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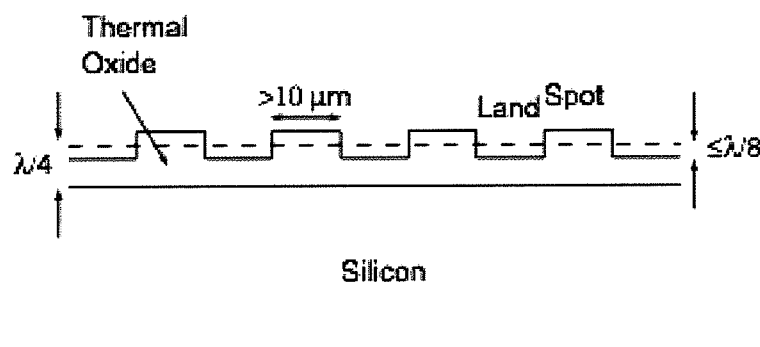


Figure 1

(57) Abstract: A land contrast platform for interferometrically detecting the presence of a target analyte in a sample is disclosed. The land contrast platform includes a spatially patterned substrate with a layer of recognition material immobilized thereon. The spatially patterned substrate includes a plurality of high regions and low regions; the high regions being at a substantially uniform first height and the low regions being at a substantially uniform second height, the first and second heights being different. The layer of recognition material is designed to bind with the target analyte. The high and low regions have approximately the same reflectance but complementary quadrature conditions such that equal target analyte binding on the high and low regions produces opposite responses; target binding on the high regions producing a positive or negative reflectance difference and target binding on the low regions producing the other of the positive or negative reflectance difference.

Balanced-Quadrature Interferometric Protein Microarray

[001] BACKGROUND

[002] The present invention generally relates to systems for detecting the presence of one or more target analytes or specific biological material in a sample, and more particularly to a microarray for detecting the presence of biological materials and/or analyte molecules bound to target receptors by sensing changes in the optical characteristics of a probe beam reflected from the surface by the materials and/or analytes.

[003] In many chemical, biological, medical, and diagnostic applications, it is desirable to detect the presence of specific molecular structures in a sample. Many molecular structures such as cells, viruses, bacteria, toxins, peptides, DNA fragments, and antibodies are recognized by particular receptors. Biochemical technologies including gene chips, immunological chips, and DNA arrays for detecting gene expression patterns in cancer cells, exploit the interaction between these molecular structures and the receptors. These technologies generally employ a stationary chip or other structure prepared to include the desired receptors (those which interact with the target analyte or molecular structure under test). Since the receptor areas can be quite small, chips may be produced which test for a plurality of analytes. When the receptors are exposed to a biological sample, only a few may bind a specific protein or pathogen.

[004] One such technology for screening for a plurality of molecular structures is the so-called immunological compact disk, which simply includes an antibody microarray. Conventional fluorescence detection can be employed to sense the presence in the microarray of the molecular structures under test. Other approaches to immunological assays employ traditional Mach-Zender interferometers that include waveguides and grating couplers. Interferometric optical biosensors have the intrinsic advantage of interferometric sensitivity, but are sometimes characterized by large surface areas per element, long interaction lengths, or complicated resonance structures. They also can be susceptible to phase drift from thermal and mechanical effects.

[005] While the abovementioned techniques have proven useful for producing and reading assay information within the chemical, biological, medical and diagnostic application industries, developing improved fabrication and reading techniques with improvement in performance over existing technology is desirable.

[006] SUMMARY

[007] Traditional protein microarrays use the structure of spots printed on a homogenous substrate (the surrounding land) to produce the contrast for imaging molecular binding. However, physical differences between the spot and the background can lead to systematic shifts in the data from these protein-patterned biological platforms. A land-contrast (LC) platform can use a spatially patterned substrate rather than protein patterns to produce image contrast. An embodiment of the protein-patterned platform utilizes surface-enhanced reflectometry to detect protein immobilized on silicon wafers with 140 nm thermal oxide to maximize the sensitivity. The oxide layer establishes an interferometric quadrature condition, and a 1 nm protein layer (at a spot) induces 2.4% reflectance change ($\Delta R/R$) at a 633 nm wavelength and normal incidence.

[008] There have been other approaches to fabricating protein microarrays using patterned substrates, most notably the BIND® biosensor of SRU Biosystems, Woburn, MA, USA (see for instance, U.S. Patent No. 7,371,562) and the Epic® System of Corning Inc., Life Sciences Division, Lowell, MA, USA (see for instance, U.S. Patent No. 7,349,080). However, these systems function by fabricating distributed diffraction elements that diffract incident light to propagate along the surface of the structure in either a guided wave mode, or as an evanescent mode. The LC platform does not rely on this diffraction or guided wave process. The function of the substrate patterning for the LC platform is to provide imaging or laser scanning contrast between two quadrature conditions.

[009] The LC platform utilizes two complementary quadratures between the spot and the land to enhance the signal. The spot and the land have equal reflectance but opposite quadrature responses for the protein layer. This condition is called Land Contrast (LC). Protein can be evenly immobilized on the entire chip and detected by reflectometry. The contrast between spot and land can be directly converted to protein thickness. This is achieved because equal binding on the spot and the land produces equal but opposite responses. The LC platform is sensitive for protein detection in either dry or liquid environments.

[010] One embodiment of the present invention uses micro-patterning of thermal oxide on silicon to establish two opposite quadrature conditions for in-line common-path interferometry. Of course, other materials could be used for the LC platform. The micro-patterns eliminate the

need for protein printers. This is realized by etching spot patterns into a silicon wafer. In one embodiment, the SiO₂ thickness within the spots is 140 nm while the SiO₂ thickness on the land is 77 nm. Immunoassay tests have been done on LC platforms read respectively by Molecular Interferometric Imaging (MI2) and Spinning Disc Interferometry (SDI). The sensitivity for protein layer height is on the order of 3 pm.

[011] A land contrast platform for interferometrically detecting the presence of a target analyte in a sample is disclosed herein. The land contrast platform can include a spatially patterned substrate and a layer of recognition material immobilized on the spatially patterned substrate. The spatially patterned substrate can include a plurality of high regions and a plurality of low regions, where the height of the plurality of high regions is substantially uniform at a first height, and the height of the plurality of low regions is substantially uniform at a second height. The first height is different from the second height. The recognition material immobilized on the spatially patterned substrate is designed to bind with the target analyte. The land contrast platform is designed such that the high regions and the low regions have approximately the same reflectance but have complementary quadrature conditions. This complementary quadrature condition causes an equal target binding on the high and low regions to produce opposite responses; the target binding on the high regions producing one of a positive or negative reflectance difference, and the target binding on the low regions producing the other of a positive or negative reflectance difference.

[012] The average of the first height and the second height can be an antinode condition that maximizes the phase contrast interferometric response. The average height can be approximately $\lambda/4$, the first height of the high regions can be approximately $\lambda/8$ above the average height and the second height of the low regions can be approximately $\lambda/8$ below the average height, where λ is the wavelength of a probe beam used for interferometric detection of the target analyte.

[013] The substrate of the land contrast platform can include a silicon layer covered by a thermal oxide layer. The thickness of the thermal oxide layer in the high regions can be approximately 140 nm and the thickness of the thermal oxide layer in the low regions can be approximately 77 nm.

[014] The land contrast platform can be balanced such that the surface area of the plurality of high regions is approximately equal to the surface area of the plurality of low regions of the land contrast platform. The land contrast platform can be in the shape of a disc, a chip or other shape. The land contrast platform can also include an identifier, such as a barcode. The barcode can be formed using a portion of the plurality of high regions and the plurality of low regions such that the barcode can be read interferometrically. The identifier can be used to indicate the type of recognition material immobilized on the land contrast platform. While barcodes have been incorporated into other optical disc platforms (see for instance, U.S. Patent No. 7,157,049), such barcodes are not readable interferometrically such as can be done by the embodiments of the present invention.

[015] A method of producing a land contrast platform for interferometrically detecting the presence of a target analyte in a sample is also disclosed. The method can include steps of obtaining a silicon wafer; creating an oxide surface on the silicon wafer; covering the oxide surface with a photomask; exposing the oxide surface covered by the photomask to create surface patterns on the oxide surface; etching the oxide surface to create a lower region and a higher region; and immobilizing recognition material on the lower region and the higher region. The recognition material is designed to bind with the target analyte. The etching of the oxide surface creates a lower region and a higher region. Both the lower region and the higher region can have approximately the same reflectance but complementary quadrature conditions such that an equal target binding on the lower region and the higher region produces opposite responses, the target binding on the lower regions producing one of a positive or negative reflectance difference and the target binding on the higher regions producing the other of a positive or negative reflectance difference. Ultraviolet light can be used in the exposing step. The oxide surface can be made to have a surface thickness of approximately 140 nm, and the etching step can be used to create the lower region with a thickness of approximately 77 nm. The method can also include a cleaning step following the etching step, where the cleaning step includes a wash with Piranha solution.

[016] A method of detecting one or more target analyte in a sample using land contrast chips is also disclosed. The method includes a first step of making a plurality of land contrast chips having a spatially patterned substrate which includes a plurality of high regions and a plurality of low regions. The height of the high regions is substantially uniform at a first height, and the

height of the low regions is substantially uniform at a second height, the first height being different from the second height. The high regions and the low regions have approximately the same reflectance but have complementary quadrature conditions such that an equal target binding on the high and low regions produces opposite responses; the target binding on the high regions producing one of a positive or negative reflectance difference and the target binding on the low regions producing the other of a positive or negative reflectance difference. The method further includes steps of placing an identifier on each of the land contrast chips; immobilizing a layer of recognition material designed to bind with a first target analyte on the chips; exposing at least one chip to the sample; and interferometrically reading the at least one exposed chip to determine the presence or absence of the first target analyte. The method can also include placing the at least one exposed chip on the surface of a disc; and interferometrically reading the disc using spinning disc interferometry. The identifier can be a barcode formed using a portion of the high regions and low regions such that the barcode can be read interferometrically.

[017] The method can also be used to detect multiple target analytes by immobilizing a layer of different recognition materials on different land contrast chips, where each of the recognition materials is designed to bind with a particular target analyte. Land contrast chips having different recognition materials immobilized thereon can then be exposed to the sample; and interferometrically read to determine the presence or absence of the multiple target analytes. The identifier on each chip can identify the type of recognition material immobilized on the individual chip. The identifier can be a barcode formed using a portion of the high and low regions such that the barcode can be read interferometrically. The exposed chips can be placed on the surface of a disc, and the disc interferometrically read using spinning disc interferometry to determine the presence or absence of one or more of the multiple target analytes. The barcode of any chip indicating the presence of its associated target analyte can be read to determine the type of recognition material immobilized on the chip.

[018] Additional features and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of illustrated embodiments.

[019] BRIEF DESCRIPTION OF THE FIGURES

[020] Aspects of the present invention are more particularly described below with reference to the following figures, which illustrate exemplary embodiments of the present invention:

[021] Fig. 1 illustrates a surface relief of an embodiment of a land-contrast platform showing the two surface heights associated with opposite quadratures;

[022] Fig. 2 illustrates a “spot-based” pattern that can be etched into a land-contrast platform for spots and land;

[023] Fig. 3 illustrates some exemplary alternative land-contrast patterns;

[024] Fig. 4 illustrates the uniform immobilization of recognition molecules on the two surface heights which removes the need for protein printers;

[025] Fig. 5 shows a sequence of experimental measurements for a land-contrast chip: image (1) shows the spot and land patterns etched on a silicon thermal oxide chip by photolithography under white light; image (2) shows a reflectance image of a blank chip (the spot and land regions have the same reflectance); image (3) shows a reflectance image after evenly applying an antibody layer on the entire chip; and image (4) shows a reflectance image after sandwich assay;

[026] Fig. 6 shows a graph of the reflectance at 633 nm of a land-contrast chip in both air and water environments, and shows that the working conditions at 77nm and 140nm thicknesses for this embodiment is suitable for both environments;

[027] Fig. 7 shows a graph illustrating that the reflectance changes at 77nm and 140nm thicknesses to a protein layer are opposite on the spot and land regions in both air and water environments;

[028] Fig. 8 is a schematic of an exemplary layout of 8x8 spot patterns on a 100-mm diameter silicon wafer;

[029] Fig. 9 shows the kinetic curves for the complete process of immunoassays of rabbit IgG forward and sandwich assays at five concentrations monitored in real time using molecular interferometric imaging;

[030] Fig. 10 shows a graph of the forward assay portions of the kinetic curves in Fig. 9 fitted by the real-time binding equation;

[031] Fig. 11 shows the response curve of a rabbit IgG sandwich assay read in a dry state using a spinning disk interferometry system, the differences of contrast images (ΔC) before and after the sandwich are shown for 30, 100, 300, 1000, and 3000 ng/ml concentration groups;

[032] Fig. 12 shows an embodiment of a land contrast chip with a land-contrast pattern and a unique land-contrast barcode;

[033] Fig. 13 illustrates the dispersing of N different types of the land-contrast chips of Fig. 12 into a sample solution; and

[034] Fig. 14 illustrates a plurality of the land-contrast chips shown in Figs. 12 and 13 arranged randomly on a disc for scanning.

[035] DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

[036] For the purposes of promoting an understanding of the principles of the invention, reference will now be made to the embodiments illustrated in the drawings and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the illustrated device, and such further applications of the principles of the invention as illustrated therein being contemplated as would normally occur to one skilled in the art to which the invention relates.

[037] This application is related to U.S. Patent No. 6,685,885, issued February 3, 2004, entitled "Bio-Optical Compact Disk System," the disclosure of which is incorporated herein by this reference. This application is also related to U.S. Patent Application Serial No. 11/345,462 entitled "Method and Apparatus for Phase Contrast Quadrature Interferometric Detection of an Immunoassay," filed February 1, 2006; and U.S. Patent Application Serial No. 11/675,359 entitled "In-Line Quadrature and Anti-Reflection Enhanced Phase Quadrature Interferometric Detection," filed February 15, 2007; and U.S. Patent Application Serial No. 11/744,726, entitled "Molecular Interferometric Imaging Process and Apparatus," filed May 4, 2007, the disclosures of which are all incorporated herein by this reference.

[038] Prior to describing various embodiments of the invention the intended meaning of quadrature in the interferometric detection system(s) described below is further explained. In some specific applications quadrature might be narrowly construed as what occurs in an interferometric system when a common optical "mode" is split into at least 2 "scattered" modes that differ in phase by about $N\pi/2$ (N being an odd integer). However, as used in the present application, an interferometric system is in quadrature when at least one mode "interacts" with a

target molecule or varying surface height to produce a phase difference of about $N\pi/2$ (N being an odd integer). This definition of quadrature is also applicable to interferometric systems in which the “other mode(s),” referring to other reference waves or beams, interact with a different molecule or surface height. The interferometric system may be considered to be substantially in the quadrature condition if the phase difference is $\pi/2$ (or $N\pi/2$, wherein N is an odd integer) plus or minus approximately twenty or thirty percent. The phrase “in-phase” is intended to describe in-phase constructive interference, and “out of phase” is intended to describe substantially 180-degree-out-of-phase destructive interference. This is to distinguish these conditions, for both of which the field amplitudes add directly; from the condition of being “in phase quadrature” that describes a relative phase of an odd number of $\pi/2$.

[039] Traditional protein microarrays use the format of spots printed on a homogenous substrate (the surrounding land) to produce the contrast for imaging molecular binding. However, protein printing is subject to spot defects (washing tails, spot unevenness, donut shapes, etc.) which cause inter-spot differences and spot-background unevenness leading to systematic shifts in the data. Moreover, printing is costly because of the need for expensive printers and considerable post-processing (spot incubation, land passivation, protein stabilization, etc.).

[040] A land-contrast (LC) platform produces the image contrast by a spatially patterned substrate rather than by the protein pattern. Some embodiments of the original in-line BioCD utilize surface-enhanced reflectometry to detect protein immobilized on silicon wafers with 140 nm thermal oxide to maximize the sensitivity. In these embodiments, the oxide layer establishes an interferometric quadrature condition, and a 1 nm protein layer (in a spot) induces 2.5% reflectance change ($\Delta R/R$) at a 633 nm wavelength and normal incidence. The LC platform extends this concept by utilizing two complementary quadrature conditions between the spot and the land to enhance the signal. This is achieved because equal protein binding on the spot and the land produces equal but opposite responses. One way of doing this is to etch patterns in the upper surface of the LC platform to create two surface heights having equal reflectivity but opposite quadrature responses to the protein layer. A land contrast platform can be created out of many different materials.

[041] The surface height profile of an exemplary embodiment is shown in Fig. 1 for a thermal oxide layer on silicon, where the patterns are etched in the thermal oxide using photolithography. The average thermal oxide thickness is $\lambda/4$, which is an antinode condition that maximizes the phase-contrast interferometric response. The two surface heights are roughly (but not exactly) an eighth-wavelength above or below the average height, placing both surface heights in the condition of in-line phase quadrature, but with opposite signed responses to additional surface height.

[042] A standard pattern that can be etched into the surface is a spot pattern, as shown in Fig. 2. In this case, the starting thermal oxide thickness is 140 nm (a quadrature condition) and the land is etched down to 77 nm (the opposite quadrature condition). However, there are many possible alternative patterns; some exemplary alternative patterns are shown in Fig. 3. The spot pattern in the upper-left of Fig. 3 is the most similar to conventional spotted arrays, but is not balanced between spot and land areas. The other 3 patterns in Fig. 3 can be easily balanced between spot and land areas. The checkerboard and stripe patterns have equal area component structures at each of the two heights. The unit cell pattern in the lower-left balances the spot-based patterns. For data processing, equal areas of “spot” and “land” can be helpful for optimizing signal-to-noise performance. In addition, a striped pattern can be compatible with chemical surface modification protocols. It is also possible to build up positive-negative versions of the spot pattern to balance the areas of the two surface heights, while retaining the spot-based pattern that is more conventional for array image analysis.

[043] One of the benefits of this land-contrast structure is the ability to eliminate the need for protein printers (typically spot-based, but also soft lithography). Antibodies, or other recognition molecules, can be uniformly immobilized on both surfaces, as shown in Fig. 4.

[044] Experiments were performed using the spot-based pattern in a chip format to demonstrate the principle of a land-contrast structure. This embodiment, shown in Fig. 5, uses spot regions of 140 nm thickness SiO_2 and a land region that has been etched to 77 nm thickness SiO_2 . Fig. 5, image (1) shows this embodiment of a land-contrast chip under white light.

[045] The reflection coefficients of the spot and land regions are conjugate to each other: $0.07-0.383i$ and $0.07+0.383i$, respectively. The reflectances of both areas are equal to 0.152 at a 633 nm wavelength (normal incidence). As shown in Fig. 5, image (2), the spot-land contrast is zero.

Black circles in image (2) are due to light scattering on the edge of the spots. Fig. 6 shows that the reflectances are equal at a 633 nm wavelength in both air and water for heights of 77 and 140 nm. Fig. 7 shows that the protein response of the two regions has opposite signs: $\Delta R/R = +2.55\%$ on the spot region and $\Delta R/R = -2.55\%$ on the land for a 1 nm protein layer ($n = 1.48$) in air. Fig. 7 also shows a contrast-protein conversion factor of 0.51% in water.

[046] Fig. 5, image (3) shows a reflectance image of the land contrast chip after antibody molecules are evenly immobilized across an entire well of the array, forming a dielectric layer. The spot-land contrast continues to increase after a sandwich assay. The antibody changes in layer thickness after a sandwich immunoassay, shown in Fig. 5, image (4), are measured by monitoring the spot-land contrast induced by the patterned substrate.

[047] The initial contrast of a LC structure, such as a chip or disc, is not necessarily always to be zero. In practice, the non-zero value occurs for the unprinted LC structure because of the thickness variation of the thermal oxide layer. However, this is not a problem because the initial contrast can be measured before carrying out the assay. The contrast increment is the quantity which can be converted to protein thickness, not the contrast value itself.

[048] The conversion between the spot-land contrast and protein thickness is derived using a general model of a biomaterial on a substrate. If the substrate has reflection coefficient r in an ambient medium with refractive index n_m under conditions of light wavelength λ (in vacuum) and incidence angle θ_0 , then after applying a biomaterial layer with thickness d ($d \ll \lambda$) and refractive index n_p on the substrate, the reflection coefficient is changed to r' and

$$r' = r + \left[\frac{(r_p - r)(1 - rr_p)}{(1 - r_p^2)} + r \frac{\tan \theta_p}{\tan \theta_0} \right] \frac{4\pi i n_p d \cos \theta_p}{\lambda} \quad (1)$$

where r_p is the reflection coefficient at the interface between the ambient medium and the protein, θ_p is the refraction angle in the protein layer.

[049] For the case of s-polarization (including normal incidence), Eq. 1 can be simplified to:

$$r' = r + \frac{(1 + r)^2}{\cos \theta_0} \frac{(n_m^2 - n_p^2)}{n_m} \frac{\pi i}{\lambda} d \quad (2)$$

Based on Eq. 2, the reflectance change ratio due to the biomaterial layer is approximately

$$\frac{\Delta R}{R} = \frac{|r'|^2 - |r|^2}{|r|^2} = \text{Im} \left[\frac{(n_p^2 - n_m^2)(1+r)^2}{n_m r} \frac{2\pi d}{\lambda \cos \theta_0} \right] \quad (3)$$

For a land-contrast platform, the reflection coefficients are r on the spot region and $\bar{r}$ (conjugate value of r) on the land region. The spot-land contrast is defined as

$$C = (R_{spot} - R_{land}) / (R_{spot} + R_{land}).$$

[050] Contrast is zero for a land-contrast platform without a protein layer. The contrast increment due to a thickness d of biomaterial is:

$$\Delta C = \frac{\Delta R}{R} = \text{Im} \left[\frac{(n_p^2 - n_m^2)(1+r)^2}{n_m r} \frac{2\pi d}{\lambda \cos \theta_0} \right] \quad (4)$$

Therefore, the 140/77 nm land-contrast platform has conversion factor $\Delta C = 2.55\%$ for adding 1 nm of protein ($n = 1.48$) in air or 0.51% in water ($n = 1.33$) under the condition of 633 nm light and normal incidence.

[051] A LC platform can be made by photolithography. For the embodiment of a 77/140 nm land contrast silicon wafer, positive photoresist (AZ1518, MicroChemicals Company) can be spun on 140-nm oxide on silicon wafer (Addison Engineering, Inc.), covered by a photomask, exposed under ultraviolet light and developed to create spot patterns on the wafers. A plasma etcher can be used to etch the land to 77 nm SiO₂ film.

[052] The photomask pattern can be designed by AutoCAD2000 software. In an exemplary embodiment shown in Fig. 8, 96 wells (each well consisting of 8x8 spots) are distributed on a 4 inch diameter wafer.

[053] An exemplary procedure of pattern creation with photoresist is as follows:

- 1) Spin on AZ1518 photo resist (undiluted by toner) at 3000rpm for 1 min.
- 2) Bake at 90 degrees for 5 min.
- 3) Cover the disc with photomask and expose it with canon mask aligner for 30 sec.
- 4) Develop the photoresist with AZ 351 developer (in 1:4 dilution ratio with DI water) for 3 min.

[054] Now the disc is covered by photoresist, and the photoresist on the land (except spots) has been developed and washed. The exposed SiO₂ can be etched to the desired thicknesses, such as 140 nm and 77 nm for SiO₂ with a plasma etcher (Plasma Tech RIE in Birck, Purdue) for 125 seconds under the following conditions:

Pressure = 50 mTorr (88 on dial panel);

Power = 100 W (333 on dial panel);

SF₆ flow rate = 50 sccm (48.0 on dial); and

Ar flow rate = 5 sccm (2.0 on dial).

[055] After etching, the platform can be cleaned using a sequence of Acetone, DI water, Piranha solution, and finally DI water. The Piranha solution wash is recommended because the photoresist hardened after plasma etching is hard to clean with regular organic solution.

[056] Physical adsorption based on methylation of the silica surface through treatment with chlorodimethyl-octadecylsilane (CH₃(CH₂)₁₇Si(CH₃)₂Cl) that binds with silanol groups on the silica surface can be used for protein immobilization. The CH₃ end groups of the silanes are hydrophobic and bind with protein through hydrophobic interaction. Hydrophobic interactions have large association constants and provide for a relatively simple means of immobilizing proteins on the disc surface. The platforms are soaked in 0.02 M chloro-octadecylsilane in toluene solution for 12 hours and rinsed with toluene, acetone, methanol and deionized water and dried with dry nitrogen gas. Surface immobilization can be accomplished using other methods known in the art, such as by using butyraldehyde, isocyanate or di-iso, or by physical adsorption through covalent binding.

[057] The performance of the land-contrast platform was tested using two different reading systems: a Molecular Interferometric Imaging (MI2) system in a liquid environment and a spinning disk interferometry (SDI) system in a dry environment. For a more detailed description of MI2, see U.S. Patent Application Serial No. 11/744,726, entitled "Molecular Interferometric Imaging Process and Apparatus," filed May 4, 2007.

[058] To demonstrate the performance of a land-contrast (LC) platform and to explore its potential in protein real-time interaction study, rabbit IgG assays were performed on surface-silanized LC chips and the kinetic curves of assays were acquired at multiple concentrations. For

this experiment, the platform is in the format of 2x2 spots on chips. The diameter of each spot is 150 μm . The protein thicknesses were detected by Molecular Interferometric Imaging (MI2). This MI2 system uses a reflective microscope and a CCD to acquire images of the reflectance of chips illuminated by 630 ± 15 nm light (generated by a 10 mW LED). The MI2 system was used to take one image frame of the LC chip every 43 sec in accumulation mode to suppress shot-noise. The pixel resolution is 2.2 μm with a 7x objective lens. The contrast value is calculated for each frame and converted to protein thickness, and the protein thickness change due to immuno-binding is monitored in real time. The frame rate can be manipulated with a trade-off between shot-noise and image capture speed. A microfluidic channel is built by placing a cover-slip on the land-contrast chip with a 0.5 mm gap between the cover-slip and the chip. Protein solution is driven continuously through the channel and therefore provides a stable liquid environment with uniform protein concentrations. The contrast conversion factor is 0.51% for 1 nm protein in this situation because the detection was done in a liquid environment (see Fig. 7). In the process of assays, 10 $\mu\text{g/ml}$ polyclonal anti-rabbit IgG (R2004, Sigma Inc.) in Phosphate Buffered Saline (PBS, PH = 7.4) solution flowed across each LC chip for 1.5 hours on five chips. About 2.4 nm antibody was immobilized on the chips after this step. In step two, 0, 100, 200, 500 and 1000 ng/ml rabbit IgG (I5006, Sigma Inc.) in PBS flowed respectively across five chips for 2 hours, and the rabbit IgG antigen bound with the anchored antibody (forward assay). In the last step, 10 $\mu\text{g/ml}$ anti-rabbit IgG flowed across each chip for 1.5 hours. This secondary antibody binds with the bound antigen to amplify the protein thickness (sandwich assay). Protein thicknesses were measured in real-time.

[059] Five kinetic curves were acquired for the five antigen concentrations for the process steps of antibody printing, forward assay and sandwich assay. Fig. 9 shows these curves for the complete process of the immunoassays (including antibody immobilization, forward assay and sandwich assay) at five concentrations monitored in real time. The flow rate is $2 \text{ mL h}^{-1} \text{ mm}^{-2}$. The forward assay portions of the curves in Fig. 9 are fitted in Fig. 10 by the equation:

$$h(t) = H(1 - e^{-t/\tau}) \quad (5)$$

where

$$H = \frac{H_{\max} [Ag]}{k_D + [Ag]} \quad (6)$$

$$\tau = \frac{1}{k_{on}} \left(\frac{1}{k_D + [Ag]} \right), \text{ and } k_D = \frac{k_{off}}{k_{on}} \quad (7)$$

[060] In Fig. 10, H and τ values are found by fitting at conditions of $[Ag] = 100, 200, 500$ and 1000 ng/ml. Therefore the maximum height of the bound antigen layer H_{max} , antigen-antibody dissociation constant k_{off} , association constant k_{on} and equilibrium constant k_D can be found by further fitting H and τ into Eq. 6 and 7. The results are

$$H_{max} = 0.60 \text{ nm},$$

$$k_{off} = 4.35 \times 10^{-5} \text{ sec}^{-1},$$

$$k_{on} = 435 \text{ g}^{-1} \text{ ml sec}^{-1}, \text{ and}$$

$$k_D = 100 \text{ ng/ml}.$$

These values indicate 25% activity for the antibody molecules anchored by physical adsorption.

[061] The sandwich assays in Fig. 9 show that amplification by the secondary antibody is not a constant for all concentrations. $\Delta H_{sandwich} / \Delta H_{forward}$ equals 6.2, 4.9, 4.5, and 3.8 at 100, 200, 500, and 1000 ng/ml antigen concentrations respectively. This phenomenon may be caused by the limited space on the chip surface. One antigen has many binding sites for the polyclonal antibody, but the space around the antibody is limited. If the antigen molecules are already crowded after the forward assay at high concentration, then the secondary antibody molecules have less space to bind with the antigen, and the secondary antibody/antigen ratio would be less.

[062] The detection limit of the protein thickness is 3 pm based on the area of 4 spots and the relevant land (overall, about 0.1 mm^2). This was estimated by subtracting a fitted curve from the 100 ng/ml forward assay data (Fig. 10) and calculating the standard deviation of the data points after subtraction. A 3 pm detection limit was also found by analyzing the standard deviation of the 0 ng/ml forward assay data without curve subtraction (where the curve is flat). From the results, LC chips can be used with an MI2 system to provide an accurate and efficient tool to perform protein interaction studies.

[063] For the example of an assay read by a spinning disk interferometry (SDI) system, the ability of the LC platform to detect protein concentration was also explored in the following assay in a dry condition. A spinning disc interferometry (SDI) system was used to acquire the

reflectance image of a LC disc for $1/f$ noise suppression and high throughput on the SDI system. In this assay, the LC disc is in the format of 96 wells on a silicon thermal oxide wafer with 100 mm diameter, each well consists of 8×8 spots which can serve for one type of protein detection. All spot regions have 140 nm thickness SiO_2 film while the land is etched to be 77 nm. The disc is mounted on a motor fixed on a translational stage in a similar configuration to a CD reader. The motor is spun at 1200 rpm and the stage moves at steps of $5 \mu\text{m}$. A 633 nm wavelength laser beam is focused on the LC platform with $5 \mu\text{m}$ resolution. Reflected laser intensity is detected by a silicon detector and the signal is sent to a computer where the reflectance image of the whole disc is reconstructed.

[064] The LC disc was surface-silanized to immobilize antibodies by physical adsorption. 100 $\mu\text{g/ml}$ anti-rabbit IgG in PBS was loaded on 8 wells of a LC disc with a pipette. The wells were fully and evenly incubated in antibody solution for 30 min to form an antibody layer. The disc was then washed and dried. A pre-scan was performed to record the initial height of the antibody layer on all wells, and then the 8 wells were incubated with 0, 10, 30, 100, 300, 1000, 3000, and 10,000 ng/ml rabbit IgG (I5006, Sigma Inc.) in PBS + 0.05% Tween 20 (PBST) for 30 min, and then washed by DI water and incubated with 10 $\mu\text{g/ml}$ anti-rabbit IgG in PBST for 30 min. All incubations were static (ambient). A post-scan was performed to record the protein height after assay. The height increment due to the sandwich assay was calculated and the response curve is shown in Fig. 11. The differences of contrast images (ΔC) before and after the sandwich are shown for 30, 100, 300, 1000, and 3000 ng/ml concentration groups. The curve was fitted by the Langmuir equation. The equilibrium constant was found to be 550 ng/ml, which is larger than the 100 ng/ml acquired by the MI2 system. This is because the protein transport in diffusion-limited incubation slows down the binding rate. The k_D here is an effective value for equilibrium constant between antigen and antibody binding.

[065] The land contrast patterns etched into a wafer can be made into individual chips by dicing up the wafer. A 100-mm diameter wafer has the potential to provide up to 10,000 mm^2 chips. Each chip can be composed of a land-contrast pattern and a two-level barcode. An exemplary embodiment is shown in Fig. 12. The barcode can be a standard bar-code, but imprinted using the two heights of the etched chip and read out interferometrically. The bar-code identifies the recognition molecule immobilized on the chip. A large number of chips can be placed in a single solution so that many of the chips have the same recognition molecules immobilized on their

surfaces. These chips can all have the same bar-code, the bar-code relating to the type of immobilized species on the surface. Many different sets of these chips can be made, each with a different surface chemistry bar-coded appropriately. After chemical immobilization, they can be placed into containers, one for each kind of recognition molecule. In this way, the chips become barcoded “reagents” that can be added to a sample solution, as shown in Fig. 13. There can be L chips of type A, M chips of type B, etc. up to N different types of chips. These chips can be placed in the sample solution, incubated, and then retrieved.

[066] After retrieval from the sample solution, the chips can be placed on the surface of a disc and read interferometrically using SDI with the land contrast mechanism. Fig. 14 illustrates a random arrangement of these chips on a disc. The barcode of each chip will tell which recognition molecule the chip was prepared with. The chips can be arranged randomly and read by scanning the entire disc. There is room on a 100-mm diameter disc for over a thousand such chips.

[067] While an exemplary embodiment incorporating the principles of the present invention has been disclosed hereinabove, the present invention is not limited to the disclosed embodiments. Instead, this application is intended to cover any variations, uses, or adaptations of the invention using its general principles. Further, this application is intended to cover such departures from the present disclosure as come within known or customary practice in the art to which this invention pertains and which fall within the limits of the appended claims.

We claim:

1. A land contrast platform for interferometrically detecting the presence of a target analyte in a sample, the land contrast platform comprising:
 - a spatially patterned substrate having a plurality of high regions and a plurality of low regions, the height of the plurality of high regions being substantially uniform at a first height, and the height of the plurality of low regions being substantially uniform at a second height, the first height being different from the second height; and
 - a layer of recognition material immobilized on the plurality of high regions and the plurality of low regions, the recognition material being designed to bind with the target analyte;
 - wherein the high regions and the low regions have different quadrature conditions such that target binding on the high and low regions produces different interferometric responses
2. The land contrast platform of claim 1 wherein the high regions and the low regions have approximately the same reflectance but have complementary quadrature conditions such that equal target binding on the high and low regions produces opposite responses, target binding on the high regions producing one of a positive or negative reflectance difference and target binding on the low regions producing the other of a positive or negative reflectance difference.
3. The land contrast platform of claim 1 wherein an average height of the first height and the second height is an antinode condition that maximizes the phase contrast interferometric response.
4. The land contrast platform of claim 3 wherein the average height is approximately $\lambda/4$, the first height of the high regions is approximately $\lambda/8$ above the average height and the second height of the low regions is approximately $\lambda/8$ below the average height, λ being the wavelength of a probe beam being used for interferometric detection of the target analyte.
5. The land contrast platform of claim 1 wherein the substrate comprises a silicon layer covered by a thermal oxide layer.

6. The land contrast platform of claim 5 wherein the thickness of the thermal oxide layer in the high regions is approximately 140 nm and the thickness of the thermal oxide layer in the low regions is approximately 77 nm.
7. The land contrast platform of claim 1 wherein the surface area of the plurality of high regions is approximately equal to the surface area of the plurality of low regions.
8. The land contrast platform of claim 1 wherein the substrate is a disc.
9. The land contrast platform of claim 1 wherein the substrate is a chip further comprising a barcode, the barcode being formed using a portion of the plurality of high regions and the plurality of low regions such that the barcode can be read interferometrically.
10. The land contrast platform of claim 1 wherein the barcode indicates the type of recognition material immobilized on the chip.
11. A method of producing a land contrast platform for interferometrically detecting the presence of a target analyte in a sample, the method comprising:
 - obtaining a silicon wafer;
 - creating an oxide surface on the silicon wafer;
 - covering the oxide surface with a photomask;
 - exposing the oxide surface covered by the photomask to create surface patterns on the oxide surface;
 - etching the oxide surface to create a lower region and a higher region, both the lower region and the higher region having approximately the same reflectance but different quadrature conditions such that target binding on the lower region and the higher region produces different interferometric responses; and
 - immobilizing recognition material on the lower region and the higher region, the recognition material being designed to bind with the target analyte.

12. The method of claim 11 wherein the lower region and the higher region created in the etching step have complementary quadrature conditions such that equal target binding on the lower region and the higher region produces opposite responses, target binding on the lower region producing one of a positive or negative reflectance difference and the target binding on the higher region producing the other of a positive or negative reflectance difference.

13. The method of claim 12 wherein the creating an oxide surface step produces an oxide surface with a thickness of approximately 140 nm, and the etching step etches the oxide surface to create the lower region with a thickness of approximately 77 nm.

14. A method of detecting one or more target analyte in a sample using land contrast chips, the method comprising:

making a plurality of land contrast chips having a spatially patterned substrate; the spatially patterned substrate including a plurality of high regions and a plurality of low regions, the height of the plurality of high regions being substantially uniform at a first height, and the height of the plurality of low regions being substantially uniform at a second height, the first height being different from the second height, wherein the high regions and the low regions have different quadrature conditions such that target binding on the high and low regions produces different quadrature responses;

placing an identifier on each of the plurality of land contrast chips;

immobilizing a layer of first recognition material on the spatially patterned substrate of a first portion of the plurality of land contrast chips, the first recognition material being designed to bind with a first target analyte;

exposing at least one chip of the first portion of the plurality of land contrast chips to the sample; and

interferometrically reading the at least one exposed chip of the first portion of the plurality of land contrast chips to determine the presence or absence of the first target analyte.

15. The method of claim 14 wherein the plurality of high regions and the plurality of low regions have approximately the same reflectance but have complementary quadrature conditions such that equal target binding on the high and low regions produces opposite responses, target

binding on the high regions producing one of a positive or negative reflectance difference and target binding on the low regions producing the other of a positive or negative reflectance difference;

16. The method of claim 14 further comprising:

placing the at least one exposed chip of the first portion of the plurality of land contrast chips on the surface of a disc; and

wherein the interferometrically reading step is performed using spinning disc interferometry on the disc.

17. The method of claim 14 wherein the identifier is a barcode, the barcode being formed using a portion of the plurality of high regions and the plurality of low regions such that the barcode can be read interferometrically.

18. The method of claim 14 further comprising:

immobilizing a layer of second recognition material on the spatially patterned substrate of a second portion of the plurality of land contrast chips, the second recognition material being designed to bind with a second target analyte;

exposing at least one chip of the second portion of the plurality of land contrast chips to the sample; and

interferometrically reading the at least one exposed chip of the second portion of the plurality of land contrast chips to determine the presence or absence of the second target analyte.

19. The method of claim 18 wherein, for each individual chip of the plurality of land contrast chips, the identifier is a barcode identifying the type of recognition material immobilized on the individual chip, the barcode being formed using a portion of the plurality of high regions and the plurality of low regions such that the barcode can be read interferometrically.

20. The method of claim 19 further comprising:

placing the exposed chips of the first and second portions of the plurality of land contrast chips on the surface of a disc; and

wherein the interferometrically reading step is performed using spinning disc interferometry on the disc to determine the presence or absence of both the first target analyte and second target analyte, and further includes reading the barcode from any exposed chip indicating the presence of its associated target analyte to determine the type of recognition material immobilized on such any exposed chip.

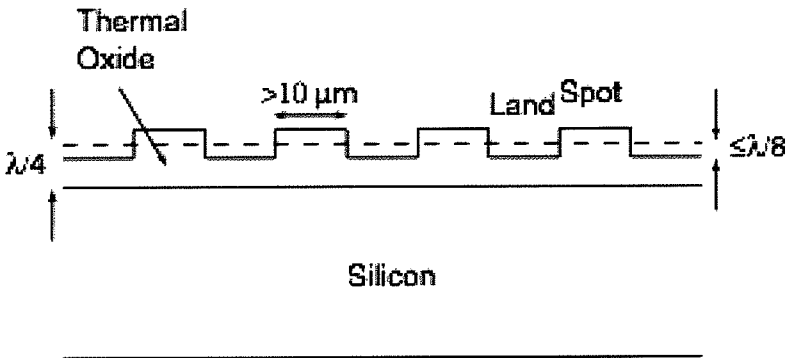


Figure 1

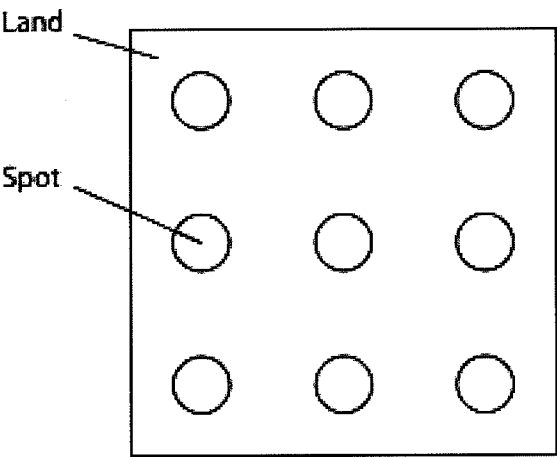


Figure 2

Land Contrast Patterns

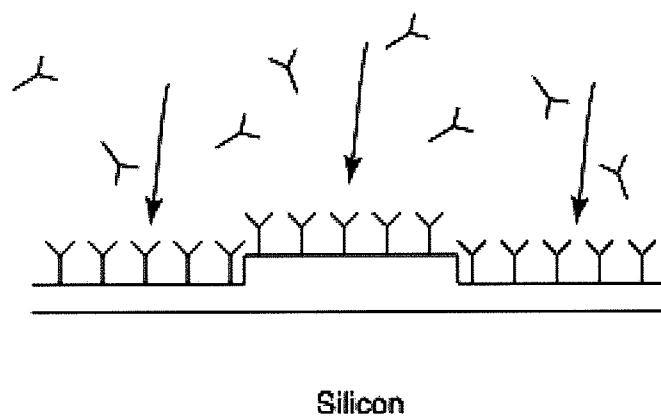
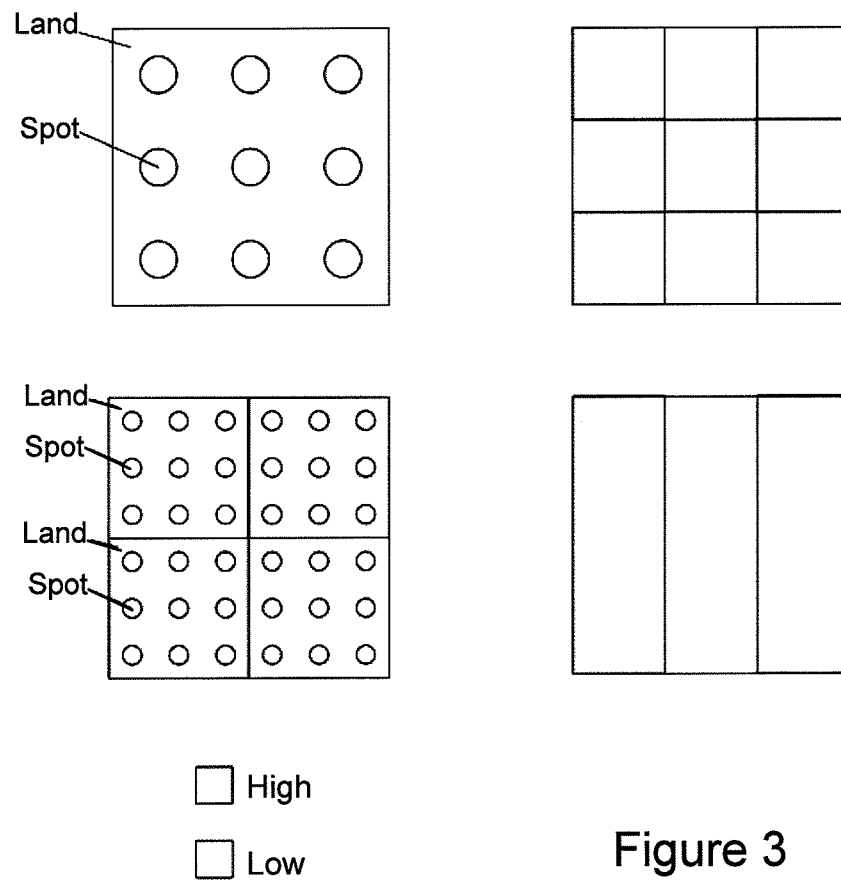


Figure 4

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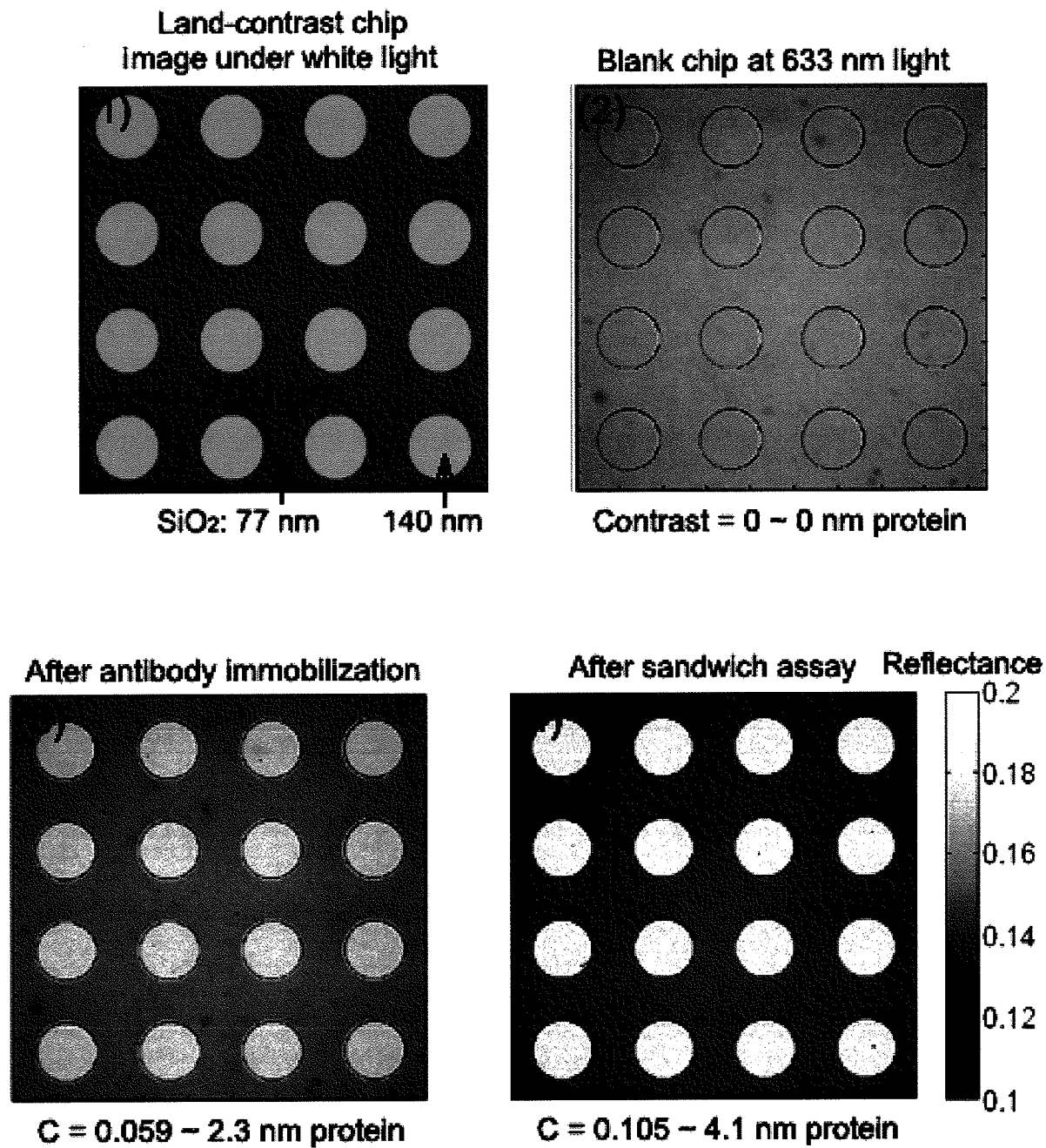


Figure 5

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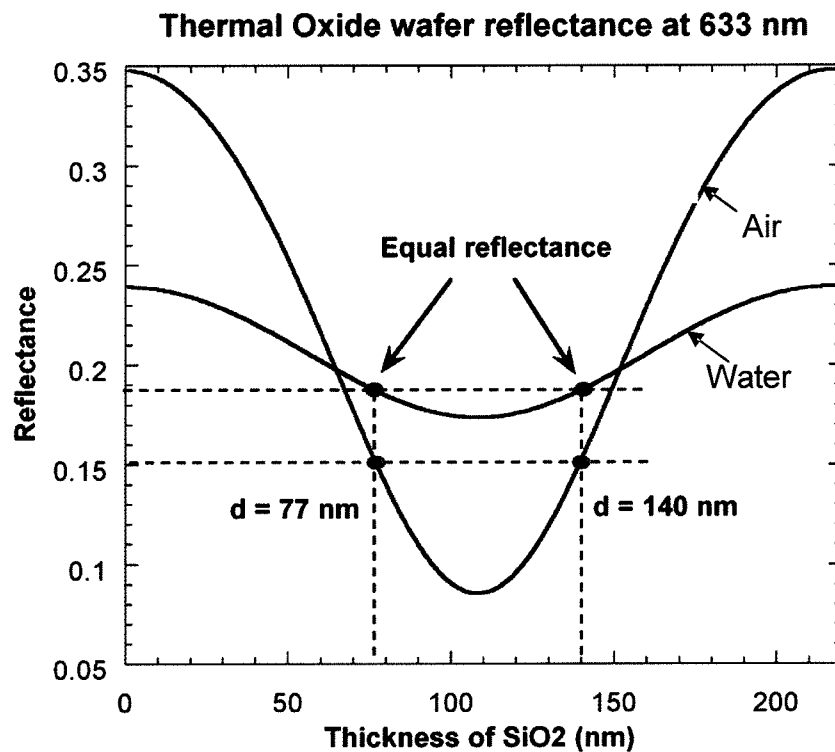


Figure 6

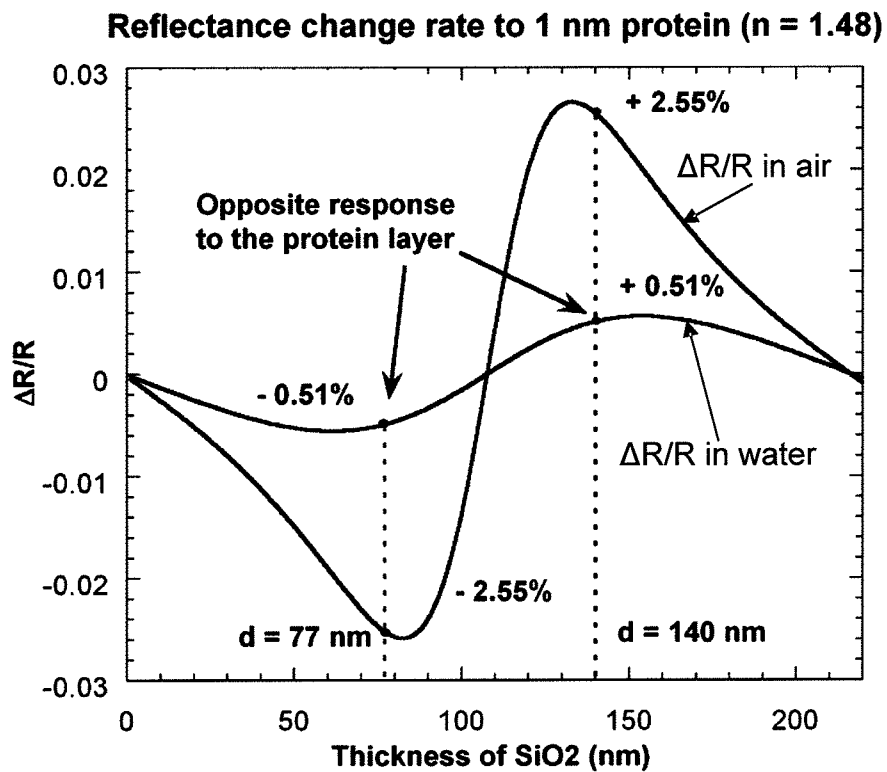


Figure 7

96-well land-contrast BioCD design

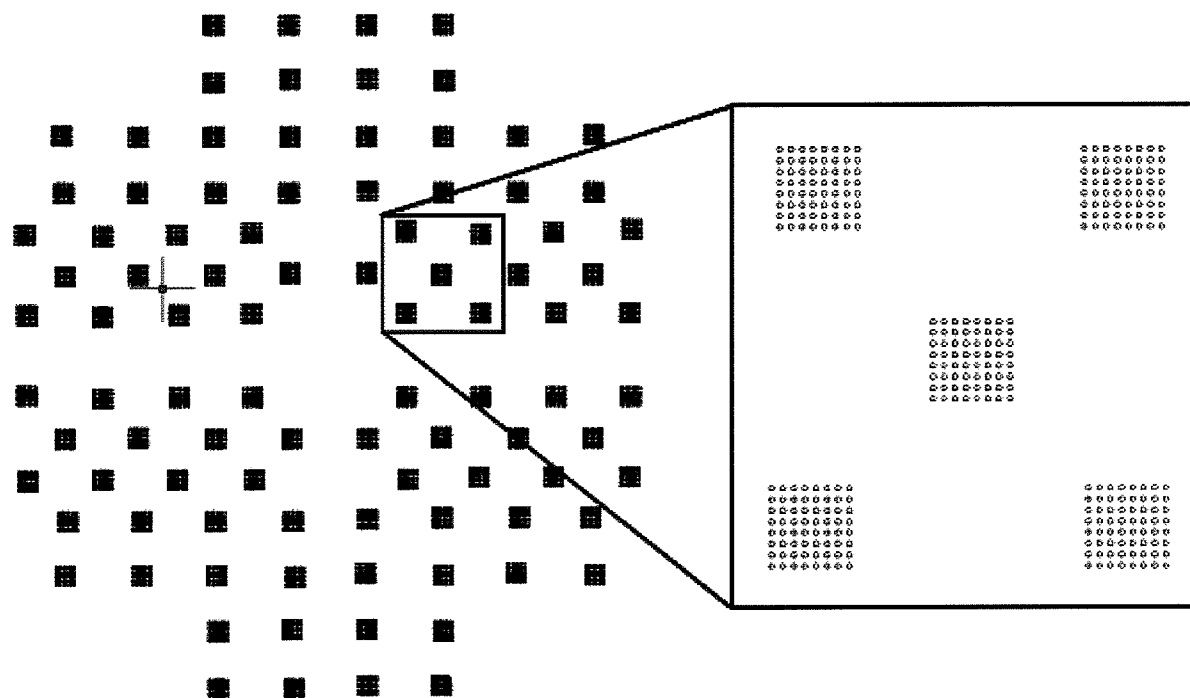


Figure 8

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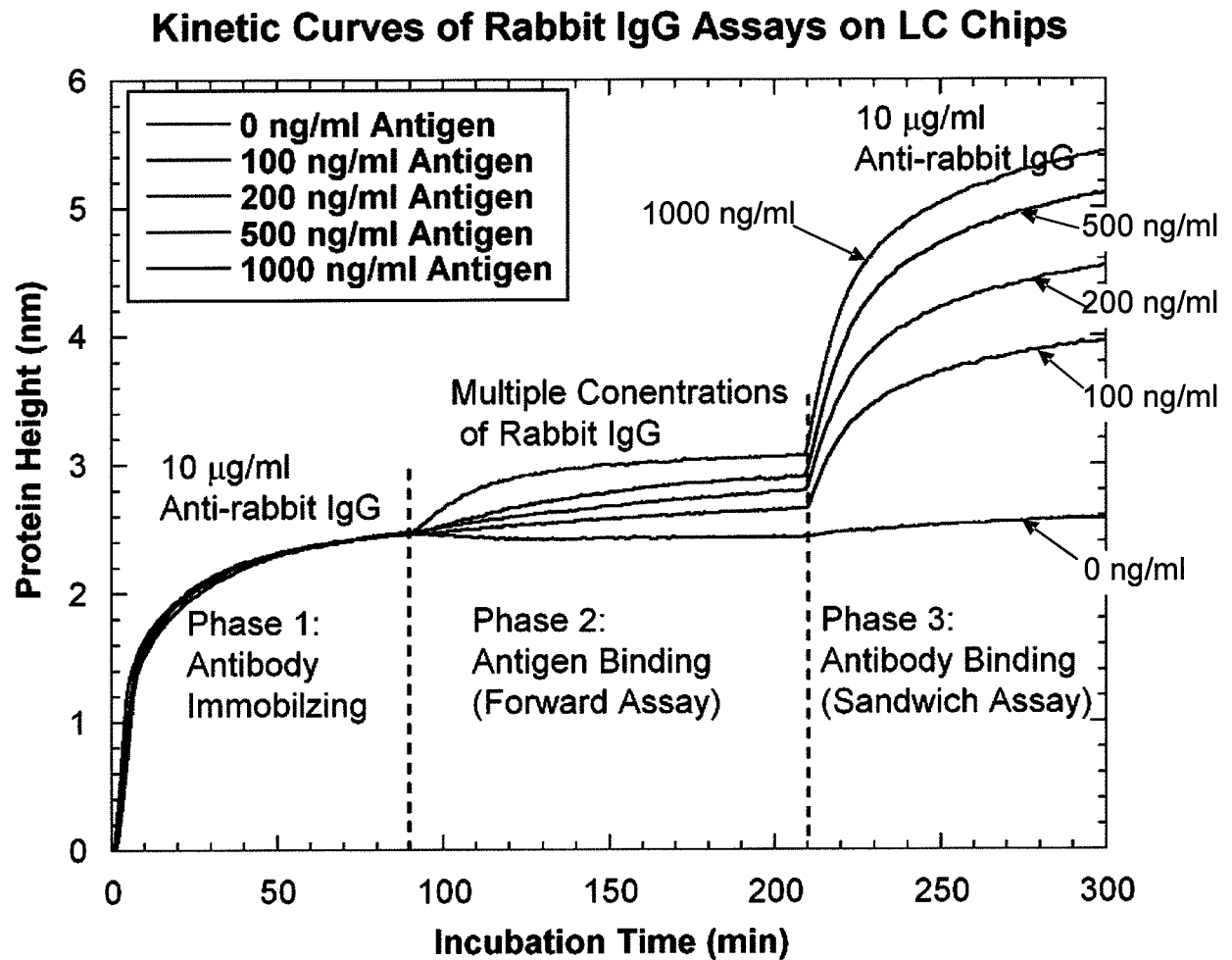


Figure 9

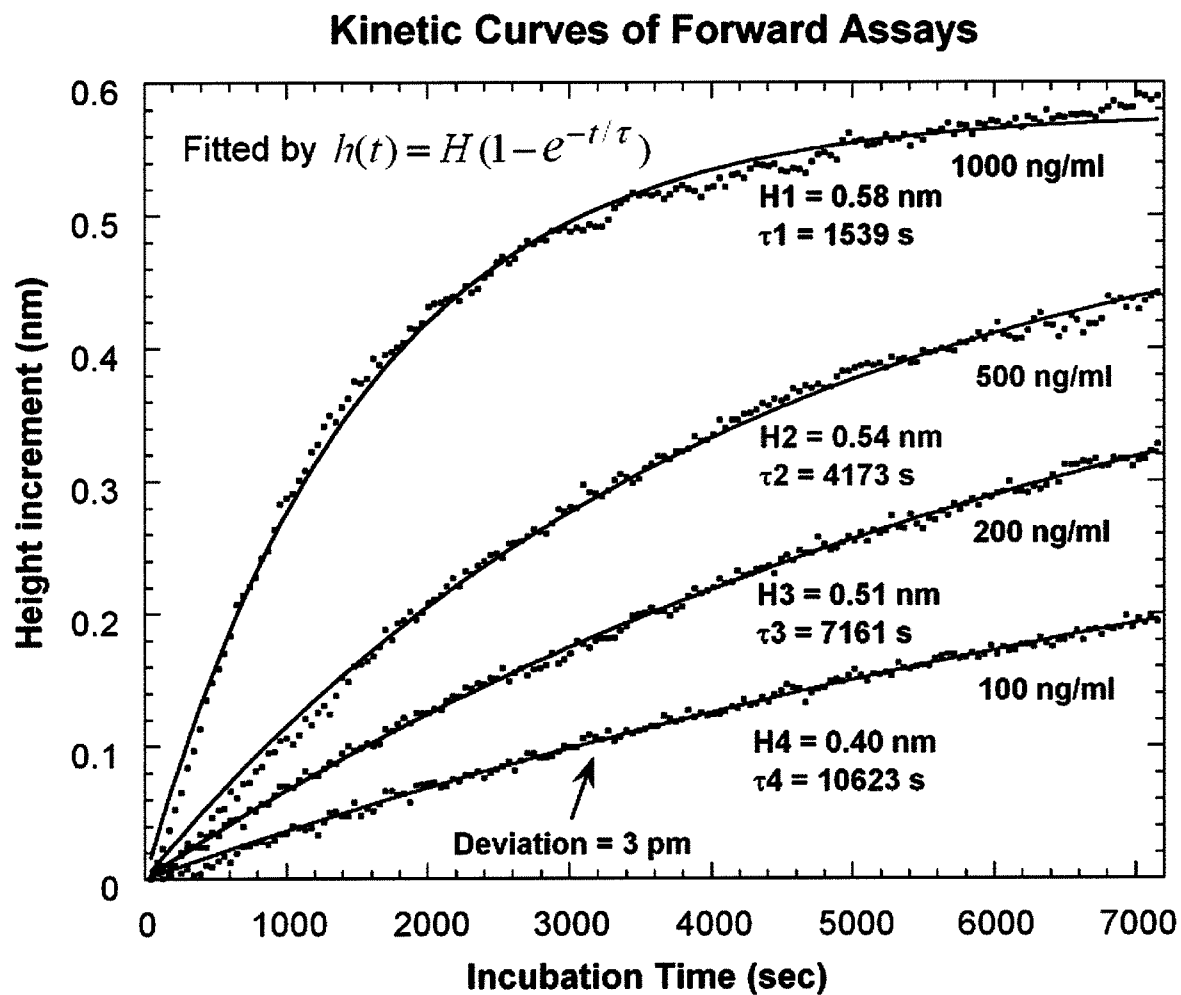


Figure 10

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Response Curve of Rabbit Sandwich Assay

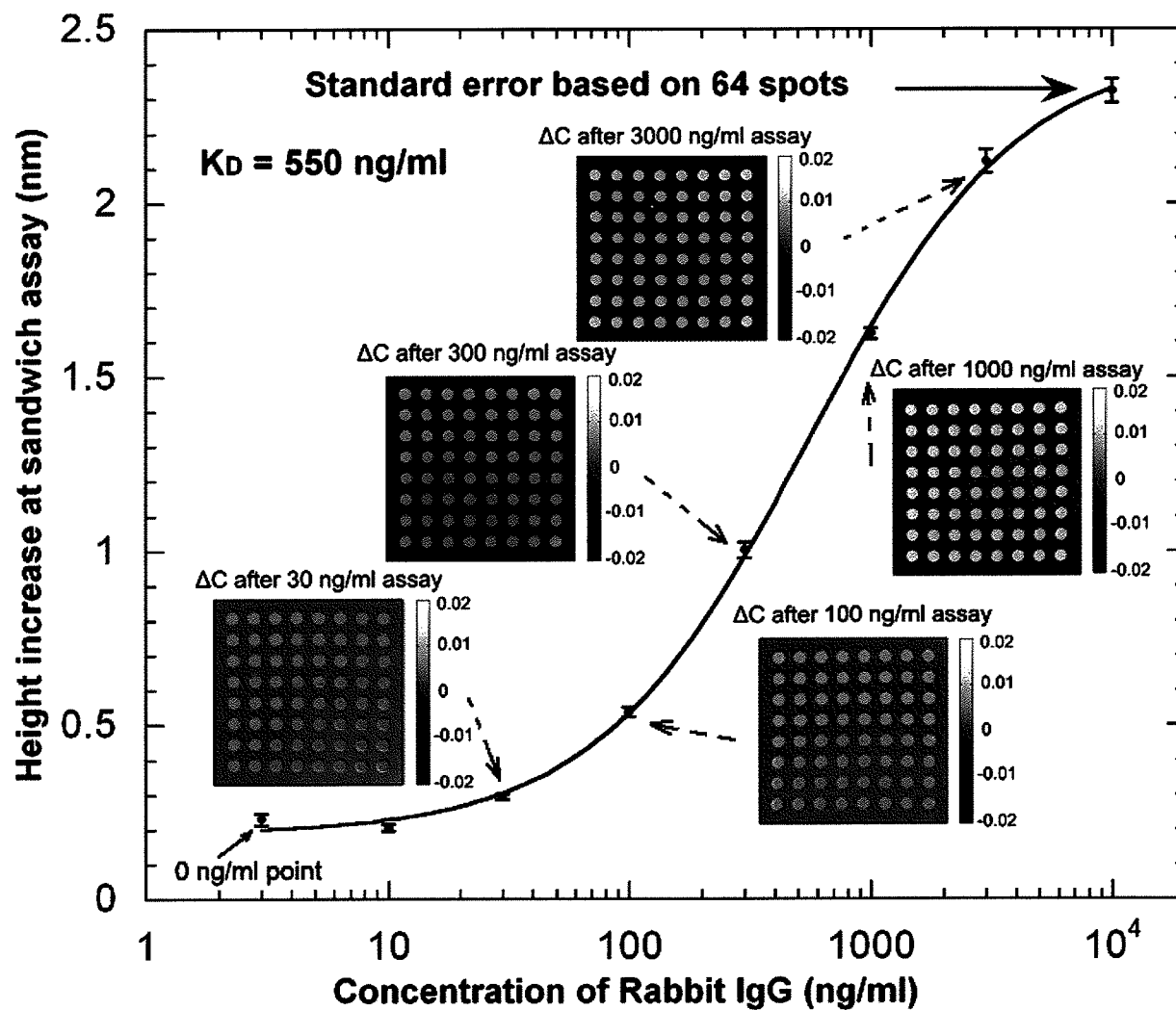


Figure 11

QuadraChip™ Array with Barcode

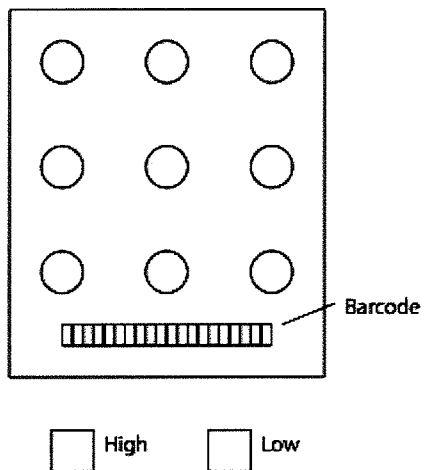


Figure 12

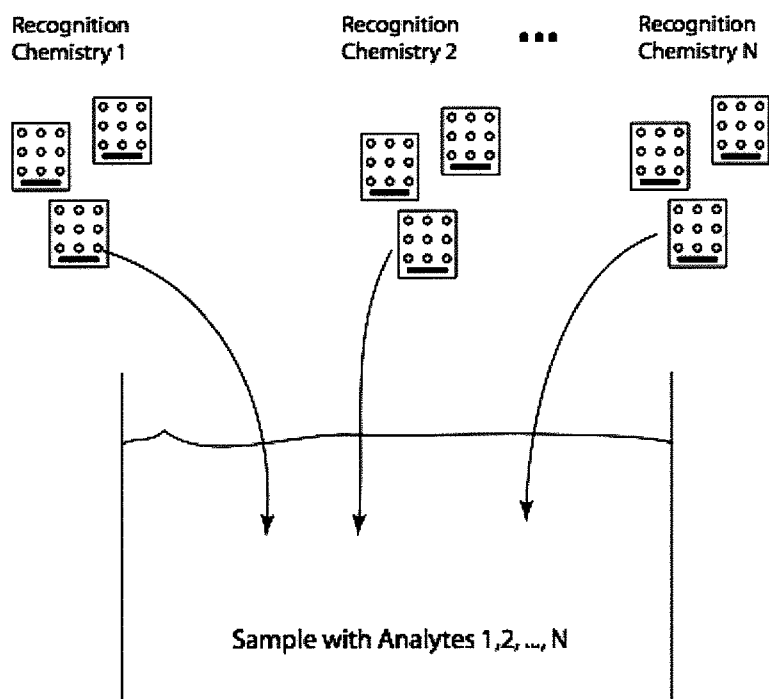


Figure 13

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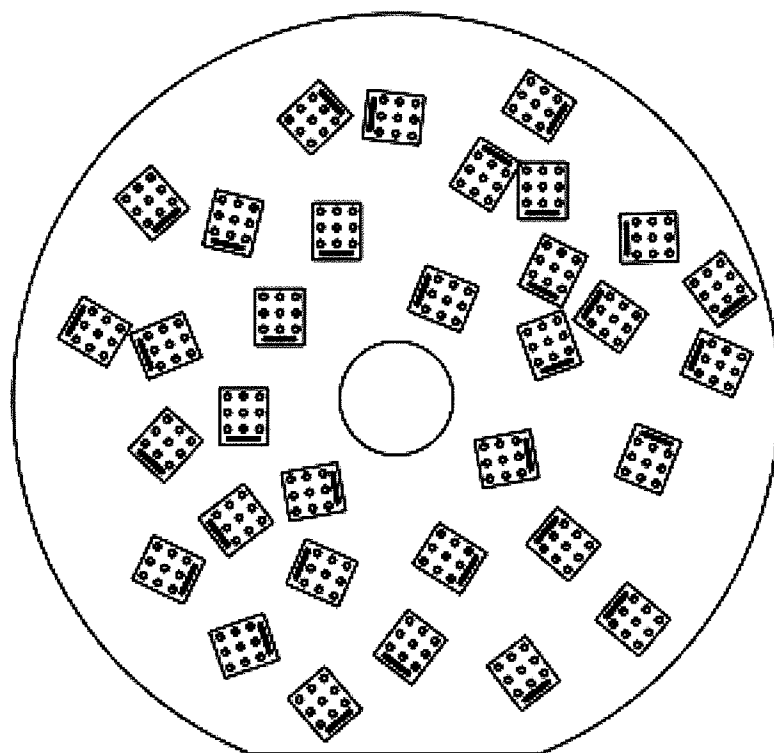


Figure 14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/78692

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 21/45 (2009.01)

USPC - 422/57

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)
422/57, 55, 50

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Pubwest (PGPB, USPT, EPAB, JPAB); Google Patents; Search Terms Used: protein micro array, land contrast platform, LC platform, protein immobilization, patterned substrate, plurality, recognition material, analyte, antibody, antigen, quadrature condition, micro patterning, etching spot pattern, barcode, silicon layer, oxide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2004/0166593 A1 (NOLTE et al.) 26 Aug 2004 (26.08.2004) para 0002, 0003, 0007, 0008, 0034, 0035, 0038, 0040, 0041, 0043, 0046-0048, 0067, 0070, 0072, 0090, 0095, 0098, 0135, 0138; claims 4; figures 1, 3A, 3B, 9, 5, 11, 12, 23	1, 2, 7, 8, 14-16, 18 ----- 3-6, 9-13, 17, 19, 20
Y	US 2008/0129981 A1 (NOLTE et al.) 5 June 2008 (05.06.2008) abstract, para 0031, 0045	3-6, 11-13
Y	US 5,867,297 A (KIANG et al.) 2 Feb 1999 (02.02.1999) col 5, ln 52-55; col 7, ln 5-12; figure 24	9, 10, 17, 19, 20

☐ Further documents are listed in the continuation of Box C.

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

16 May 2009 (16.05.2009)

Date of mailing of the international search report

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