin member 20.

Fig. 1b is a cross-section along the length of the pin member 20. The thickness $T_T$ of the illustrated pin member 20 ranges from 200 $\mu$m to 2 mm, and the thickness $T_P$ of the dispensing tip 24 ranges from 1 $\mu$m to 500 $\mu$m.

As shown in Fig. 1c, the illustrated pin member 20 further comprises a vent 32 in the form of a second channel 33 in fluid communication with the reservoir 28, which again is in fluid communication with the first channel 30 that is open at the dispensing tip 24. The length of the vent $L_V$ may be between 0.1 cm and 2 cm and the width and/or the depth may e.g. be in the range from 10 $\mu$m to 500 $\mu$m. The reservoir width $W_R$ may range from 10 $\mu$m to 1000 $\mu$m and may have a length $L_R$ typically in the range from 10 $\mu$m to 2 cm.

Preferably, the vent includes a liquid stop, which prevents the sample from either entering or passing the vent. The liquid stop may be e.g. a hydrophobic porous material, a hydrophobic or hydrophilic surface of the second channel, a geometry of the second channel or an opening in fluid communication with the reservoir that terminates capillary flow from the reservoir.

The first channel 30 and the reservoir 28 may be constructed in many ways. For example the first channel and the reservoir may be partly or fully defined in a first body, e.g. made of solid silicon. Alternatively, the first channel 30 may be partly or fully defined in a first body, and the reservoir may be partly or fully defined in a second body, wherein the first body is, e.g., a microfabricated part made of solid silicon, and the second body is a made of glass and comprises the reservoir 28. In a further embodiment of the present invention, the first channel 30 and the reservoir 28 are partly or fully defined by a first body and a second body and optionally by a third body. For example, the pin member may constitute a sandwich structure, the top and bottom parts of the sandwich structure e.g. made from a silicon wafer and the middle part being a $\text{SiO}_2$ layer.

The first channel may e.g. have a depth in the range 0.1 - 200 $\mu$m and a width in the range 0.1 - 300 $\mu$m wide. The length of the first channel 30 may range from 50 $\mu$m to 2000 $\mu$m and the volume of the first channel may range from 0.5 $\mu$l to 0.2 $\mu$l.

The reservoir 28 may have a depth in the range 1 - 1000 $\mu$m, a width in the range 10 $\mu$m - 5 mm, and a length in the range 500 $\mu$m - 20 mm. The reservoir may have a volume in the range 5 $\mu$l - 5 $\mu$l or it may even have a volume in the range 1 $\mu$l - 25 $\mu$l.
In a preferred embodiment of the present invention, the volume of the reservoir is less than or equal to 25 ml such as less than or equal to 20 ml, 15 ml, 10 ml, 5 ml, 3 ml, 2 ml, 1 ml, or 0.5 ml, such as less than or equal to 0.1 ml.

The circumference may vary along the length of the microspotting pin and is typically in the range 50 μm – 3 mm.

The microspotting pin of the present invention especially useful for printing and manufacturing high quality microarrays of proteins, DNA, RNA, polypeptides and/or oligonucleotides and microarrays of other biological materials such as antibodies, beads and cells. The microspotting pin of the present invention may also be used for printing and manufacturing high quality microarrays of other matters, such as solid semiconductor quantum dots or liquid dots containing various functional molecules, such as sensors.

Fig. 1d illustrates an embodiment of the invention wherein the first channel 30, the second channel 33, and the reservoir 28 of the pin member 20 form a single channel extending along substantially the entire length of the pin member with substantially the same cross-section along the length of the channel.

The hatching in Figs. 1c and 1d indicate a groove in the pin member 20 with a solid bottom. It should be noted that a first part of the first channel 30 communicating with the reservoir 28 has a solid bottom while a second part of the first channel is open at the top and the bottom of the channel forming a slot in the pin member 20 providing appropriate fluid communication between the first channel 30 and the end wall surfaces 34.

Fig. 2a shows the end of the pin member 20 with the dispensing tip 24 in more detail. Again, the hatching indicate a groove in the pin member 20 with a solid bottom. Along the second part of the first channel 30, the dispensing tip 24 is tapered towards the end wall surfaces 34. The length of the tapered part a6 may range from 1 μm to 10 nm. The reservoir 28 is tapered towards the first channel 30. The dimension of the tapered part a5 is optimised to enhance the fluid communication between the reservoir 28 and the first channel 30. The taper length a5 is between 0 - 3 mm. At the end wall surface 34, the first channel 30 has an opening to the surroundings of the pin member 20. The opening is dimensioned to optimise the fluid communication between the first channel 30 and the end wall surface 34 (see details on Figs. 5a-5c). The length C of the first channel 30 may range from 1 μm to 10 mm. The width B of the first channel may range from 1 μm to 300 μm. The area A of the end wall surface 34 that touches a surface when printing typically ranges from 10⁻⁶ μm² – 10 mm², such as e.g. 10⁻⁶ μm² – 10⁻⁴
μm², 10⁻⁴ μm² − 10⁻² μm², 10⁻² μm² − 1 μm², 1 μm² − 100 μm², 100 μm² − 1000 μm², 1000 μm² − 10000 μm², or 10⁵ μm² − 10 mm².

Fig. 2b shows a cross-section of the shaft 22 of hatched part of the pin member 20 showing the first channel 30 etched in the silicon. In the illustrated example, the depth D of the first channel 30 is typically from 1 μm to 300 μm and has a rectangular shape.

Fig. 3 illustrates another embodiment of the invention wherein the pin member 20 has a closed reservoir 28, a closed first channel 30, and a closed second channel 33 in order to significantly reduce evaporation of the sample contained in the pin member 20. The illustrated embodiment is made by wafer bonding of two microfabricated silicon wafers.

In another embodiment of the present invention, the first channel 30 and the reservoir 28 are partly or fully defined by a first body and a second body and optionally by a third body whereby the microspotting pin constitutes a sandwich structure, the top and bottom parts of the sandwich structure e.g. comprising material from a silicon wafer and the middle part being a SiO₂ layer. The SiO₂ layer facilitates fusion bonding of the top and bottom parts.

Fig. 3a shows a longitudinal cross section of the exemplary fusion bonded dispensing pin member 20 comprising a pin mounting head 26 and the dispensing tip 24. The thickness T_{BP} of a bonded microspotting pin is typically in the range 100 μm − 2 mm, such as e.g. 200 μm − 1.5 mm.

Fig. 3b shows a transversal cross section with the closed reservoir 28. The shape of the reservoir is not limited to any particular shape and may be located in the wafer with the dispensing tip 24 or in the wafer providing a lid a8 or in both wafers. The microspotting pin may be rectangular or quadratic. The shown cross sectional shape of the microspotting pin is achieved by etching silicon in diluted potassium hydroxide.

Preferably, the wafer is a silicon wafer.

Alternatively, the lid a8 could be made of glass or a polymer, such as a plastic, and the reservoir may be sealed by anodic bonding or by gluing. The transparency of glass makes it possible to monitor the sample contained in the pin member 20.

Fig. 4a shows such an exemplary anodic bonded dispensing pin member 20 wherein the dispensing tip 24 is integrated in the silicon wafer a2 and a glass lid a8 is bonded to seal the reservoir 28.

Fig. 4b the cross sectional view of the dispensing pin member 20. The reservoir 28 may be defined in any of the wafers.
In a preferred embodiment of the present invention, the pin member 20 tapers towards the dispensing tip 24 from at least to sides, such as from 2, 3 or 4 sides.

Figs. 5a-c illustrate various exemplary embodiments of the dispensing tip 24. The shape of the dispensing tip may be optimised to give highly reproducible and uniform spots.

In Fig. 5a, two sidewall surfaces 37 gradually taper towards the end wall surface 34. The dispensing tip 24 has two substantially flat printing end wall surfaces 34, oriented generally perpendicular to the centre line C_L of the pin member 20, such that the end wall surface 34 is generally parallel to the surface of a substrate to be printed.

Fig. 5b shows another exemplary embodiment of a dispensing tip 24. The dispensing tip is formed by two sidewall surfaces 37, each of which tapers with an angle θ_1 relative to the centre line C_L of the pin member 20 and then continues in two tapering side wall surfaces a9, each of which has a smaller angle θ_2 relative to the centre line C_L of the pin member 20. The tapering angles θ_1 and θ_2 equal range from 0° to 90°, and θ_2 < θ_1.

The end wall surface 34 is defined by two substantially point end wall surfaces a10.

In Fig. 5c θ_2 = 0° resulting in a second section 38 that is non-tapered. The length L_E of the second part of the dispensing tip 24 is 5 μm – 5 mm. The end wall surface 34 is defined by two substantially flat printing end wall surfaces 39, oriented generally perpendicular to the centre line C_L of the pin member 20, such that the surfaces 39 are generally parallel to the surface of a substrate to be printed.

The configuration and dimensions of the dispensing tip 24 can be designed so that the volume of liquid sample deposited by each pin member 20 and/or the area of the spotted liquid sample (spot) can be varied as desired. It is contemplated that for example the configuration and dimensions of the dispensing tip 24 can be designed so that the volume of liquid sample deposited by each pin member 20 can be as large as about 0.1 ml, as minute as about 10^{-4} pl, or any volume between about 0.1 ml and 10^{-4} pl. Similarly, the configuration and dimensions of the dispensing tip section 24 can be designed so that the area of the spotted liquid sample (spot) deposited by each pin member 20 can be as large as about 10 mm^2, as minute as about 10^{-6} μm^2, or any area between about 10 mm^2 and about 10^{-6} μm^2.

One of ordinary skill in the art will of course appreciate that the dispensing tip section 24 may be configured in various other ways to optimize the microspotting process. For example, the surface or surfaces making up the end wall surface 34 may be smooth,
textured, concave, convex, include one or more pores, channels, or nozzles or combinations of the same.

Figs. 6a-e illustrate exemplary embodiments of the end wall surface(s) 34. Fig. 6a is a bottom view of the end wall surfaces 34 of the dispensing tip 24 of Fig. 5a where the two substantially flat printing end wall surfaces 34 is oriented perpendicular to the centre line C1 of the microspotting pin such that the surfaces 34 are generally parallel to the surface of a substrate to be printed. The opening to the first channel 30 is shown, and it should be noted that the part of the first channel, which is proximal to the dispensing tip, may be open in this embodiment.

Figs. 6b and 6c show examples of a partly closed first channel 30 where the end wall surface is U-shaped, and Figs. 6d and 6e show embodiments of end wall surface(s) where the cross section of the closed first channel is triangular a13 and rectangular a14, respectively. By changing the shape of the first channel, it may be possible to change the surface tension of the liquid at the end wall surface 35 and thereby the release of the droplet from the dispensing tip. The cross sectional shape could be any and is not limited to the ones shown in the figures. In a preferred embodiment of the present invention, the cross sectional shape of the first channel is triangular. The triangular shape seems to facilitate the release of the liquid droplet from the dispensing tip to the substrate to be printed. The area A of the end wall surface(s) of the dispensing tip 24 that touches a surface when printing is typically $10^{-6} \, \mu m^2 - 10 \, mm^2$, such as e.g. $10^{-6} \, \mu m^2 - 10^{-4} \, \mu m^2$, $10^{-4} \, \mu m^2 - 10^{-2} \, \mu m^2$, $10^{-2} \, \mu m^2 - 1 \, \mu m^2$, $1 \, \mu m^2 - 100 \, \mu m^2$, $100 \, \mu m^2 - 1000 \, \mu m^2$, $1000 \, \mu m^2 - 10000 \, \mu m^2$, or $10^5 \, \mu m^2 - 10 \, mm^2$.

Starting at the top, Figs. 7a and 7b illustrate the processing steps providing the wafer defining the dispensing tip, channels and the reservoir.

As shown at the top of Fig. 7a, in process step 1, photoresist 82 (AZ5214e, 4.2 \mu m) is spun by conventional spin coating technique on a crystalline silicon wafer (350 \mu m thick double sided polished) 80. Below, in process step 2, photolithography is performed using an Electronic Vision EV 450 mask aligner (EV-aligner) where the mask is aligned to the flat of the wafers and the resist is exposed for 25 sec and subsequently developed for 1 min. This step defines the reservoirs, channels and venting channel and tip configuration. In process step 3, using the photoresist 82 as etching mask the pattern is transferred into the silicon by deep reactive ion etching on a STS ASE system. The photoresist is then stripped in acetone.

In process step 4, a stochiometric silicon nitride thin film b1 is deposited on the wafer 80 by low pressure chemical vapour deposition (LPCVD), layer thickness is typically
1500 Å. In process step 5 illustrated at the top of Fig. 7b, photoresist (AZ5214e, 1.5 μm) 82 is spun on the rear of the wafer and a second mask is aligned by back side alignment. Below, in process step 6, the photoresist is exposed for 8 sec and developed for 1 min defining the shape of the microspotting pin. The pattern is transferred to the wafer by reactive ion etch of the silicon nitride using the photoresist 82 as mask in STS RIE-system. The photoresist is then stripped in acetone.

In process step 7, the microspotting pins are released by etching the wafer all the way through in a diluted potassium hydroxide (28wt%) saturated with isopropanol at a temperature of 70 °C. Etching rate is measured to 0.6 μm/min.

Finally, in process step 8 illustrated at the bottom of Fig. 7b, the silicon nitride is stripped in a 160 °C warm phosphorous acid (H₃PO₄). An optional coating b2 is grown by thermal oxidation after a standard RCA-cleaning process. Oxide thickness is ranging from 100 Å - 3 μm, grown at temperatures ranging from 900 °C – 1200 °C in a dry O₂ or wet H₂O gas atmosphere.

Each microspotting pin is released from the wafer with a tweezer and is directly used for microarray printing or an adapter is mounted to optimise the fitting to the holder in the robot.

Starting at the top, Figs. 7c and d illustrate the processing steps providing a cover wafer made of crystalline silicon and including a fusion bonding step for joining the cover wafer and the wafer defining the dispensing tip, channels and the reservoir.

In process step 1 illustrated at the top of Fig. 7c, a stoichiometric silicon nitride thin film b1 is deposited on a double-sided polished 300 μm thick crystalline silicon wafer 80 by a low pressure chemical vapour deposition system (LPCVD). Layer thickness is typically 1500 Å. Photoresist 82 (AZ5214e, 1.5 μm) is spun on the wafer 80. Below, in process step 2, a mask is aligned to the flat of the wafer using an EV aligner. The photoresist 82 is exposed for 8 sec and developed for 1 min defining the shape of the microspotting pin. In process step 3, the pattern is transferred to the wafer by reactive ion etch of the silicon nitride b1 using the photoresist 82 as mask in STS RIE-system. The photoresist is then stripped in acetone.

As shown at the top of Fig. 7d, in process step 4, the wafer 80 is subsequently etched all the way through in a diluted potassium hydroxide (28wt%) saturated with isopropanol (IPA) at a temperature at 70 °C. Etching rate is measured to 0.6 μm/min.

In process step 5, the silicon nitride b1 is stripped in a 160 °C warm phosphorous acid (H₃PO₄).
An optional coating b2 is grown by thermal oxidation after a standard RCA-cleaning process. Thickness ranging from 100Å-3 µm grown at temperatures ranging from 900°C – 1200°C. in a dry O2 or wet H2O gas atmosphere.

The wafers 80 are going through a cleaning process consisting of 5 min in piranha in order to give the wafer a rough clean. Then 100 sec in an IMEC clean to loosen particles and give the wafer a gently chemical polish followed by 20 min in piranha in order to clean but mainly to make the surface highly hydrophilic. Piranha is a solution consisting of H2SO4:H2O2 in the ratio 4:1 at 80°C. IMEC consist of H2O:5%HF:IPA in the ratio 100:10:1 at room temperature.

In process step 6, the wafers 80 are pre-bonded using EV aligner with bonding chucks. The bond is strengthened in an annealing step in a N2 atmosphere for 4 hours at 1100°C.

Each microspotting pin is released from the wafer with a tweezer and is directly used for microarray printing or an adapter is mounted to optimise the fitting to the holder in the robot.

Starting at the top, Figs. 7e and 7f illustrate the processing steps providing a cover wafer made of a bulk glass wafer (borosilicate glass wafers from Schott Glass, 500 µm thick with sharp edges) and including an anodic bonding step for joining the cover wafer and the wafer defining the dispensing tip, channels and the reservoir.

In process step 1 illustrated at the top of Fig. 7e, a 1 µm amorphous silicon layer b4 is deposited on a glass wafer b3 using a Varian sputtering system. (Alternatively it could also be a metal bi-layer e.g. Ti/Au). Prior to sputtering the wafers are cleaned on a polishing wash bench with water and Triton soap followed by a piranha clean (H2SO4:H2O2 in 4:1) for 10 min at 80°C. The surface is hydrogen passivated by a 10 sec. dip in HF 5% in order to optimise the adhesion of the silicon layer b4 to the glass wafer b3.

In process step 2, thick photoresist b5 (AZ4562e, 9 µm) is spun on the glass wafer b3 by conventional spin coating technique and soft baked for 3 min at 90°C. Photolithography is performed on an EV-aligner. The photoresist is exposed for 50 sec and subsequently developed for 3.5 min., defining the shape of the microspotting pins.

In process step 3, the pattern is transferred to the amorphous silicon layer b4 using the photoresist b5 as mask in a polycrystalline silicon wet etch (HNO3:BHF:H2O in the ratio 500:25:500) for about 7 min. The photoresist b5 is hard baked in an oven for 25 min at 120° in order to dry out the photoresist and thereby make it more resistant to the
following wet-etch. The edges are protected by blue film prior to the following wet-etch. The glass wafer b3 is etched in 40% HF all the way through (etch rate~ 3 μm/min).

In process step 4, photoresist is subsequently stripped in acetone and the silicon is stripped in a polycrystalline silicon wet etch (HNO₃:BHF:H₂O in the ratio 500:25:500) for about 7 min. The wafers 80 and b3 are cleaned in a piranha solution for 10 min.

In process step 5, the wafers 80 and b3 are bonded in a home made anodic bonding equipment with integrated alignment. The wafers are placed in a chamber in a 1 bar N₂ ambient on two molybdenum plates with individual heating and temperature control positioned over one another facing the two surfaces to be bonded against each other. The wafers is separated a small distance about 20-50 μm, while the wafers are aligned, by moving the plates with x-y manipulators. Then the wafers are joined. The temperature on the plates is raised to about 350 °C - 400 °C. A voltage is applied across the wafer stack. The voltage is ramped up to about 1 kV and as long the bonding take place a current is running across the stack. When the current is sufficiently small (< 0.5 mA) the voltage is turned off and the wafers taken out ready for use.

Each microspotting pin is released from the wafer with a tweezer and is directly used for microarray printing or an adapter is mounted to optimise the fitting to the holder in the robot as explained in more details below.

The microspotting pins and pin holders may be assembled by placing a desired number of the microspotting pins into each of the holders. This may be accomplished with the aid of a vacuum tweezers, which grasps the mounting head of the microspotting pin. Each microspotting pin is dropped into a desired slot in the holder.

After the microspotting pins are placed in a corresponding holder, the holder is attached to the arm of a precision x-y-z motion control system (not shown). The microspotting pins are moved to the source plate location and the microspotting pins filled by dipping into the solution. The volume picked up by each microspotting pin is on the order of a microlitre or less of sample. The spot is actually made by the careful z motion and touching the microspotting pins to the substrate. To account for height variations on the surface of the substrate, and to prevent unnecessary wear on the pin tips, the microspotting pins "float" in their holder and rise out of the slots of the holder as they touch the surface of the substrate as shown in Fig. 8b, thereby providing a very light touch, but one that depends on the weight of the microspotting pin. After the microspotting pins are filled, they go through a "pre-spotting" procedure of 20 spottings during which time the volume deposited decreases to its steady state value.
Presumably, this is due to the removal of the liquid film that is adhering to the external surface of the shaft with subsequent print volumes replenished by drawing from the reservoir 28 and first channel 30. The microspotting pin transfers the print volume to a hydrophobic or chemically reactive surface to prevent spreading of the drop. In many cases the material being deposited is also (reversibly) covalently bound to the surface of the substrate, for example the nitrogen functionality on the DNA oligomer bound to an aldehyde group of the substrate to form a Schiff base complex that is later removable. After deposition the subsequent processing depends on the final application but in the case of DNA oligomers, the surface is used in hybridization experiments with a probe DNA molecule.

Fig. 8a-8d show an exemplary embodiment of a microfabricated spotting apparatus. The microspotting pins 20 are mounted in a pin holder 40, which is attached to a robot (not shown) with precise movement in x-y-z-directions. The pin mounting head 26 prevents the microspotting pin from falling through the holder and defines the critical length of the microspotting pin. In Fig. 8a the microspotting pins are in close proximity to the surface and vertical aligned in the holder. The microspotting pins touch the surface of the substrate in Fig. 8b, thereby creating a spot on the surface of the substrate. The microspotting pins might tip over because the silicon microspotting pin does not fit perfectly in the circular hole in the holder due to the pin shape giving raise to misaligned spots. In Fig. 8c the adapter c1 is attached to the microspotting pin and the microspotting pin stays aligned in existing pin holder, when spotting.

In Fig. 8d the spotting apparatus is moved over the vacuum stage c2 in the robot bench as part of the washing procedure used to clean the microspotting pins between print runs to avoid cross contamination. In the lid 30 are holes with same dimensions as in holder 40. Since the silicon microspotting pins are not circular as stainless steel pins the vacuum suction is not very efficient. Making the adapter sufficiently long that the adapter and microspotting pin fit the holes in the lid 30 of the conventional vacuum suction stages in Fig. 8e, and thereby gives a more effective vacuum suction of the microspotting pins.

Fig. 9a illustrates an exemplary embodiment of a microspotting pin comprising a microfabricated pin member 20 and an adapter m1. The adapter m1 provides easier handling of the microspotting pin and may improve the alignment of the microspotting pin in the pin holder attached to the apparatus (not shown). The adapter comprises an adapter head m3 and an adapter shaft m2. The diameter $D_h$ of the adapter head may be at maximum 4.5 mm and the outer diameter $D_s$ of the adapter shaft may e.g. be 0.5
mm to 3 mm. The height $H_n$ of the head may e.g. be in the range 0.5 mm - 10 mm. The length $L_S$ of the shaft may e.g. be in the range 0.5 mm - 5 cm. The pin mounting head 26 is aligned in the adapter head m3, a1 is a second channel which may be used as a vent of the microspotting pin in the pin mounting head 26.

Fig. 9b illustrates the cross sectional view of the adapter. The trench or aperture in the head m3 is dimensioned to align and fit the pin mounting head 26 of the microspotting pin. The tapered slot facilitates centring of the microspotting pin. $D_m$ is the inner diameter of the lower end of the adapter shaft m2 and may equal the width of the pin shaft 100 μm - 2 mm. DC is the inner diameter of the upper part of the adapter shaft m2 and may be larger or equal to $D_m$. The width $D_k$ of the adapter head aperture for receiving and holding the pin mounting head 26 may range from 1 mm to 4.5 mm.

Fig. 9c is a top view of the adapter head m3 with the pin mounting head 26 of the microspotting pin placed in the aperture in the adapter head m3. The vent 32 of the pin member 20 having a closed reservoir 28 is open so that the capillary forces are allowed to force sample into the pin member channels and reservoir.

Fig. 9d shows a cross section of the shaft of a preferred embodiment of a microspotting pin comprising the adapter and shows an example of the distribution of material m4 that attach the microspotting pin to the adapter. The attaching material m4 may be one of an adhesive such as epoxy glues like e.g. EPO-TEK77, UV-cured glues like e.g. Dymax, Araldit or 10 seconds glues like e.g. Attak, underfills, a glass frit. The adapter m1 may be comprise one or more of following materials e.g. metals, stainless steel, brass, polymers such as plastics, or ceramics. For instance the adapter shaft m2 could comprise a stainless steel pipe m2 and the adapter head may comprise a polymer, e.g. PMMA.

Figs. 10a - 10c illustrates an exemplary embodiment of the microspotting pin comprising the adapter wherein the adapter comprises two parts m11 and m12. The parts m11 and m12 may be made of e.g. metals, stainless steel, brass, polymers such as plastics, ceramic.

Fig. 10a illustrate how a part m11 could be outlined seen from the front side with the recess m13. The outer shape of the adapter may be like the one illustrated in Fig. 9a-c. The diameter $D_n$ of the head may e.g. be less than or equal to 4.5 mm and the outer diameter of the shaft $D_S$ is 0.5 mm to 3 mm. The height $H_n$ of the head may be in the range 0.5 mm - 10 mm. The length $L_S$ of the adapter shaft may be in the range 0.5 mm to 5 cm. Inside each adapter part a recess m13 is made to receive the pin shaft 22 and the pin mounting head 26. The width $B_R$ of the recess to adapt the pin shaft may be in
the range 100 μm - 2mm and the width $D_R$ of the recess to adapt the pin mounting head may be in the range 1 mm - 4.5 mm. Furthermore, Fig. 10a illustrates where to dispense the glue m10 for assembly as described later.

Cross sectional views of the adapter is illustrated on Figs. 10b - 10c. The depth $D_R$ of the recess m13 is half the pin shaft thickness i.e. anywhere between 100 μm - 1 mm and the height of each part is the half of the width $D_S$. The adapter parts and the microspotting pin are easily joined by pick and place as illustrated on Figs. 10b - 10c. First the lower part m11 is positioned facing the recess upward, secondly glue is dispensed in the bottom of the recess. Thirdly the pin member 20 is placed on the glue in the recess. Glue m10 is dispensed on top of microspotting pin and on the upward facing sides of the adapter part m11. Finally the other adapter part m12 is placed on top of the assembly facing the recess downwards. Optionally the space between the microspotting pin and the adapter walls can be filled with e.g. a underfiller, a glass frit or a silicone for sealing.

Fig. 11 shows another exemplary embodiment a microspotting pin comprising an adapter. The illustrated adapter comprises a cylindrical pipe m20 wherein the pin shaft 22 is placed. The length $L_N$ of the adapter has to be long enough to guide and align the microspotting pin in the holder and make a seal in the vacuum station, but not so long that it is dipped into the sample, when loading the microspotting pin. Typical $L_N$ is anywhere between 0.5 - 5 cm. The diameter $D_N$ of the adapter m20 is dimensioned to fit any hole of the holder in the robot, typical having a diameter $D_N$ anywhere between 0.5 - 3 mm and with a wall width $T_W$ between 50-300 μm. The microspotting pin may be sealed in the adapter by a material m4 e.g. glue, filler, silicone or glass frit. In this case the microspotting pin rest on the holder 40 on the edge of the pin mounting head 26. The cylindrical pipe could be a commercial needle pipe. The cylindrical pipe may comprise a material selected from the group consisting of metals, stainless steel, brass, plastic, polymers, ceramic. Optionally, an adapter head can be mounted on the pin mounting head 26 for increased support and handling of the microspotting pin. An exemplary embodiment of an adapter head is described in Fig. 12.

Figs. 12a - 12c show an exemplary embodiment of a microspotting pin comprising an adapter m30 capable of receiving the pin mounting head 26 of the microspotting pin. The adapter is for easier handling and support of the microspotting pin in the pin holder attached to the robot (not shown). The adapter shown on Fig. 12a-b comprises a head m30 with a recess to adapt the pin mounting head 26 and a venting hole m31 in fluid communication with the vent 32 in the pin member 20. The diameter $D_{AH}$ of the head is
at maximum 4.5 mm and the length $L_{AH}$ of the head is anywhere between 3 mm - 10 mm.

Fig. 12c illustrates the cross sectional view of the adapter. The recess in the adapter head m30 is dimensioned to align the microspotting pin and fit to the pin mounting head 26. The depth m32 of the recess may equal the length ($L_{v}$) of the pin mounting head 26, that is to say, in the range 100 µm – 1 cm. The width $W_{AH}$ of the recess may be in the range 200 µm – 2 mm in order to match the thickness of the pin mounting head. $D_{v}$ is the diameter of the venting hole m31 and is anywhere between 100 µm - 2 mm.

The pin mounting head 26 is mounted in the adapter m30 either by gluing with glue e.g. EPO-TEK77, UV-cured glues like e.g. Dymax, Araldit or 10 seconds glues like e.g. Attak or if the material is flexible, by squeezing the microspotting pin in to stick. The adapter m30 might be made of one or more of following materials e.g. metals, stainless steel, brass, plastic, polymers, ceramics. The shape is not limited to the one shown. It could as well be cubic. The adapter can optionally be combined with the adapter illustrated in Fig. 12, in order to combine guidance and handling of the microspotting pin.

Fig. 13a - 13c shows an exemplary embodiment for a microspotting pin comprising an adapter that uses the edges m42 of the pin member 20 as well as the adapter m40 itself for guidance in the holder hole m41. Fig. 13a and b shows the microspotting pin and adapter from the front and the side respectively with the adapter m40 mounted. The length $L_{1}$ of the adapter may be in the range 0.5 cm - 5 cm and $L_{2}$ may be in the range 0.3 cm - 1 cm. $L_{2}$ may be used for handling the microspotting pin and $L_{1}$ may be used as the guidance of the microspotting pin in the holder hole m41. In the upper end there may be a venting hole m43 e.g. with a diameter in the range 100 µm - 800 µm in fluid communication with the vent in the pin member 20. The width $W_{1}$ of the adapter may e.g. be in the range 300 µm – 1 mm. In Fig. 13b is a side view of the microspotting pin comprising the adapter and it can be seen that the adapter is split into two legs m44. The distance between the two leg m44 may e.g. be in the range 300 µm – 2 mm.

The pin member 20 is placed and fixed between the two legs. The total width $W_{2}$ of the adapter shaft may e.g. be in the range 0.5 mm - 2 mm.

Fig. 13c is a cross section of the pin shaft 22 placed between the two legs m44 of the adapter m40 in a holder hole m41 to show the idea of this type of adapter. The cross sectional shape of a leg m44 can be either as illustrated on the drawing or rectangular.

In the latter case the microspotting pin with adapter may be guided in the holder hole
m41 by the corners m45 of the legs and the edges m42 of the microspotting pin itself. The microspotting pin is mounted in the adapter either by gluing with glue e.g. EPO-TEK77, UV-cured glues like e.g. Dymax, Araldit or 10 seconds glues like e.g. Attak or if the material is flexible, by squeezing the microspotting pin in to stick. The adapter m30 might be made of one or more of following materials e.g. metals, stainless steel, brass, plastic, polymers, ceramics.

Fig. 14 illustrates a pin holder 40 for mounting of microspotting pins. The illustrated pin holder 40 is constituted by a planar member 44 having an array of circular holes m41 extending there through, each of the holes m41 accepting a microspotting pin according to the present invention. Pin holders of the illustrated type may accommodate up to 5000 microspotting pins 20 according to the present invention. The illustrated embodiment has a width Wph of app. 16 cm and a depth Dph of app. 10 cm. The array pattern, pitch, and dimensions of the holes m41 defines a pin density. The pin density of the holder 40 is important since it determines the time for making the microarray by the holder 40 with the microspotting pins. In one embodiment, the shafts 22 of the microspotting pins 20 have a cross-section that allows the shafts 22 to be slip-fitted into the holes m41 in a frictionless manner with no lateral movement and be suspended from their mounting heads 26, which rest on the upper surface 44 of the pin holder 40. In another embodiment, the adapters of the microspotting pins 20 have a cross-section that allows the adapters to be slip-fitted into the holes m41 in a frictionless manner with no lateral movement and be suspended from their adapter heads m30, which rest on the upper surface 44 of the pin holder 40, while preventing rotation of the microspotting pins 20 in the holes m41. As also illustrated in Fig 14, the holder has protrusions 42 adjacent respective holes, the adapter head abutting the protrusion when mounted in the hole whereby rotation of the microspotting pins is eliminated.

Fig. 15 shows arrays of spots produced by a microspotting pin (pin B) according to the present invention and a prior art microspotting steel pin (pin A), and Fig. 16 shows plots of spot sizes of the arrays shown in Fig. 15. The pins are tested using a 1x MSS solution (Genpak, Genetix, UK) prepared with 2 uM Cy3- labelled 25-mer oligonucleotide (TAG Copenhagen). 10 ul is loaded into a 384 microtiterplate and placed in the microarraying robot (Q-Array, Genetix, UK). Pins are installed in the respective holder, slides (cel associates, US) are put in the slide holder and the lid is closed. The program initiates by a washing step followed by drying in the vacuum station. Here after the microspotting pin pick up liquid from the microtiterplate and
starts dispensing on the slides. Slides are scanned in a laser scanner (Scanarray lite, Packard).

Fig. 15 shows arrays of 400 spots with pitch sizes of 200 μm, 150 μmm and 100 μm, respectively. Each array is printed twice for each pit size. It is evident that the prior art steel pin only print 200 spots per sample load and for pitch size 100 μm, the spots printed by the prior art pin are overlapping. The microspotting pin according to the present invention is capable of printing far more than 400 spots per sample load and easily prints an array of dots with a 100 μm pitch.

The higher resolution and improved repeatability of the microspotting pin according to the present invention is further illustrated in Fig. 16.

Although the invention has been described in terms of exemplary embodiments, it is not limited thereto. Rather, the appended claims should be construed broadly, to include other variants and embodiments of the invention, which may be made by those skilled in the art without departing from the scope and range of equivalents of the invention.
CLAIMS

1. A microspotting pin for dispensing a volume of a liquid on a substrate, comprising
   a pin member with
   a dispensing tip at a first end thereof with an end wall surface,
   a reservoir defined by at least one reservoir wall,
   a first channel defined by at least one channel wall and extending from the
   reservoir to the dispensing tip providing fluid communication between the
   reservoir and the dispensing tip, and wherein
   the reservoir is a closed reservoir.

2. A microspotting pin according to claim 1, further comprising an adapter for
   receiving and holding the pin member facilitating handling of the pin member.

3. A microspotting pin for dispensing a volume of a liquid on a substrate, comprising
   a pin member with
   a dispensing tip at a first end thereof with an end wall surface,
   a reservoir defined by at least one reservoir wall,
   a first channel defined by at least one channel wall and extending from the
   reservoir to the dispensing tip providing fluid communication between the
   reservoir and the dispensing tip, and
   an adapter for receiving and holding the pin member facilitating handling of the pin
   member.

4. A microspotting pin according to claim 3, wherein the reservoir is a closed
   reservoir.

5. A microspotting pin according to any of the preceding claims, wherein the pin
   member further comprises a head facilitating handling of the microspotting pin.

6. A microspotting pin according to any of claims 2-5, wherein the adapter further
   comprises a head for handling of the microspotting pin.

7. A microspotting pin according to any of claims 2-6, wherein the pin member is
   movably positioned in the adapter.

8. A microspotting pin according to any of claims 2-7, wherein the pin member is
   fixed to the adapter.
9. A microspotting pin according to any of claims 2-8, wherein the adapter prevent rotation of the microspotting pin in the pin holder.

10. A microspotting pin according to any of the preceding claims, wherein at least a part of the pin member is made of silicon.

11. A microspotting pin according to any of claims 2-10, wherein at least a part of the adapter is made of steel.

12. A microspotting pin according to any of the preceding claims, wherein the pin member further comprises a venting means in fluid communication with the reservoir.

13. A microspotting pin according to any of the preceding claims, wherein the venting means comprises a second channel in fluid communication with the reservoir.

14. A microspotting pin according to any of the preceding claims, wherein the first channel is a closed channel.

15. A microspotting pin according to claim 13 or 14, wherein the second channel is a closed channel.

16. A microspotting pin according to any of the preceding claims, wherein at least one wall for contacting liquid comprises a protection layer.

17. A microspotting pin according to claim 16, wherein the protection layer comprises a silane.

18. A microspotting pin according to any of the preceding claims, wherein the pin member has a cross-section that varies along the longitudinal extension of the pin member.

19. A microspotting pin according to any of the preceding claims, wherein the dispensing tip of the pin member has the smallest cross-section of the pin member.

20. A microspotting pin according to any of the preceding claims, wherein outer surface of the dispensing tip comprises a hydrophobic layer for inhibiting wetting of the outer surface.

21. A microspotting pin according to any of claims 13-20, wherein the reservoir, the first channel, the second channel, and the reservoir form a channel extending along substantially the entire length of the pin member with substantially the same cross-section along the length of the channel.
Fig. 4a

Fig. 4b
Fig. 7c
Fig. 7d
Fig. 7e
Fig. 7f

12/23
Fig. 16

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<th>Pin</th>
<th>Average spot diameter</th>
<th>Percentage deviation</th>
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<td>Pin A</td>
<td>130 um</td>
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<td>Pin B</td>
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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 BOIL 3/00

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 BOIL

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

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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search: 21 July 2005

Date of mailing of the international search report: 29/07/2005

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Authorized officer: Wypolsz, N
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