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(54) Title: HIGHLY REFLECTIVE BIOGRATINGS AND METHOD

(57) Abstract

A reflective biograting consists of an optically flat layer of a transparent composition such as silicon dioxide having a first and second surface, spatially periodic zones of active and inactive binding agent on the first surface, and a reflective metal layer having a thickness sufficient to prevent transmission of substantially all light therethrough. The reflective metal layer can be supported on an optically flat surface of metal wafer, and the reflective metal can be aluminum, silver, gold, chromium, nickel, titanium or platinum coating on a polished wafer. Preferably, the silicon dioxide layer is formed either by direct sputtering of silicon dioxide or by coating an alkali metal silicate solution on the surface of the reflective metal, optionally containing an aminoalkysilane and a water-soluble hydroxylated polymer such as a dextran. Alternatively, the reflective support comprises a multilayer dielectric mirror on an optically flat surface.

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Description

Highly Reflective Biogratings and Method

Technical Field

This invention relates to improved biogratings for use in a reflective diffraction immunoassay and their method of manufacture. In particular, this invention relates to multilayer biogratings having high reflectivity and high protein binding capacity.

Background Art

Many solid-phase binding assays involve surface illumination and consequent light emissions from molecules attached to the surface or are masked by forward scattering. Generally, these emissions travel in all directions. Either these divergent emissions must be collected with expensive and awkward light collection optics to achieve sensitivity, the inherent inefficiencies and consequent low signal to light level ratios must be accepted, or the signal must be measured against a strong background.

Diffraction gratings cause light to be diffracted into specific angles as contrasted to being scattered in all directions. The original diffraction gratings were prepared by ruling a number of straight, parallel grooves in a surface. Incident light was diffracted by each of the surfaces and was principally directed in directions in which light from each groove interferes constructively with light scattered by the other grooves. This constructive light interference property of a grating allows efficient collection of light. Preformed diffraction gratings of this type have been used in binding assay systems.

Many assay systems have been developed using different physically measurable properties of reagents to provide a measurement of an analyte concentration in a sample. Radioimmunoassay (RIA), immunofluorescence, chemiluminescence, enzyme immunoassays (EIA), free radical immunoassays (FRAT), light scattering nephelometry, transistor bridge probes, indium reflective surfaces, and ultrasonic probes have been applied. These systems use the highly selective reaction between a primary member of a binding pair such as an antibody or antigen and an analyte selectively binding therewith. These techniques require expensive measurement equipment and often involve very complicated test procedures.

Reflective and transmissive biograting immunoassay systems and methods were disclosed in U.S. Patent 4,647,544. One embodiment described in the patent uses a biograting, a substantially flat surface having a coating thereon and having substantially uniform light scattering properties. The coating comprises a diffraction grating pattern of alternating parallel linear zones of an active and deactivated binding agent. The zones form a diffraction grating when the active binding agent binds with its opposite member of the binding pair. In the absence of such binding, no significant light diffraction occurs, that is, light energy detected at the diffraction angles is at a minimum value, approaching zero. When the binding occurs, the accumulation of bound material in the patterns of a diffraction grating creates a light disturbing grating, and light detected at the light diffraction angles increases to a larger value which correlates to the presence and quantity of the binding partner (analyte) in the sample. The flat surfaces upon which the biograting is formed in the patent include glass, plastic, plastic coating on a solid surface, gel

or other suitable inert material onto which specific antibody molecules can be attached.

U.S. Patent 4,876,208 describes transmissive and reflective diffraction binding assays and biograting systems of the type described in U.S. Patent 4,647,544. The biograting supports disclosed in this patent include a smooth upper surface of any material to which a primary hybridizing reagent can be adhered by physical or chemical bonding and which will not interfere with the reactions which are used to determine the presence and extent of the hybridizing reaction. Organic and inorganic polymers, both natural and synthetic, are described. Examples of polymers listed include polyethylene, polypropylene, polybutylene, poly(4-methylbutylene), butyl rubber, silastic polymers, polyesters, polyamides, cellulose and cellulose derivatives (such as cellulose acetate, nitrocellulose and the like), acrylates, methacrylates, vinyl polymers (such as polyvinyl acetate, polyvinyl chloride, polyvinylidene chloride, polyvinyl fluoride, and the like), polystyrene and styrene graft copolymers, rayon, nylon, polyvinylbutyrate, polyformaldehyde, etc. Other materials which are listed are silicon wafers, glasses, insoluble protein coatings on a smooth insoluble surface, metals, metalloids, metal oxides, magnetic materials, materials used in semiconductor devices, cermets and the like. The supports disclosed as preferred include polished single crystalline silicon, aluminum, epitaxial silicon coatings, silicon nitride coatings, silicon dioxide coatings, and polysilicon coatings.

Disclosure of the Invention

This invention is directed to improved reflective diffraction biogratings suitable for use in the apparatus and methods of U.S. Patents 4,647,544 and 4,876,208, the entire contents of which are hereby incorporated by reference. These biogratings have a higher reflectivity, a high binding capacity and optical flatness.

In summary, the biograting consists of an optically flat layer of silicon dioxide having a first and second surface, alternating zones of active and inactive binding agent on the first surface, and a reflective metal layer having a thickness which is sufficient to prevent transmission of substantially all light (less than one percent) therethrough. The reflective metal layer can be supported on an optically flat surface of metal wafer, and the reflective metal can be aluminum, gold, silver, chromium, platinum, titanium or nickel coating on a polished wafer. Preferably, the silicon dioxide layer is formed by sputtering a layer of silicon dioxide or by coating an alkali metal silicate solution on the surface of the reflective metal. The sputtering can be carried out using conventional sputtering devices and processes, and the thickness of the silicon dioxide can be controlled by varying the discharge time. The alkali metal silicate solution optimally contains from 1 to 20 wt.% and preferably from 5 to 10 wt.% silicon dioxide; from 0.5 to 15 wt.% and preferably from 5 to 10 wt.% of an aminoalkyltrialkoxysilane; and from 1 to 20 and preferably from 5 to 10 mg/ml of a water-soluble polysaccharide. The method for making the biograting comprises uniformly adhering a binding agent to one surface of an optically flat layer of silicon dioxide, the reflective metal layer being on the second surface;

and selectively deactivating zones of the binding agent to form spatially periodic patterns of active and deactivated binding agent by exposing the surface to a deactivating amount of UV light through a transparent mask having a spatially periodic pattern of opaque zones thereon.

An alternative reflective biograting embodiment for a diffraction bioassay device of this invention comprises a spatially periodic pattern of zones of active and inactive binding agent on an optically flat surface of a multilayer dielectric mirror.

Brief Description of the Drawings

Fig. 1 is a fragmentary, magnified cross-sectional view of one embodiment of this invention.

Fig. 2 is a schematic representation of the process for manufacturing an insoluble support with the spatially periodic pattern of Fig. 1.

Fig. 3 is a cross-sectional view of a dipstick having mounted thereon, a plurality of insoluble supports with spatially periodic pattern of binding reagents on the surfaces thereof.

Fig. 4 is a fragmentary, magnified cross-sectional view of an alternate embodiment of this invention with a spatially periodic pattern of binding agent on a multilayer dielectric mirror.

Fig. 5 is a dose response curve showing data obtained in Example 11.

Best Mode for Carrying Out the Invention

In an effort to increase the sensitivity of diffraction immunoassay systems using reflective biogratings, a wide variety of surfaces were investigated. The optimum reflective biograting combines high protein binding capacity, optical flatness, a high reflectivity, and selected thickness of the transparent layers. Examples of typical normal incidence reflectivities of biogratings found were bare silicon (23%), silicon oxide on silicon (12%), silicon nitride on silicon (1%), glass microscope slide (%4), and polystyrene (7%). With the improved biogratings of this invention, reflectivities greater than 60 percent have been obtained, substantially increasing the sensitivity of the bioassays developed using the improved biograting.

The term "biograting", as used herein, is defined to be a substantially flat surface having a coating thereon and having substantially uniform light scattering properties. The coating comprises a spatially periodic pattern of active and deactivated binding agent. One suitable pattern comprises alternating, parallel linear zones of an active and deactivated binding agent. Any other spatially periodic pattern including those described in U.S. Patent 4,647,544 can be used. The zones form a diffraction grating when the active binding agent binds with its opposite member of the binding pair. In the absence of such binding, no significant light diffraction occurs, that is, light energy detected at the diffraction angles is at a minimum value, approaching zero. When the binding occurs, the accumulation of bound material in the spatially periodic patterns create a spatially periodic light disturbing pattern, and light detected at the light diffraction angles increases to a

large value which correlates to the presence and quantity of the binding partner (analyte) in the sample.

The term "binding reagent" is used herein to designate one member of any binding pair of compounds or materials which selectively bind to form a conjugate. The members of the binding pair are generically denoted by the terms "ligand" and "antiligand", either one of which can be a binding reagent. The binding reagent can be a member of the well-known antibody-antigen or antibody-hapten pairs wherein the antibody binds selectively with the respective antigen or hapten, or combinations where the antibody is replaced with an Fab, Fab', $F(ab')_2$ fragment or hybrid antibody. The binding reagent can also be a member of other types of binding pairs such as biotin-avidin; lectin-sugar; IgG antibody Fc portion with protein A or protein G; enzyme-enzyme substrate; DNA or RNA binding with DNA, DNA fragments or other nucleotide sequences; enzyme-enzyme inhibitor; protein-protein receptor; chelating agent-ligand; and the like. Also included are specific binding pairs wherein a mercapto group binds specifically with a dithio or disulfide group (-S-SH or -S-S-) or with a N-substituted-2,4-diketo-3-pyrroline group, and other molecules with functional groups that will bind each other specifically. In general, the binding reagent is selected to bind specifically or selectively with the analyte, the material for which a sample is assayed. A non-light disturbing layer or coating of binding reagent is applied to an insoluble surface and is transformed into a spatially periodic design of non-light disturbing material for use in the method of this invention.

The term "binding assay", is used herein to designate an assay using any binding reaction between a

binding reagent and the other member of the binding pair which is selectively bindable therewith.

The term "light disturbing", as used herein, is defined to include all ways in which light is affected including light absorbing, reflecting, scattering, refracting and phase changing.

The term "spacially periodic pattern", as used herein, is defined to include gratings having spatially periodic designs which are formed in one or more immunological steps. For the method of this invention, the patterns are formed directly by the conjugation of the non-light disturbing binding reagent on the insoluble surface with a light disturbing analyte. Types of patterns formed in the process of this invention include reflection amplitude gratings, transmission amplitude gratings, reflection phase gratings, and transmission phase gratings. In reflection amplitude gratings, light is reflected from the grating, and the amplitude of the reflected light is modulated by the spatially variable reflection of the grating. In transmission amplitude gratings, light is transmitted through the grating, and the amplitude of the transmitted light is modulated by the spatially variable transmission of the grating. In the reflection phase grating, the light is reflected from the grating, and the phase of the reflected light is modulated by the spatially variable refractive index of the grating. In the transmission phase gratings, light is transmitted through the grating, and the phase of the transmitted light is modulated by the spatially variable refractive index of the grating. In the method of this invention, the diffraction grating may function as one or more of these types of gratings concurrently, and all of these grating types are included within the diffraction

gratings made in the method of this invention.

The term "optically flat", as used herein, is defined to be a surface with a maximum height variation of less than 600 Å over a surface area of 4 mm^2 or the area illuminated by the laser beam, whichever is smaller.

The term "wafer", as used herein, is defined to be a flat plate of insoluble solid having optically flat areas.

The term "alkyl", as used herein includes saturated and unsaturated, straight, branch-chained and cyclic hydrocarbon groups. The term "lower alkyl" is defined to include alkyl groups having from 1 to 6 carbon atoms.

Fig. 1 is a fragmentary, magnified cross-sectional view of one embodiment of this invention. It consists essentially of an optically flat layer of a transparent material 2 having a first surface 4 and a second surface 6. It has a spatially periodic pattern of zones of active binding agent 8 and inactive binding agent 10 on the first surface. It also has a reflective metal layer 12 on the second surfaces 6 having a thickness sufficient to prevent transmission of substantially all light (less than one percent) therethrough. For aluminum, for example, the reflective metal layer 12 should have a thickness of at least about 1000 Å. The active binding agent zones 8 have a width, a, and a distance between centers of the binding agent or "period", d.

The reflective metal layer 12 can be any reflective metal which has the stability required for the processing steps and an inherent reflectivity (for polished or optically flat surfaces) of at least 40%. Examples of

suitable reflective metals include aluminum, gold, silver, chromium, titanium, nickel and platinum.

The transparent layer or coating 2 can be any transparent material which can bind protein and can be applied as a coating. It can be an organic material such as an organic polymer such as nitrocellulose. It can also be an inorganic material such as a silicon dioxide. The invention is hereinafter described with the use of silicon dioxide for purposes of clarity of description and not by way of limitation. Any transparent material satisfying the above requirements can be used and are considered to be within the scope of this invention.

The silicon dioxide layer can be any plate with optically flat areas of transparent glass containing silicon dioxide, preferably treated with a suitable silane to increase its protein binding capacity. If the silicon dioxide is a self-supporting layer such as a microscope slide or coverslip, for example, the reflective metal can be applied to the surface opposite to the side carrying the biograting with a mirroring, vapor deposition, sputtering or other metallization process.

The combination yielding the highest optical flatness and reflectivity comprises a silicon dioxide coating formed on a reflective metal coated, optically flat area of wafer 14 of silicon or silicon dioxide. The wafer is the supporting layer and should have the physical and chemical stability to undergo the metallization process without significant change. A convenient source of wafers are polished plates of semiconductor materials such as silicon wafers typically used in semiconductor manufacture. These are readily

available in a polished, optically flat form and have the thermal and chemical stability for metallization by vapor deposition or metal sputtering, both conventional and well known processes commonly used in semiconductor manufacture. However, polished glass would be equally suitable as a substrate since no radiation penetrates the metal layer.

The optimum reflective metal coating process depends upon the particular metal used. Aluminum, gold and silver coatings can be directly applied to one surface of the support wafer in a sputtering process carried out in an inert atmosphere, usually in a partial vacuum. The coating thickness is controlled to be sufficient to reflect all (at least 99 %) of the incident light. The thickness of reflective aluminum, for example, required to achieve this result is about 1000 Å. Suitable processes for depositing the metal coatings, for example sputtering and vapor deposition, are described in VLSI TECHNOLOGY, Edited by S.M. Sze, New York: McGraw-Hill (1983).

The silicon dioxide coating is then applied to the reflective metal surface by a process which yields a product having a high reflectivity and an optically flat surface. The silicon dioxide coating can be applied by sputtering, as described in VLSI TECHNOLOGY (supra, p 358). Alternatively, the silicon dioxide coating can be applied by spin coating the reflective surface with an alkali metal silicate solution. Spin coating is a conventional process, well known to a person skilled in the coating art. The thickness of the coating is determined by the viscosity of the alkali metal silicate solution, spinning speed, temperature and evaporation rate. In general, the surface is spun around an axis

perpendicular to the surface, and the solution is applied either before or during the spinning. If the coating is applied to a conventional circular wafer disk having a diameter of 4 inches, the spinning speed should be from 1500 to 8000 rpm and preferably is from 2500 to 4000 rpm.

The alkali metal silicate solution can be made of any alkali metal (sodium, potassium, lithium, etc) and is preferably a conventional sodium silicate (water glass) solution containing from 1 to 20 wt.% and preferably from 5 to 10 wt.% alkali metal silicate.

The protein binding capacity of the silicon dioxide product is increased if the surface is treated with a protein binding agent such as an aminosilane. If the silicon dioxide coating is formed from an alkali metal silicate solution, the protein binding agent can be incorporated directly in the coating solution. Suitable aminosilanes include aminoalkylsilanes having the formula:

$$R_2R_3R_3SiCH_2R_1$$

wherein,

R_1 is hydrogen, an aminoalkyl group having from 1 to 18 carbons, or an aminoalkylamino group having from 1 to 18 carbons; and

R_2, and R_3 are each, individually, a lower alkyl or alkoxy group.

Examples of suitable aminoalkylsilanes include aminopropyltriethoxysilane, aminopropyltrimethoxysilane, aminobutyltriethoxysilane, N-(2-aminoethyl-3-aminopropyl)triethoxysilane, ω-aminoundecyltrimethoxy-

silane, and aminopropylmethyldiethoxysilane, for example. A preferred aminoalkyltrialkoxysilane is N-(2-aminoethyl-3-aminopropyl)triethoxysilane. The alkali metal silicate solution can contain from 0.5 to 15 wt.% and preferably from 5 to 10 wt.% of the aminoalkylsilane.

The binding capacity is further increased if the alkali metal silicate solution also includes from 1 to 20 mg/ml and preferably from 5 to 10 mg/ml of a water soluble hydroxylated polymers, preferably polysaccharides. Suitable polysaccharides include water-soluble gums, hydrolyzed starches, cellulose derivatives, and other conventional water-soluble hydroxylated polymers. A particularly suitable polysaccharide are the dextrans having a molecular weight of from 5000 to 500,000 and preferably from 10,000 to 75,000.

The silicon dioxide coating thickness is determined by the sputtering time in the sputter coating process or by the speed of rotation in the spin coating process.

If the silicon dioxide coating is applied as an alkali silicate solution, the coated support is cured by heating in an oven at a temperature of from 90° to 200°C and preferably from 120° to 150°C for a time sufficient to cure the coating. The heating time will depend upon the thickness of the coating and the concentration of the coating solution. A heating time of from 0.5 to 16 hours is sufficient. A heating time of from 1 to 3 hours is preferred.

The method for making the biograting comprises a first step of uniformly adhering a binding agent to the silicon dioxide surface. This followed by a step of selectively deactivating zones of the binding agent to

form a spatially periodic pattern of active and deactivated binding agent by exposing the surface to a deactivating amount of UV light through a transparent mask having a diffraction grating pattern of opaque zones thereon.

The binding reagent applied to the silicon dioxide surface of an insoluble support is selected to bind with the analyte to be determined in the assay. It can be any member of the binding pairs described above. It can be an antibody; antibody fragment selected from the group consisting of Fab, Fab', or $F(ab')_2$ fragments; hybrid antibody; antigen; hapten; protein A; protein G; lectin; biotin; avidin; chelating agent; enzyme; enzyme inhibitor; protein receptor; nucleotide hybridizing agent; or a bacteria, virus, Mycoplasmatales, spore, parasite, yeast, or fragment thereof; or combinations thereof.

The combined optical thickness or optical path length of the transparent layer 2 and the binding layers (8 and 10) applied thereto is preferably about one-fourth the wavelength ($\lambda/4$) of the laser light to be used, measured in the direction of the laser light.

Fig. 2 is a schematic representation of the process for manufacturing an insoluble support with the diffraction grating design of Fig. 1. One member of the binding pair can be applied to the silicon dioxide surface 20 (Step A) by covalent bonding or adsorption in solution 22 in Step B. For covalent bonding, the surface, after being coated with an aminosilane, can be reacted with the protein.

One procedure for conjugating aminosilane groups

with proteins can be achieved with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI). This is a water-soluble carbodiimide which is used for coupling antibodies or proteins with haptens or solid phases through functional groups such as carboxy and/or amino groups. EDCI reactions can be carried out as follows: To a solution of antigen or antibody in 0.01 M phosphate buffered saline, pH 6.0 at 4° C, is added an excess amount (normally 100 times the stoichiometric amount) of EDCI. The insoluble support having amino groups thereon, is added to the solution. After addition, the mixture is stirred at refrigerated temperatures for 16-24 hours to complete the reaction.

If the protein to be coupled to the support is an antibody, the conjugation is preferably carried out with a soluble periodate such as an alkali metal periodate. To a solution of antibody in 0.2 M acetate buffer, pH 5, is added a solution of the periodate (11.2 mg of periodate per 1 mg of antibody). The mixture is stirred at 4-8 °C for 1 to 1.5 hours. It is then dialyzed against 0.1 M carbonate buffer, pH 8.9, and the resulting solution is incubated with the insoluble support in a refrigerator overnight.

The antibodies can also be coupled to the insoluble surface through a thioether linkage. In this procedure, the aminopropyltriethoxysilane activated surface is allowed to react with an excess amount of iodoacetic anhydride or bromoacetic anhydride in anhydrous dimethylformamide at room temperature overnight while protecting the reactants from light. The activated surface is then washed thoroughly with deionized water and kept protected from light exposure until it is reacted with the antibody. Before being reacted with the

iodoacetyl or bromoacetyl activated surface, the antibody is treated with 2-aminoethanethiol at 37°C for 2 hours in a degassed 0.1 M phosphate buffer solution, pH 6.0. After the reaction with 2-aminoethanethiol, the solution is chromatographically purified with a Sephadex column (Pharmacia) to remove the excess amount of 2-aminoethanethiol. The antibody reaction product has mercapto groups. It can be coupled to the solid surface by reacting the solution with the iodoacetyl or bromoacetyl activated surfaces at refrigerated temperatures overnight.

Non-covalent bonding can be achieved by immersing the surface in an aqueous buffer solution. The buffered binding reagent solution is placed in a container containing the silicon dioxide surface and incubated at room temperature until adsorption occurs, for example for from 0.5 to 18 hours and preferable from 1 to 3 hours, at temperatures of from 4 to 40°C and preferable from 20 to 26°C. The surface is then rinsed with a buffered saline solution and dried.

The concentration of binding reagent in the buffer solution is selected to provide the desired reagent density on the silicon dioxide surface. The binding reagent solution can contain from 0.02 to 100 micrograms/ml of the binding reagent and preferably contains from 10 to 50 micrograms/ml of the binding reagent in a buffered solution having a pH of from 6.0 to 9.5 and preferably from 7.0 to 8.5. The surface with the coating 26 thereon is then rinsed and dried.

A suitable rinse solution is an aqueous phosphate buffer solution such as is described in U.S.Patent 4,528,267 having a phosphate molarity of from 0.0001 to

0.05, a pH of from 6 to 8 and containing from 0.001 to 0.1 weight percent non-ionic surfactant and from 0.0001 to 0.5 weight percent of an animal serum albumin. Suitable non-ionic surfactants include polyoxyethylene ethers (BRIJ) such as lauryl, cetyl, oleyl, stearyl, and tridecyl polyoxyethylene ethers; polyoxyethylenesorbitans (TWEEN) such as polyoxyethylenesorbitan monolaurate, monopalmitate, monostearate, monooleate and trioleates; and other polyoxyethylene ethers (TRITON), for example. Preferred non-ionic surfactant are the polyoxyethylene-sorbitans such as polyoxyethylenesorbitan monolaurate (TWEEN 20).

A mask is prepared by photographic methods conventional in semiconductor manufacturing. For example, a mask for a linear spatially periodic pattern can be prepared on a quartz glass or other UV-transparent plate through a photoresist process similar to photography. The linear dark zones of the mask correspond to active binding reagent areas desired on the ultimate surface.

In Step C, the mask 24 is mounted in a suitable support 25 of a UV light focusing apparatus such as a Karl Suss Model 40 Mask Aligner (Karl Suss, Waterbury Center, Vermont 05677). The mask 24 is placed over the silicon dioxide surface 20 having a coating 26 of binding reagent, and the surface is exposed to ultraviolet radiation from UV radiation source 28 until the binding capability of the portions of the binding reagent exposed to the radiation is substantially reduced or preferably eliminated. To manufacture a precision grating design, the radiation should form a sharp image on the coated surface. Penumbrae should be minimized. Preferably, the ultraviolet light passing through the mask is focused to

a sharp image on the surface coating using conventional projection alignment techniques without contact with the coated surface.

The ultraviolet radiation exposure required to deactivate coating exposed thereto depends upon the binding reagent. For antibody binding reagents, exposure times of from 30 sec to 30 min and preferably from 1 to 5 min is sufficient with a ultraviolet radiation having a wavelength such as 254 nm and a power of from 8 to 14 milliwatts per cm^2. Some adjustment in time of exposure and/or power may be necessary to deactivate the binding sites of other binding agents.

To alter the epitopes of antigenic binding reagents such as human IgG, exposure times of from 5 to 30 min and preferably from 5 to 10 min are sufficient with a ultraviolet radiation having a wavelength of 254 nm and a power of from 8 to 14 milliwatts per cm^2. Some adjustment in time of exposure and/or power may be necessary to alter or destroy the antigenic sites of other binding agents.

This treatment reduces or eliminates the binding properties of the binding reagent in zones 10, leaving active binding reagent in a spatially periodic pattern or design as the zones 8 (Fig. 1).

In Step D, the coated substrate containing areas having binding protein in a diffraction grating design is cut into smaller area chips 30, each chip having a size sufficient to perform a binding assay. These chips are then mounted on a suitable diagnostic support such as the dipstick shown in Fig. 3.

Fig. 3 is a cross-sectional view of a dipstick having mounted thereon, a plurality of insoluble supports with non-light disturbing spatially periodic patterns or designs of binding reagents on the surfaces thereof. The dipstick body 32 has a plurality of insoluble support surfaces 34 having a spatially periodic pattern or design of binding reagent coated thereon such the biogratings shown in Fig. 1 made by the process shown in Fig. 2. The materials from which the dipstick 32 are made are preferably non-binding to minimize non-specific binding during the binding assay procedure. Suitable dipstick surface materials include polyolefins such as polyethylene and polypropylene, hydrophilic polysilicon and polysiloxane polymers, and the like. Also suitable are polymers which have been treated to render the surfaces non-binding to proteinaceous materials. The silanes can be applied to the silicon dioxide surface in a vapor phase, for example.

The support for the diffraction grating supports can be any articles upon which the diffraction grating support surface can be mounted. The description of dipsticks are provided by way of example, and not as a limitation. Other articles such as microwells, plates, cavities and the like can be used. For many applications, dipsticks are a preferred embodiment.

Fig. 4 is a fragmentary, magnified cross-sectional view of an alternate embodiment of this invention with a spatially periodic pattern of binding agent on a multilayer dielectric mirror. This highly reflective device comprises alternating layers of two transparent materials having different refractive indexes. Multiple dielectric mirrors and suitable transparent materials for manufacturing them are described in Born, Max et al,

PRINCIPLES OF OPTICS. 6th ed. New York:Pergamon Press, pp 66 et seq (1980) and Hecht, Eugene, OPTICS. 2nd ed. Reading:Addison-Wesley, pp 377 et seq, the entire contents of which are hereby incorporated by reference. The preferred optical thickness of each layer is approximately one-quarter wavelength ($\lambda/4$) of the light to be reflected measured in the direction of the light. The mirrors are thus manufactured for use with collimated light of a selected frequency, usually a selected laser frequency.

The multilayer dielectric mirror comprises alternating layers of units of a layer of one transparent material such as silicon dioxide 70 and a layer of another transparent material such as titanium dioxide 72 having the quarter wavelength optical thicknesses described above. The bottom reflective layer unit is supported on the optically flat surface of a wafer 74. Each successive reflective layer unit provides an incremental increase to the reflectivity. Although no theoretical limit to the number of reflective layer units exists, the amount of the reflectivity increase provided with each additional unit is incrementally smaller.

As described above with respect to the embodiment of Fig. 1, the binding layer provides a spatially periodic design or pattern of active binding agent. The periodic design comprises a plurality of zones of non-light disturbing active binding agent 76 separated by zones of non-light disturbing deactivated binding agent 78, for example, binding reagent which has been deactivated by exposure to ultraviolet radiation, other deactivating radiation, or other deactivation energy. The binding agent is a member of a binding pair as described above.

This invention is further illustrated by the following specific but non-limiting examples. Examples which have been reduced to practice are stated in the past tense, and examples which are constructively reduced to practice herein are presented in the present tense. Temperatures are given in degrees Centigrade and weight as weight percents unless otherwise specified.

EXAMPLE 1

Aluminum Coated Silicon Surface

Bare silicon wafers are placed in a high frequency magnetron sputtering chamber, the chamber evacuated to 5×10^{-6} torr, argon gas is introduced, and the plasma glow discharge is initiated to deposit aluminum on the silicon wafer surface using the procedures of VLSI TECHNOLOGY (supra) and MICROCHIP FABRICATION: A PRACTICAL GUIDE TO SEMICONDUCTOR PROCESSING, (supra). After 35 minutes, the plasma is terminated, the chamber vented, and the aluminum coated wafer removed.

EXAMPLE 2

Silicon Dioxide Coated Al/Si Support

The product of Example 1 is placed in a high frequency magnetron sputtering chamber, the chamber evacuated to 5×10^{-6} torr, and plasma gas is introduced. A plasma glow is initiated to clean the aluminum surface. Then a silicon dioxide target is introduced or exposed, and after the desired silicon dioxide coating is formed on the aluminum surface, about 30 minutes, the plasma is terminated, the vessel vented, and the aluminum-on-silicon wafers with a 1000 Å coating of silicon dioxide

are removed.

EXAMPLE 3

APTS coated SiO_2/Al/Si Support

Aminopropyltriethoxysilane (APTS) is coated onto a silicon dioxide/aluminum/silicon wafer by vapor deposition. The wafer is placed in a vacuum oven, the oven heated to 170°C, and the chamber evacuated to about 0.1 torr. The aminopropyltriethoxysilane is introduced into the oven, allowed to vaporize, and the coating process is continued for 4 hr. The oven is evacuated to remove remaining aminopropyltriethoxysilane vapor, and the wafer is retained in the oven for 8 hr to complete the coating reaction. The oven is then vented, and the APTS coated SiO_2/Al/Si wafer is removed.

EXAMPLE 4

Sodium Silicate Coating on Al/Si Support

A 3.1 molar sodium silicate solution (VMR Scientific Catalog No. AL 68330) was diluted 1:4 with deionized water, and pipetted onto an aluminum coated silicon wafer. The wafer was spun with a Model 6000 spin coater (Integrated Technologies, Inc. Asushnet, MA 02743) at 3000 rpm for one min. The silicate coated wafer was then cured in an oven at 135°C for 2 hr and allowed to cool to room temperature.

EXAMPLE 5

APTS/SILICATE Coating on Al/Si Support

A mixture of 3.1 molar sodium silicate solution, aminopropyltriethoxysilane, and deionized water (1:0.5:3.5 v/v/v) was prepared. The solution was pipetted onto an aluminum coated silicon wafer. The wafer was spun with a Model 6000 spin coater (Integrated Technologies, Inc. Asushnet, MA 02743) at 3000 rpm for one min. The silicate coated wafer was then cured in an oven at 135°C for 2 hr and allowed to cool to room temperature.

EXAMPLE 6

APTS-DEXTRAN-SILICATE Coating on Al/Si Support

To each ml of a mixture of 3.1 molar sodium silicate solution, aminopropyltriethoxysilane, and deionized water (1:0.5:3.5 v/v/v) was added 5 mg of dextran (50,000 daltons). The mixture was pipetted onto a aluminum coated silicon wafer. The wafer was spun with a Model 6000 spin coater (Integrated Technologies, Inc. Asushnet, MA 02743) at 3000 rpm for one min. The silicate coated wafer was then cured in an oven at 135°C for 2 hr and allowed to cool to room temperature.

EXAMPLE 7

Reflectivities of Coated Al/Si Supports

The reflectivities of the silicon surfaces prepared in Examples 1-6 were determined. The silicon surfaces

were illuminated with a Model 1107P Helium-Neon Laser (Uniphase, Sunnyvale, CA 94086) at an incident angle of 75°. The reflected light intensity was measured with a Model 61 Optometer (United Detector Technology, Hawthorne, CA). A reflectivity of 80% or higher was found on all surfaces.

Surface Coating	Reflectivity
Silicon Dioxide	0.92
APTS/Silicon Dioxide	0.84
Silicate	0.85
APTS/Silicate	0.80
APTS/Silicate/Dextran	0.80

EXAMPLE 8

Monoclonal Anti-β-hCG (Fab) Coating

A silicon dioxide/aluminum/silicon wafer, coated by vapor deposition with APTS, was incubated with a solution of monoclonal anti-β-hCG (Fab) in 0.01 M phosphate buffer, pH 7.4 (100 μg/ml) at 4-8°C for 4 hr. The surface was briefly washed with 0.05 M Tris buffer, pH 8.5, containing 2.5% sucrose. It was then incubated with 0.05 M Tris buffer, pH 8.5, containing 2.5% sucrose and 0.5 wt.% human serum albumin (HSA) at 4-8°C for 30 min. The residual liquid was removed by spinning the wafer to yield an anti-β-hCG coated silicon dioxide surface.

EXAMPLE 9

Monoclonal Anti-β-HCG (IgG) Coating

Monoclonal anti-β-hCG antibody was diluted in 0.2 M of acetate buffer, pH 5.0, to a concentration of 2 mg/ml. The solution was cooled in an ice bath, and a solution of sodium periodate (22.4 mg/ml) in 0.1 M acetate buffer was slowly added. The mixture was then stirred at 4-8°C for 1 to 1.5 hr and then dialyzed against 2 L of 0.1 M carbonate buffer, pH 8.9, at 4-8°C for 4-6 hr. The antibody solution obtained was incubated with a silicon dioxide/aluminum coated silicon wafer surface which has been coated with APTS by vapor deposition in a refrigerator overnight. The surface was briefly washed with 0.05 M Tris buffer, pH 8.5, containing 2.5% sucrose. It was then incubated with 0.05 M Tris buffer, pH 8.5, containing 2.5% sucrose and 0.5 wt.% human serum albumin (HSA) at 4-8°C for 30 min. The residual liquid was removed by spinning the wafer to yield an anti-β-hCG coated silicon dioxide surface.

EXAMPLE 10

Biograting Preparation

An anti-β-hCG coated silicon dioxide surface prepared by the procedure of Example 8 was placed under a photomask using a Karl Suss Model 40 Mask Aligner. The photomask has parallel opaque lines having a center-to-center distance, d, of 10 μm. The surface was illuminated with UV light at 254 nm for 6 min. After illumination, the surface was diced into 4 x 6 mm chips.

EXAMPLE 11

Anti-β-hCG (Fab) Biograting Immunoassay

The 4 x 6 mm chips prepared in Example 10 were mounted on a microscope slide and incubated with 600, 300, 150, 75 and 0 mIU/ml of β-hCG solutions (test samples) at room temperature for 5 min. The surface was washed with deionized water and dried with a stream of air. The diffraction intensities of the surfaces were determined with a Model 61 optometer to give a dose response curve shown in Fig. 5.

Claims

1. A reflective biograting consisting essentially of an optically flat layer of a transparent composition having a first and second surface, a spatially periodic pattern of zones of active and inactive binding agent on the first surface, and a reflective metal layer on the second surface, the reflective metal layer having a thickness sufficient to prevent transmission of substantially all incident light therethrough.

2. The reflective biograting of Claim 1 wherein the reflective metal layer is supported on an optically flat surface.

3. The reflective biograting of Claim 1 wherein the reflective metal is aluminum, gold, silver, chromium, platinum, nickel or titanium.

4. The reflective biograting of Claim 1 wherein the transparent composition is silicon dioxide.

5. The reflective biograting of Claim 4 wherein the layer of silicon dioxide is formed by sputtering of silicon dioxide.

6. The reflective biograting of Claim 4 wherein the silicon dioxide layer is formed by coating an alkali metal silicate solution on the surface of the reflective metal.

7. The reflective biograting of Claim 4 wherein the layer of silicon dioxide is bonded with an aminoalkylsilane having the formula:

$R_2R_3R_3SiCH_2R_1$

wherein,
R_1 is hydrogen, an aminoalkyl group having from 1 to 18 carbons, or an aminoalkylamino group having from 1 to 18 carbons; and

R_2, and R_3 are each, individually, a lower alkyl or alkoxy group.

8. The reflective biograting of Claim 7 wherein the silicon dioxide layer is formed by coating an alkali metal silicate solution on the surface of the reflective metal, and the alkali metal silicate solution contains from 1 to 20 wt.% silicon dioxide and from 0.5 to 15 wt.% of the aminoalkylsilane.

9. The reflective biograting of Claim 8 wherein the aminoalkylsilane is aminopropyltriethoxysilane or N-(2-aminoethyl)-3-aminopropyltriethoxysilane.

10. The reflective biograting of Claim 7 wherein the aminoalkylsilane is applied and bonded to the layer of silicon dioxide by vapor deposition.

11. The reflective biograting of Claim 10 wherein the aminoalkylsilane is aminopropyltriethoxysilane or N-(2-aminoethyl)-3-aminopropyltriethoxysilane.

12. The reflective biograting of Claim 8 wherein the alkali metal silicate solution contains from 1 to 20 mg/ml of a water-soluble hydroxylated polymer.

13. The reflective biograting of Claim 12 wherein the hydroxylated polymer is a dextran having a molecular weight in the range of from 5000 to 500,000.

14. The reflective biograting of Claim 1 wherein the binding agent is a member of a binding pair, one member of which is an antibody; antibody fragment selected from the group consisting of Fab', Fab, $F(ab')_2$ fragments; hybrid antibody; antigen; hapten; protein A; protein G; lectin; biotin; avidin; chelating agent; enzyme; enzyme inhibitor; protein receptor; nucleotide hybridizing agent; or a bacteria, virus, Mycoplasmatales, spore, parasite, yeast, or fragment thereof; or combinations thereof.

15. A method for making a biograting comprising

a) uniformly adhering a binding agent to a first surface of an optically flat layer of a transparent composition having a first and second surface, a reflective metal layer on the second surface having a thickness sufficient to prevent transmission of substantially all light therethrough; and

b) selectively deactivating zones of the binding agent to form a diffraction grating pattern of alternating zones of active and deactivated binding agent by exposing the surface to a deactivating amount of UV light through a transparent mask having a spatially periodic pattern of opaque zones thereon.

16. The method of Claim 15 wherein the reflective metal layer is supported on an optically flat surface.

17. The method of Claim 15 wherein the reflective metal is aluminum, gold, silver, chromium, platinum, nickel or titanium.

18. The method of Claim 15 wherein the transparent composition is silicon dioxide.

19. The method of Claim 18 wherein the layer of silicon dioxide is formed by sputtering of silicon dioxide.

20. The method of Claim 18 wherein the silicon dioxide layer is formed by coating an alkali metal silicate solution on the surface of the reflective metal.

21. The method of Claim 18 wherein the layer of silicon dioxide is bonded with an aminoalkylsilane having the formula:

$$R_2R_3R_3SiCH_2R_1$$

wherein,
R_1 is hydrogen, an aminoalkyl group having from 1 to 18 carbons, or an aminoalkylamino group having from 1 to 18 carbons; and

R_2, and R_3 are each, individually, a lower alkyl or alkoxy group.

22. The method of Claim 21 wherein the silicon dioxide layer is formed by coating an alkali metal silicate solution on the surface of the reflective metal, and the alkali metal silicate solution contains from 1 to 20 wt.% silicon dioxide and from 0.5 to 15 wt.% of the aminoalkylsilane.

23. The method of Claim 22 wherein the aminoalkylsilane is aminopropyltriethoxysilane or N-(2-aminoethyl)-3-aminopropyl-triethoxysilane.

24. The method of Claim 21 wherein the aminoalkylsilane is applied and bonded to the layer of silicon dioxide by vapor deposition.

25. The method of Claim 24 wherein the aminoalkylsilane is aminopropyltriethoxysilane or N-(2-aminoethyl)-3-aminopropyl-triethoxysilane.

26. The method of Claim 22 wherein the alkali metal silicate solution contains from 1 to 20 mg/ml of a water-soluble hydroxylated polymer.

27. The method of Claim 26 wherein the hydroxylated polymer is a dextran having a molecular weight in the range of from 5000 to 500,000.

28. The method of Claim 15 wherein the binding agent is a member of a binding pair, one member of which is an antibody; antibody fragment selected from the group consisting of Fab', Fab, $F(ab')_2$ fragments; hybrid antibody; antigen; hapten; protein A; protein G; lectin; biotin; avidin; chelating agent; enzyme; enzyme inhibitor; protein receptor; nucleotide hybridizing agent; or a bacteria, virus, Mycoplasmatales, spore, parasite, yeast, or fragment thereof; or combinations thereof.

29. A reflective biograting consisting essentially of alternating zones of active and inactive binding agent on the surface layer of a multilayer dielectric mirror comprising alternating layers of first and second

transparent materials supported on an optically flat support, each of the first and second transparent materials having differing diffraction indexes.

30. A reflective biograting of Claim 29 wherein the surface layer is the first transparent material and is silicon dioxide.

31. A reflective biograting of Claim 30 wherein the second transparent materials is titanium dioxide.

32. The reflective biograting of Claim 29 wherein the binding agent is a member of a binding pair, one member of which is an antibody; antibody fragment selected from the group consisting of Fab', Fab, $F(ab')_2$ fragments; hybrid antibody; antigen; hapten; protein A; protein G; lectin; biotin; avidin; chelating agent; enzyme; enzyme inhibitor; protein receptor; nucleotide hybridizing agent; or a bacteria, virus, Mycoplasmatales, spore, parasite, yeast, or fragment thereof; or combinations thereof.

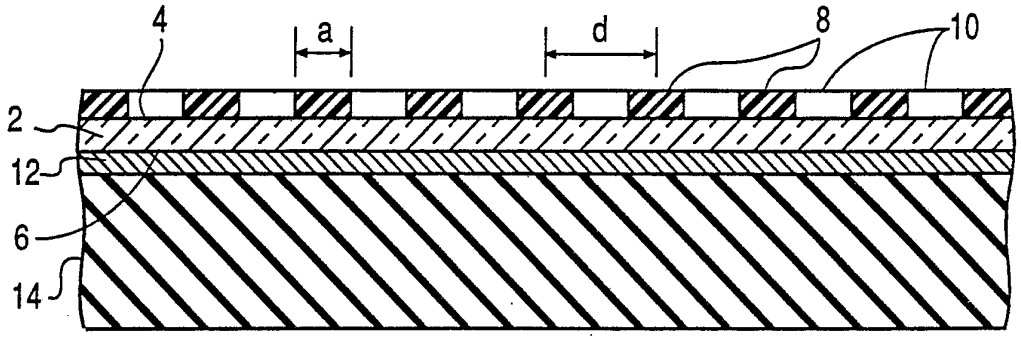

FIG. 1

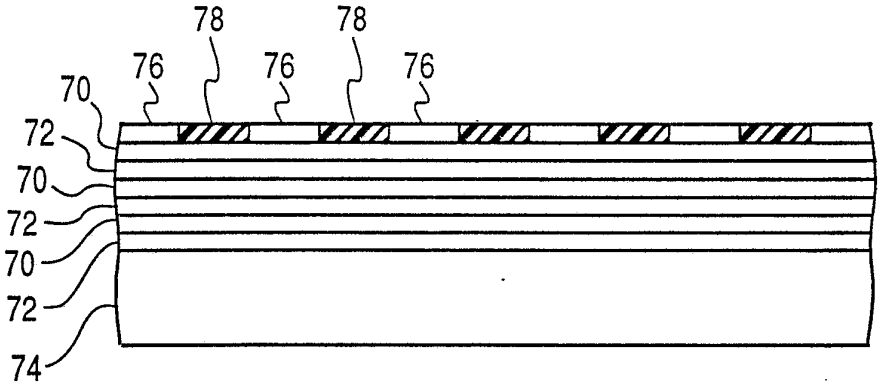

FIG. 4

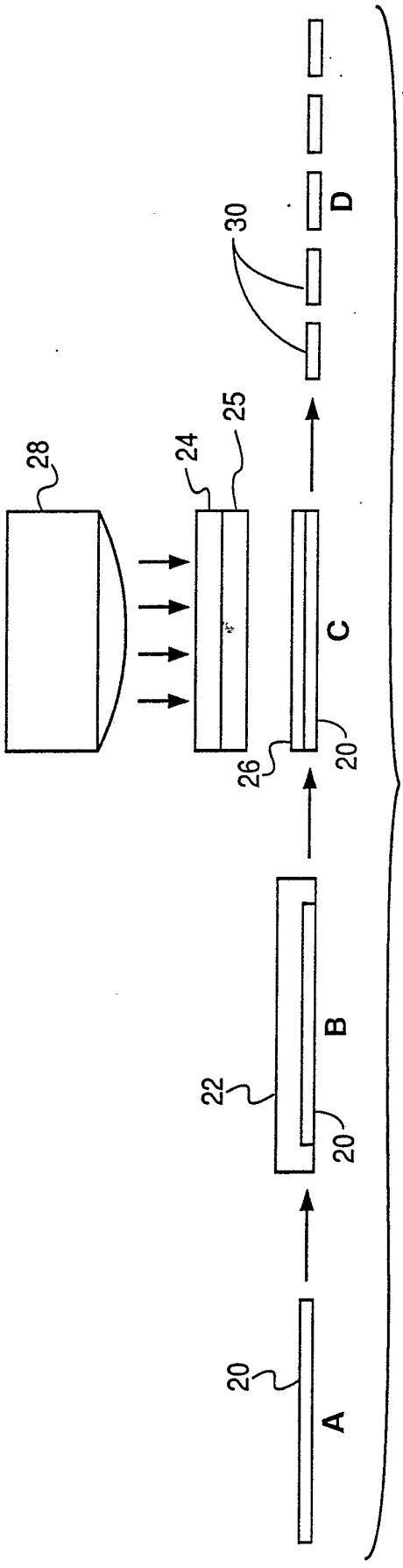

FIG. 2

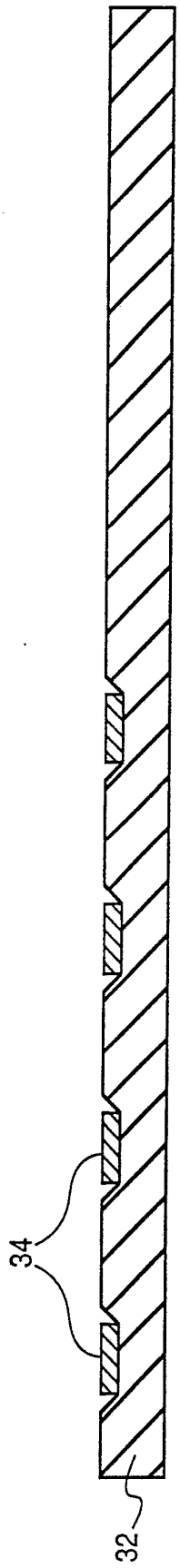

FIG. 3

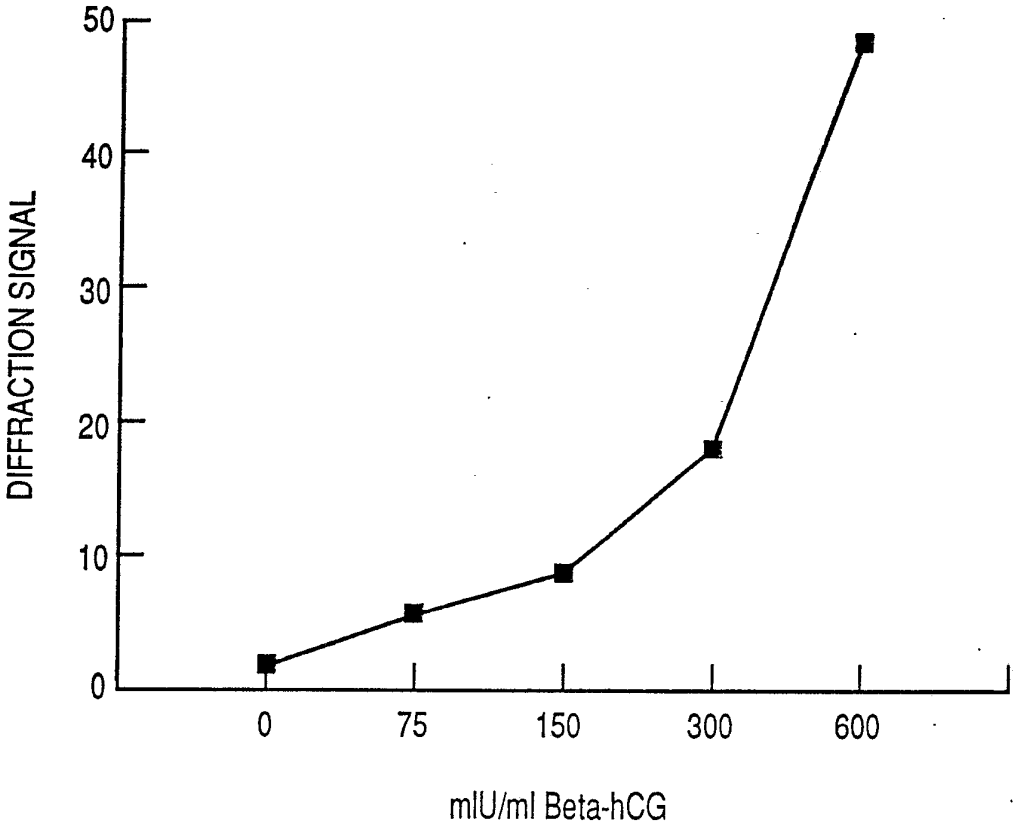
50
40
30
20
10
0
DIFFRACTION SIGNAL
0
75
150
300
600
mIU/ml Beta-hCG

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/03311

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G01N 33/552

U.S.Cl.: 436/527; 435/7

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.Cl.	436/527, 807, 519, 531; 428/433, 434; 435/7

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS and Dialog computer data bases were searched.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,647,544 (NICOLE ET AL.) 03 MARCH 1987, see the Abstract and col. 11, line 62 through col. 19, line 40.	1-32
Y	US, A, 4,876,208 (GUSTAFSON ET AL.) 24 OCTOBER 1989, see the Abstract, col. 7, lines 32-37, col. 8, line 19 - col. 9, line 43.	1-32

* Special categories of cited documents: [10]

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

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

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

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

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

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

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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 June 1991	05 SEP 1991

International Searching Authority	Signature of Authorized Officer
ISA/US	Amelia B. Yarbrough