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(54) MICRODEVICE SUBSTRATE AND METHOD FOR MAKING MICROPATTERN DEVICES

(71) We, DIOS INC., a corporation of the State of Delaware, United States of America, of 9412 Wooden Bridge Road, Potomac, Maryland 20854, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

This invention relates to methods of producing micropatterns on substrate bases and to substrate bases having micropatterns produced thereon.

Rapidly expanding usage of electronic microcircuits has resulted in a rapid advance in the art of producing articles in the nature of a micropattern of a functional material supported on or in a substrate base of a dissimilar material. Recently developed procedures employ, e.g., as a substrate for the microdevice, a base of silicon covered by silicon dioxide, and an electron resist covering the silicon dioxide, the resist being selectively irradiated by an electron beam in accordance with the micropatterning step to be performed, the irradiated resist then being removed to expose selected areas of the silicon dioxide, the exposed areas then being treated, as by etching, if necessary, the functional material then being applied, and the resist then being removed in the area which has not been irradiated, bringing the substrate to a condition suitable for the next micropatterning step. Typical requirements include diffusion of dopants into the silicon base in one or more selected areas, and provision of a conductive metal on defined areas of the silicon dioxide, and such requirements are met by different procedures each requiring use of an electron resist on which a portion of the micropattern is written by electron beam irradiation. It is common to use as the electron resist a synthetic polymer which is partially depolymerized by electron beam irradiation, the partially depolymerized material being removable by dissolution in, e.g., the corresponding monomer.

Though such prior-art procedures have achieved marked success, they are limited to those objectives which can be accomplished by treatments controlled by the presence or absence of the resist on the base. Further, such procedures require relatively extensive electron beam irradiation to "write" the pattern desired, and writing time has become recognized as a primary limiting factor. Finally, while such methods are not commonly used to produce micropatterns of very small size, with, e.g., conductive path widths as small as 1000 Å, further reduction in the dimensions of the micropattern is highly desirable.

It is an object of the invention to provide an improved method for producing micropatterns on substrate bases, which will have not only the advantages of prior-art methods but also the advantage of increased flexibility with regard to the nature and size of the micropatterns produced.

From one aspect of the invention consists in a method of producing a micropattern on a substrate base, the method including the steps of:- establishing on the surface of the base a protein layer comprising at least a compressed monolayer of denatured non-fibrous protein; establishing on said protein layer a film of masking material; directing radiant energy on said film in an area predetermined by the micropattern desired; and removing said film from only said predetermined area to expose said protein layer for further treatment.

From another aspect of the invention consists in a method of producing a micropattern on a substrate base, including the steps of:- establishing on the base a protein layer comprising at least a compressed monolayer of denatured non-fibrous protein in which the protein molecules each have a specific reactive site, the molecules of the monolayer

being spatially arranged in a predetermined pattern and said specific reactive sites therefore occupying predetermined locations with reference to said pattern; establishing over said monolayer a film of masking material; selectively removing said making film in areas predetermined with reference to said pattern; and reactively attaching to the resulting exposed portions of said protein layer pattern-building material to establish on the support the desired micropattern in configuration and location dependent upon the spatial arrangement of the molecules of said monolayer.

From yet another aspect the invention consists in a substrate base having a micropattern produced thereon by a method in accordance with either of the two preceding paragraphs.

As is usual in the art the term 'monolayer' is used herein synonymously with the term 'monomolecular layer' to mean any layer which is one molecule thick. The term 'masking material' is used to mean any resist material, and in particular a radiation-sensitive organic material, which is capable of being removed selectively, for example, by dissolution, after selective irradiation or bombardment. As is well known, such materials include negative photoresists which are materials that form polymers on exposure to radiation and positive photoresists which are polymers that are depolymerised by the irradiation. Further examples of masking materials are given hereinafter.

In a simple embodiment of the invention, the exposed area of the protein layer is a positive of the desired micropattern and the further treatment comprises removal of the protein in the exposed area, and the next micropatterning step is carried out on the exposed area, with the remaining electron resist material and protein serving as a negative mask and being subsequently removed. In another embodiment, an area of the protein monolayer is exposed as a negative of the desired micropattern, and a second protein is linked to the protein of the monolayer, the entire surface of the substrate is then irradiated, the electron resist film and monolayer are then removed from the base in all but the negative area, the next micropatterning step is then carried out on the area of the base thus exposed with the second protein linked to the monolayer serving as a negative mask, and the protein and remaining electron resist film then removed from the negative area. In other embodiments, the protein of the monolayer is such that the molecules each have a specific reactive site and thus form a predetermined spatial pattern, the resist or masking film is removed in areas which are predetermined with reference to the pattern of the protein molecules, and pattern building material, typically an enzyme or a metal-containing protein, is reactively attached to the exposed areas of the monolayer.

Methods of performing the invention will now be described with reference to the accompanying diagrammatic drawings, in which:

Figures 1 and 2 are semi-diagrammatic transverse cross-sectional views of microdevice substrates according to the invention;

Figure 3 is a semi-diagrammatic transverse cross-sectional view of a finished micropattern produced according to the invention;

Figures 4 and 4A combine to provide a flow diagram illustrating one embodiment of a method in accordance with the invention;

Figure 5 is a diagrammatic illustration of a stereo-specific protein monolayer employed in another method in accordance with the invention;

Figure 6 is a diagrammatic side elevational view of the manner in which a pattern-building molecule is attached to the monolayer of Figure 5;

Figure 7 is a diagrammatic view showing the manner in which the product of Figures 5 and 6 is attached to a substrate base;

Figure 8 is a semi-diagrammatic transverse cross-sectional view of a microsubstrate embodying the product of Figures 5-7; and

Figures 9 and 9A are views similar to Figure 8 but illustrating manners in which portions of the masking film of the microsubstrate can be selectively removed.

Simple microdevice substrates according to the invention comprise a substrate base, a protein layer comprising at least a compressed monolayer of a denatured non-fibrous protein overlying the surface of the substrate base and secured thereto, and a thin film of masking material covering the protein layer. The protein layer can be made up entirely of the protein monolayer, with the structure then consisting of the substrate base material 1, Figure 1, a thin inorganic insulating film 2 on the surface of the base, the protein monolayer 3, and the masking film 4. Alternatively, the protein layer can be made up of a compressed monolayer 13, Figure 2, of denatured non-fibrous protein and a second layer 13*a* of protein which can be adhered to the monolayer in such quantity as to provide a layer having a thickness of e.g. 1000 Angstrom units. In this embodiment, the masking film 14 is applied to the surface of protein layer 13*a*. Protein layer 13*a* can be of a single protein in undenatured form, or can be made up of a plurality of protein layers, or can be a single additional protein monolayer. While, for simplicity of illustration, the masking films 4 and 14 have been shown as discrete films overlying the protein layer, films 4 and 14 advantageously include material which impregnates the protein layer and is present

in all of the intramolecular and intermolecular interstices of the protein layer.

The substrate base 1 can be of any solid material which is different from the functional material or materials of which the micropattern is to be formed and has adequate strength and other physical properties to support the micropattern under the conditions of use. In the case of electronic microcircuits, the substrate base can be silicon, typically in the form of a wafer with a diameter of 1-2 in., the silicon being covered with an insulating layer 2 of silicon dioxide.

Monolayer 3 is a layer of denatured protein which is one molecule thick, the protein being a non-fibrous protein having a maximum molecular dimension not greater than 500 Angstrom units and a ratio of greatest molecular dimension to smallest molecular dimension of at least 2:1. The molecules of the denatured protein of the monolayer are further characterized by having a hydrophilic side and a hydrophobic side facing in generally opposite directions and separated by a distance which is small relative to the maximum length of the molecule. Such monolayers are prepared conventionally by flowing a molecular solution of the protein onto the surface of water in a Langmuir trough and laterally compressing the film under the influence of a glass bar moved laterally across the water surface until a surface tension of 0.1-0.3 dyne/cm. is obtained as measured by, e.g. a Wilhelmy balance. The usual procedure is described by Kleinschmidt, A.K. and Zahn, R.K., *Ztschr. Naturforschg* 14b, 1959, commencing at page 770. Typically, the protein can be dissolved in an aqueous ammonium acetate solution at dilutions on the order of 10^{-4} gram of the protein per ml. While the usual practice is to lift the protein monolayer from the water-air interface with a grid, it is advantageous to lift the monolayer directly with the substrate base, so that the compressed monolayer is recovered and applied to the base in the same step, the monolayer then being dehydrated with ethanol. While many flobular proteins haivng the aforementioned characteristics can be employed, specific examples of suitable proteins include cytochrome c, chymotrypsin, bovine serum albumin, and trypsin.

The masking or resist film 4 can be of any masking material which responds to radiant energy in such fashion as to be significantly more easily removable after irradiation, so that irradiation of a predetermined area of the masking film renders the film selectively removable in the irradiated area. Particularly advantageous are those materials which have a marked response to sub-atomic particle radiation, including electron beam, proton beam and neutron beam radiation. The masking material can also be one which has a specific response to electromagnetic radiation, including X-ray, microwave, infra red, ultra violet and visible radiation. The masking material can also be one which is removable by an ion beam, typically by a focussed ion beam. It is particularly advantageous to employ as film 4 an *in situ* polymerized film of a synthetic polymeric material which is partially depolymerized by electron beam irradiation. Of the synthetic polymeric materials, those derived from the acrylic esters, especially methyl acrylate and methyl metacrylate, are particularly useful, those polymers being soluble in the respective monomers so that selective removal of the masking film from irradiated areas is easily achieved by dissolution in the monomer. Advantageously, film 4 is established by flowing methyl methacrylate, containing both a free-radical initator type polymerization catalyst and a cross-linking agent, onto the protein monolayer and accomplishing polymerization to the solid state *in situ* by time or time and moderate heat. The *in situ* polymerized masking film has a maximum thickness (measured from the surface of the protein monolayer) of from 10 Angstrom units to 1000 Angstrom units, best results being achieved when the thickness does not exceed 30 Angstrom u is The polymerized *in situ*, masking film 4 not only completely covers the protein monolayer but also fills the molecular interstices of the monolayer.

The following example is illustrative of preparation of microdevice substrates according to Figure 1:

Example 1

A solution of cytrochrome c is prepared by introducing the protein into a 1-molar aqueous solution of ammonium acetate to provide 10^{-4} gram of protein per ml. in molecular solution. The protein solution is flowed onto the surface of a 0.1 molar solution of ammonium acetate in a Langmuir trough at the rate of about 0.2 ml per minute, using a pipette, until 2.5 ml of the protein solution has been flowed on, the film then being allowed to spread freely on the surface for 10 minutes under covered, clean, quiescent conditions. The resulting protein monolayer is then compressed to 0.1 dynes/cm., using a square glass rod. At this point, it must be recognized that the protein of the monolayer is denatured as a result of the surface tension at the air-water interface and that each molecule of the protein of the monolayer is oriented with its hydrophilic side toward the water and its hydrophobic side toward the air. A silicon wafer covered by a film of silicon dioxide and of smaller plan extent than the compressed protein monolayer is now lowered, silicon dioxide surface down, slowly into engagement with the monolayer. The

monolayer adheres to the silicon dioxide surface and, when the wafer is withdrawn, the monolayer covers and is adhered to the silicon dioxide film on the wafer, with the predominently hydrophilic side of each protein molecule directed away from the wafer. The wafer is then immersed in an ethanol solution and ethanol added in sequential steps of a graded series, to dehydrate the protein monolayer.

Liquid methyl methacrylate is catalyzed by addition of benzoyl peroxide and divinyl benzene, as a cross-linking agent, in amounts equal to 1% and 5%, respectively, of the weight of the monomer. The methyl methacrylate is then flowed over the protein monolayer on the silicon wafer and the substrate then allowed to stand, protein layer up, at room temperature for 10 hours, yielding a finished substrate having the configuration shown in Figure 1, with the cross-linked polymethacrylate constituing film 4. In this substrate, the monolayer 3 of cytochrome c will have a thickness of 12-14 Angstrom units, and the polymethyl methacrylate film 4 can have a thickness, in addition to that of the monolayer, on the order of 10-1000 Angstrom units or thicker.

In native form, globulins such as cytochome c can be viewed as made up of helically arranged amino acid molecules, usually referred to as residues, interrupted by changes of direction at the junctures between amino acid molecules, by cross-linking at disulfide sites, and by the presence of metal ions linked internally in the folded structure, the globulin macromolecule being a relatively compact structure. The cytochome c of monolayer 3, however, has been denatured by the surface tension at the air-water interface, during formation of the monolayer, and no longer possesses the compact macromolecular form. Denaturing can be considered as a partial uncoiling of the helices of the macromolecule to such an extent that the denatured macromolecule is in the form of a relatively flat structure with hydrophilic sites exposed at one side and hydrophobic sites exposed at the other. With the silicon wafer lowered into engagement with the monolayer, the hydrophobic sites of the protein are exposed to the silicon dioxide surface of the wafer and the protein monolayer is secured to the silicon dioxide by surface adhesion.

Denaturation of the cytochrome c of the monolayer has the effect of making the protein molecule less compact, so that the denatured macromolecule is characterized by intramolecular interstices of considerable size and number. When the liquid methyl methacrylate is applied to establish film 4, the liquid enters and fills the intramolecular interstices so that the polymethyl methacrylate of the cured electron resist film 4 is integral with the polymethyl methacrylate within the denatured protein monolayer.

Microdevice substrates according to Figure 2 can be made in a fashion similar to that of Example 1, save that a solution of the protein for layer 13*a* is flowed onto the exposed surface of the monolayer 13, glutaraldehyde is then added to the protein solution as a cross-linking agent, and the device allowed to stand for a few minutes, yielding layer 13*a* as a solid layer of glutaraldehyde-protein complex secured to monolayer 13 by cross-linking between amino groups of the two proteins. The electron resist film 14 is then applied as explained in Example 1.

DETAILED DESCRIPTION OF MICRODEVICE OF FIGURE 3 AND ITS PRODUCTION ACCORDING TO METHOD EMBODIMENT OF FIGURES 4 AND 4A

In this embodiment of the invention, the finished article comprises a micropattern 25, Figure 3, of conductive metal supported by the substrate base, the surface of those portions of the substrate base not overlain by micropattern 25 being exposed. For simplicity of explanation, micropattern 25 is considered to be a metal element having a width of approximately 100 Angstrom units and extending in a straight line from the doped, semiconductor area 26 of silicon wafer 21 across the silicon dioxide film 22, the metal element constituting a conductive lead to the semiconductor area. As illustrated by the flow diagram of Figures 4 and 4A, the method is commenced by preparing a substrate base according to Example 1, consisting of a silicon wafer carrying a silicon dioxide film, a compressed monolayer of denatured cytochrome c overlying the silicon dioxide film, and an electron resist film of polymethyl methacrylate overlying the cytochrome c monolayer. As the second step, the substrate base is irradiated with a focussed electron beam, as by using a computer controlled scanning electron microscope, over the area 26 to be doped to form the active semiconductor area. Such irradiation can be at a dosage level of from 10^{-7} to 10^{-5} coulomb per square centimeter at 10,000 EV. The substrate base is then washed with methyl methacrylate monomer, as the third step, to dissolve all of the polymethyl methacrylate layer which has been irradiated, thus exposing the protein monolayer in the area 26. For the fourth step, the exposed protein monolayer is then removed, either by dissolving the protein with an aqueous solution having a pH equal to the isoelectric point of the cytochrome c or by digesting the protein with an aqueous solution of a proteolytic enzyme, to expose the silicon dioxide film in the area of the wafer which is to be doped. In the fifth

step, the exposed silicon dioxide is removed, as by etching with hydrofluoric acid. The sixth step of the method is carried out by irradiating the substrate overall, as with X-ray irradiation or electron beam irradiation, removing the remaining polymethyl methacrylate as in step 3, and then removing the remaining protein monolayer, as in step 4, so that the substrate now presents the exposed predetermined area of the silicon wafer, with the remainder of the wafer covered by silicon dioxide as a negative mask. In step 7, the dopant desired for the active semiconductor area 26 is introduced in conventional fashion with a diffusion furnace and, if plural dopants are involved, the doping step is conventionally repeated. Using a diffisuion furnace, additional silicon dioxide film 22 is built up, so that the overall silicon dioxide film 22 is thickened and a thin film of silicon dioxide also now extends over the doped area 26.

The eighth step of the method requires reestablishment of the composite resist according to Example 1, including the compressed denatured cytochrome c monolayer and the overlying film of cross-linked polymethyl methacrylate as the electron resist. In step 9, a predetermined area smaller than the doped area and located therewithin is irradiated with a focussed electron beam, and the irradiated composite resist removed as in steps 3 and 4, the area of silicon dioxide thus exposed then being removed as in step 5. The entire substrate is then irradiated for step 10, as with X-ray radiation or electron beam radiation, and the composite resist then removed overall as in step 6, so that the surface of the substrate now appears as a predetermined exposed area of the silicon wafer with the remainder covered by silicon dioxide as a negative mask. Step 11 consists of vapor depositing aluminium, by conventional techniques, over the entire substrate. The method is then continued in step 12 by applying a new composite resist, again including the compressed denatured cytochome c monolayer and polymethyl methacrylate resist film, in accordance with Example 1. Step 13 then consists of irradiating the entire substrate except for the area to be occupied by aluminium in the final product, this irradiation being carried out by use of a focussed electron beam irradiation in known fashion. In step 14, the areas of the composite resist thus irradiated are removed, as in steps 3 and 4, leaving composite resist as a positive mask overlying the area to be occupied by aluminum in the finished article. The undesired metal is then removed by chemical etching, as step 15, and the remaining composite resist removed as in steps 3 and 4 to yield the product seen in Figure 3.

Though the case of a straight conductive lead has been described for simplicity, it will be understood that micropatterns of usual complexity can be produced in the same fashion. And, while a simple metal deposition in the positive area has been described, it will be understood that, using conventional procedures, other applications of a functional material or materials can replace simple metal deposition. Such other applications include e.g., deposition of metal oxide films or alloy films as resistive circuit elements, the introduction of dopants, and the application of dielectric materials.

The article illustrated in Figure 1 can be considered as a microsubstrate comprising a base substrate supporting a composite electron resist, the composite resist being made up of the polymeric masking film 4 and the protein monolayer 3, and it is significant that the two components of the composite are removable by different procedures each specific to a different one of the two Components. The method described with reference to Figures 4 and 4A demonstrates that such a microsubstrate can be used to produce a micropattern in a manner generally similar to currently employed single electron resist procedures. An advantage of the article and method embodiments so far described is an increase in electron beam writing speed, arising from the fact that the electron resist film 4, being part of a composite resist, is substantially thinner than the usual polymeric resists, as well as an improvement in edge definition. In this regard, it is to be noted that the polymethyl methacrylate of electron resist layer 4, Figure 1, also extends within the interstices of the protein of monolayer. When an electron beam is focussed onto the substrate, the electron dosage required to render the corresponding area of film 4 easily removable depends upon the thickness of that layer. However, the polymethyl methacrylate present in the interstices of the protein monolayer is disperse, as the interstices are disperse, and a much smaller electron dosage is expended within the monolayer, yet the irradiated polymethyl methacrylate in the interstices of the protein is readily removable by dissolution. Removal of the protein itself in the irradiated area does not depend upon irradiation, and a smaller dosage is therefore permissible than if, for example, the total thickness of films 3 and 4 were made up of solid polymethyl methacrylate. Further, the presence of the polymethyl methacrylate in the interstices of the protein monolayer, and the fact that the aqueous liquid used to dissolve the protein after removal of the polymethyl methacrylate does not dissolve polymethyl methacrylate, tend strongly to minimize undercutting, and loss of edge definition, during dissolution of the exposed protein.

The microdevice substrate of Figure 2 is

employed when it is desirable that the invention be compatible with conventional techniques and procedures. Using the additional protein layer 13*a*, the composite resist comprising the protein monolayer 13, the additional protein layer, and the masking film 14 can have a total thickness which approximates that of the thinner prior-art resists. Yet the embodiment of Figure 2 provides distinct improvements in writing speed and edge definition in the manner earlier described with reference to Figures 1 and 3-4A.

EMBODIMENTS BASED ON STEREOCHEMICALLY SPECIFIC SUBSTRATES

Another advantage of the invention lies in the fact that the substrate can be made stereochemically specific, with molecular components of the substrate arranged in a spatial configuration which is predetermined precisely and upon which the desired micropattern can be based.

Employing a compressed monolayer of denatured cytochrome c as the protein monolayer, the fact that the cytochrome c molecules are spiral-like in plan, relatively thin or flattened in edge elevation, and have diametrically opposite positive and negative sites makes it possible to align the cytochrome c molucules into the plan array shown diagrammatically in Figure 5, with the molecules 20 aligned in rows. This can be accomplished by subjecting the monolayer, while it is being formed, to the action of a strong, high voltage, low amperage, direct current electric field. Essentially identical and of the same size, the denatured cytochrome c molecules 20 each have an antigenic site, indicated for simplicity at 21 in the central area of the molecule. In the array established by the influence of the electric field, the location of each antigenic site 21 is known with some precision, so that it is practical to attach another component of the substrate at points determined by the location of the antigenic sites.

Typically, the component to be so attached can be the antigen-binding fraction of gamma globulin, recovered by electrophoresis or column chromatography, from an ionic aqueous solution of gamma globulin which has been treated with the enzyme papain to cleave the gamma globulin molecules into the Fab (antigen-binding) and F_c (crystalline) fragments. To accomplish attachment of one F_{ab} fragment of gamma globulin to each antigenic site 21 presented by the cytochrome c monolayer, an aqueous medium containing the F_{ab} fraction recovered from the papain treatment is simply flowed into the aqueous liquid upon which the cytochrome c monolayer has been formed. In this connection, it will be understood that the antigenic sites 21 appear on the hydrophilic sides of the cytochrome c molecules and are therefore immediately available for reaction with the F_{ab} fragments in the aqueous liquid, and the reaction proceeds immediately upon introduction of the F_{ab} fragments, with attachment fo the F_{ab} fragments occurring as indicated diagramtically in Figure 6. At this stage, the cytochrome c monolayer, with an F_{ab} fragment of gamma globulin attached at each antigenic site 21, is secured to a silicon wafer or other substrate base 22 by lowering the base 22 downwardly onto the monolayer and lifting the monolayer from the liquid, the hydrophobic side of the monolayer adhering to the base. Inverted, the resulting assembly is as shown in Figure 7.

The microdevice substrate is then completed by applying a non-proteinaceous resist film 23, Figure 8, of a thickness to completely cover the F_{ab} fractions. Film 23 is of a resist material with a marked response to electron irradiation. Typically, the film is of cross-linked polymethyl methacrylate, produced as hereinbefore described.

Since the cytochrome c molecules 20 are arranged in a predetermined grid-like array, and the antigenic sites 21 occupy positions of known location on the molecules, the F_{ab} fragments are disposed in a grid-like array predetermined by the cytochrome c monolayer. Accordingly, reactive ends of the F_{ab} fragments can be selectively exposed by subjecting the resist film 23 to irradiation by focussed electron beam only in the immediate locations of one, or a predetermined group, of the F_{ab} fragments and then removing the irradiated resist material, as by dissolving in methyl methacrylate monomer when the film is of polymethyl methacrylate. This step yields the structure shown in Figure 9, if the irradiated areas 24 are registered precisely with individual F_{ab} fragments, or the structure shown in Figure 9A, when the irradiated areas 24*a* are registered with a predetermined group of the F_{ab} fragments.

The now exposed ends of the F_{ab} fragments constitute antigenic sites, making it possible to attach to the substrate at each of those sites any additional component capable of reacting with an antigenic site. Suitable additional componnets include the proteins and peptides, either unaltered or with other elements, compounds or complexes added thereto by prereaction.

A typical example of a component that can be added directly at the antigenic sites presented at the exposed ends of the F_{ab} fragments is ferritin, and the addition can be accomplished from an aqueous solution containing m-xylylene diisocyanate or toluene 2,4 -diisocyanate as a cross-linking agent, following the procedures of Singer, S.J. and Schick, A.F. (1961), *J. biophys. biochem*

Cytol., Vol. 9 page 519. Particularly useful components to be added at the exposed antigenic sites of the F_{ab} fragments are enzymes, especially peroxidase and phosphatase, which provide a convenient route to deposition of metal on the substrate in the micropattern specifically defined by the exposed antigenic sites. Thus, for example, phosphatase can be reacted with an organic phosphate, such as creatinine phosphate or adenosine triphosphate, to yield creatinine plus the phosphate radical or adenosine diphosphate plus the phosphate radical, the liberated phosphate then being reacted with e.g., lead nitrate to yield insoluble lead phosphate. The lead phosphate can then be reduced by heat to yield metallic lead which as the protein is destroyed thermally, deposits on the substrate at the site occupied by the phosphatase. Since the spaces between the exposed antigenic sites of the F_{ab} fragments are of the order determined by the molecular dimensions of the cytochrome c, a group of the exposed antigenic sites will predetermine a continuous precipitate of lead in the procedure just described, assuming that a corresponding amount of the organic phosphate is employed. Thus, if a plurality of adjacent rows of the exposed antigenic sites are treated as just described, the resulting lead deposit can constitute a straight conductor portion of finite width and length determined by the rows so treated.

In order to selectively expose the reactive sites of the stereospecific monolayer, the repetitive molecular pattern can be detected by monitoring the back-scattered image of the molecules, using an electron beam in the scanning electron microscope mode. The beam spot size can be selected from a range from smaller than the protein molecule of the monolayer, e.g., one fourth the maximum dimension of the molecule, to a diameter large enough to expose a significant number of the molecules. The beam intensity, diameter and position can then be predetermined to optimally expose the desired number and locations of sites for the pattern being written.

Instead of employing the sequence of steps just described in order to arrive at a micropattern defined by, e.g., phosphatase, such a pattern can be obtained by attaching an enzyme labelled macromolecule to the antigenic sites presented by the protein monolayer after the monolayer has been subjected to the electric field. Thus, a compressed monolayer of denatured cytochrome c can be established on the surface of a body of aqueous liquid as described in Example 1, and the molecules of cytochrome c brought into a grid-like plan array by subjecting the monolayer, during formation, to the action of a strong, high voltage, low amperage, direct current electric field. A dilute, e.g., 1% by weight, solution of anticytochrome c phosphatase labelled antibody is then introduced into the liquid below the monolayer, so that one molecule of the antibody is attached to each antigenic site presented by the cytochrome c monolayer. The monolayer is then lifted with a silicon wafer as described in Example 1 and the exposed surface of the monolayer then covered with a cross-linked polymethyl methacrylate masking film by *in situ* polymerization as described in Example 1. The resulting product is a microsubstrate presenting phosphatase molecules in the grid-like plan array determined by the cytochrome c monolayer, the phosphatase being covered by polymethyl methacrylate. The microsubstrate can thus be characterized as comprising a supported layer of protein in which the macromolecules have a known, stereospecific, spatial disposition, and also macromolecules of a pattern-building protein, the phosphatase labelled antibody, arranged in the pattern determined by the supported layer. Further patterning steps can now be carried out with the microsubstrate, again relating those steps to the grid-like plan array of the cytochrome c monolayer by monitoring the back-scattered image with the scanning electron microscope.

For example, the phosphatase can be deactivated in a selected negative area or areas by subjecting the area or areas of the microsubstrate to irradiation with a focussed electron beam at a heavy dosage level, e.g., 10^{-5} coulombs/cm. sq. at 10 KV. In addition to deactivation of the enzyme, such heavy dosage electron beam writing also partially depolymerizes the polymethyl methacrylate in the selected negative area or areas, rendering the same selectively removable by dissolution in the monomer. Lead can then be introduced at the phosphatase sites, as by reacting the phosphatase with creatinine phosphate to yield phosphate ions then reacting the liberated phosphate with lead nitrate, and thermally reducing the resulting lead phosphate to yield metallic lead.

WHAT WE CLAIM IS:-

1. A method of producing a micropattern on a substrate base, the method including the steps of:-

establishing on the surface of the base a protein layer comprising at least a compressed monolayer of denatured non-fibrous protein;

establishing on said protein layer a film of masking material;

directing radiant energy on said film in an area predetermined by the micropattern desired; and

removing said film from only said predetermined area to expose said protein layer for further treatment.

2. A method according to claim 1, wherein the micropattern consists of an elec-

trically conductive material, and the base consists of an electrically insulating material.

3. A method according to claim 2, including the further steps of:-

removing said protein layer from only said exposed predetermined area; and

applying the electrically conductive material to the area of the base thus exposed.

4. A method according to any of the preceding claims wherein said masking film is an *in situ* polymerized film of polymeric material which has the property of being significantly depolymerized by irradiation.

5. A method according to claim 4, wherein said masking film includes polymeric material which extends into the molecular interstices of said monolayer.

6. A method according to claim 3, including the further steps of:-

directing radiant energy on the remaining portion of said film; and

removing said film and said monolayer from said remaining portion.

7. A method according to any of the preceding claims wherein the protein of said monolayer is a protein having a maximum molecular dimension not greater than 500 Angstrom units and a ratio of greatest molecular dimension to smallest molecular dimension of at least 2:1, the molecules of said protein having a hydrophilic site spaced from a hydrophobic site by a distance which is small relative to the maximum dimension of the molecule.

8. A method according to claim 7, wherein the base is of an inorganic electrical insulating material; and wherein said monolayer is established on the base by first forming said monolayer at the air-liquid interface of a body of aqueous liquid and then lowering the support into physical contact with the compressed monolayer while the monolayer is at the air-liquid interface.

9. A method according to any of the preceding claims wherein the protein of said monolayer is cytochrome c.

10. A method according to any of claims 1 to 8, wherein the protein of said monolayer is chymotrypsin.

11. A method according to any of claims 1 to 8, wherein the protein of said monolayer is bovine serum albumin.

12. A method according to any of claims 1 to 8, wherein the protein of said monolayer is trypsin.

13. A method according to claim 5, wherein the portion of said masking film which does not extend into said monolayer has a maximum thickness of from 10 to 1000 Angstrom units.

14. A method according to claim 13, wherein said thickness does not exceed 30 Angstrom units.

15. A method according to any of the preceding claims, wherein each molecule of said protein layer has a specific reactive site, and wherein reactive molecules of an additional material other than the protein of said monolayer are attached to the reactive sites of respective molecules of said monolayer.

16. A method as claimed in claim 15, wherein the step of establishing said monolayer further includes the step of treating the monolayer, before contact with the support, to arrange the molecules of the monolayer in a predetermined pattern in order to predetermine the arrangement of said reactive molecules of additional material;

wherein the film of masking material covers not only said monolayer but also said reactive molecules of additional material; and wherein

the step of directing radiant energy is carried out by directing the radiant energy on said film only in a plurality of localized areas each occupied by at least one of said reactive molecules of additional material,

whereby the step of removing said film results in exposure of only the ones of said reactive molecules of additional material which occupy discrete locations related to a desired micropattern.

17. A method according to claim 16, wherein said step of establishing said protein layer on the base comprises forming said monolayer at the air-liquid interface of a body of liquid,

subjecting said monolayer to the action of an electric field to arrange the molecules of the monolayer in said predetermined pattern,

introducing said reactive molecules of additional material into said body of liquid and thereby causing said reactive molecules to attach at said reactive sites on the surface of said monolayer which faces the body of liquid, and

engaging the base with the surface of said monolayer which faces away from the body of liquid to attach the monolayer to the base.

18. A method according to claim 16 or claim 17 further comprising

flowing onto the reactive molecules of additional material in said exposed discrete locations in a liquid solution of a component capable of reacting with said reactive molecules of additional material.

19. A method according to claim 18, wherein said reactive molecules of additional material are of an antigen-binding protein.

20. A method according to claim 19, wherein said component capable of reacting with said reactive molecules of additional material is a protein or a peptide.

21. A method according to claim 20, wherein said component capable of reacting with said reactive molecules of additional material is a metal-containing protein.

22. A method according to claim 21, wherein said metal-containing protein is fer-

ritin.

23. A method of producing a micropattern on a substrate base, including the steps of:- establishing on the base a protein layer comprising at least a compressed monolayer of denatured non-fibrous protein in which the protein molecules each have a specific reactive site, the molecules of the monolayer being spatially arranged in a predetermined pattern and said specific reactive sites therefore occupying predetermined locations with reference to said pattern;

establishing over said monolayer a film of masking material;

selectively removing masking film in areas predetermined with reference to said pattern; and

reactively attaching to the resulting exposed portions of said protein layer pattern-building material to establish on the support the desired micropattern in configuration and location dependent upon the spatial arrangement of the molecules of said monolayer.

24. A method according to claim 23, wherein said pattern-building material comprises an enzyme.

25. A method according to claim 23, wherein said pattern-building material comprises at least one metal containing protein.

26. A method according to claim 25, wherein sadi metal-containing protein is ferritin.

27. A method according to claim 23, wherein said pattern-building material comprises a complex of protein with an elemental metal or a complex of protein with an inorganic compound of a metal.

28. A method according to any of Claims 23 to 27, wherein the molecules of the monolayer are arranged in a grid-like array.

29. A substrate base having a micropattern produced thereon by a method according to any of the preceding claims.

30. A substrate base according to claim 29, wherein said substrate base is of silicon and said surface thereof is of silicon dioxide.

31. A base according to claim 30, wherein said film of masking material is of an *in situ* polymerized polymeric material and includes a protion overlying said protein layer and a portion occupying the molecular interstices thereof.

32. A substrate base according to claim 31, wherein said film of masking material is adhered directly to said monolayer.

33. A substrate base according to claim 29, wherein said monolayer is adhered directly to said substrate base; and said protein layer comprises an additional layer of protein overlying said monolayer.

34. A substrate base according to claim 33, wherein said film of masking material comprises a portion which overlies said additional layer of protein and a portion which occupies the intramolecular and intermolecular interstices of said protein layer.

35. A substrate base according to claim 33, wherein said additional layer of protein is thicker than said monolayer.

36. A method of producing a micropattern on a substrate base substantially as hereinbefore described with reference to, and as illustrated in, the accompanying diagrammatic drawings.

37. A substrate base having a micropattern produced thereon by a method substantially as hereinbefore described with reference to, and as illustrated in, the accompanying diagrammatic drawings.

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1572510 COMPLETE SPECIFICATION
4 SHEETS This drawing is a reproduction of the Original on a reduced scale
Sheet 1

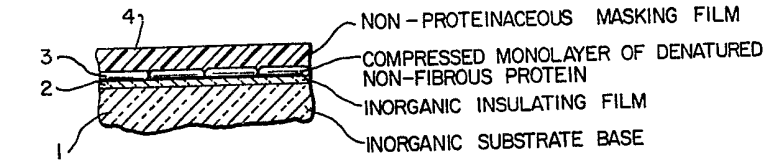


FIG. 1

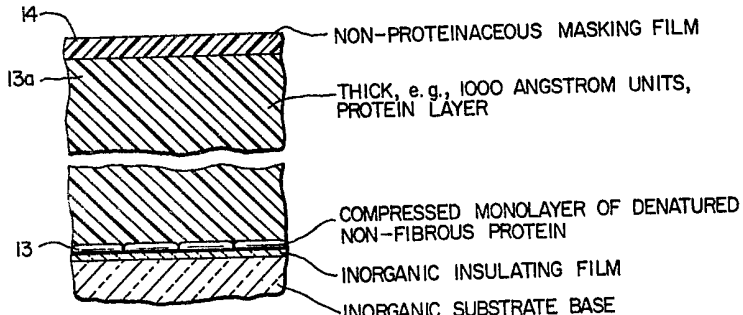


FIG. 2

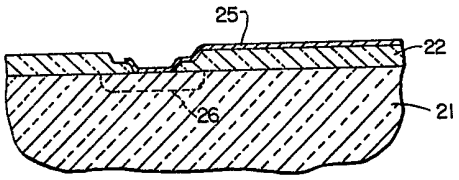


FIG. 3

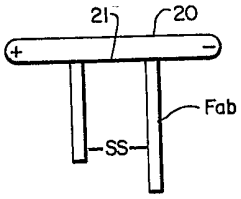


FIG. 6

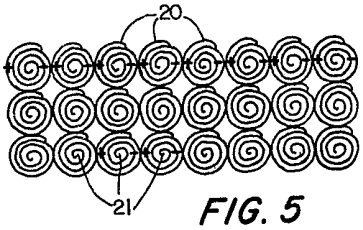
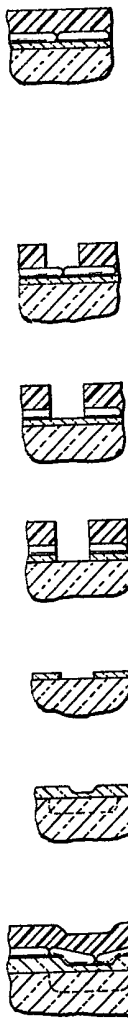


FIG. 5



STEP 1.
PROVIDE SUSTRATE AS IN FIG. 1.

STEP 2.
IRRADIATE WITH AN ELECTRON BEAM A SPECIFIC AREA OF THE RESIST FILM ACCORDING TO DESIRED MICROPATTERN.

STEP 3.
REMOVE IRRADIATED RESIST FILM, THUS EXPOSING PROTEIN MONOLAYER IN IRRADIATED AREAS.

STEP 4.
REMOVE EXPOSED PROTEIN MONOLAYER, EXPOSING SILICON DIOXIDE IN THE IRRADIATED AREA AND LEAVING PROTEIN MONOLAYER AND RESIST FILM AS A NEGATIVE MASK IN THE AREA NOT IRRADIATED.

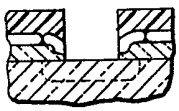
STEP 5.
REMOVE EXPOSED SILICON DIOXIDE, THUS EXPOSING SILICON IN IRRADIATED AREA

STEP 6.
IRRADIATE OVERALL AND REMOVE REMAINING COMPOSITE RESIST, LEAVING SILICON DIOXIDE AS A NEGATIVE MASK.

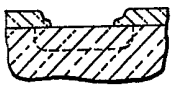
STEP 7.
INTRODUCE DOPANT INTO SILICON OF EXPOSED AREA, USING DIFFUSION FURNACE, ADDITIONAL SILICON DIOXIDE BEING BUILT UP BY THERMAL OXIDATION, INCLUDING A FILM OF SILICON DIOXIDE OVER DOPED AREA.

STEP 8.
APPLY NEW COMPOSITE RESIST OVER ENTIRE SUBSTRATE AS IN FIG. 1

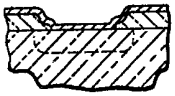
FIG. 4



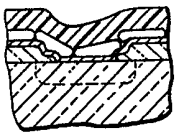
STEP 9.
IRRADIATE PREDETERMINED AREA WITHIN DOPED AREA AND REMOVE COMPOSITE RESIST AND NEW SILICON DIOXIDE FROM IRRADIATED AREA.



STEP 10.
IRRADIATE OVERALL AND REMOVE REMAINING COMPOSITE RESIST.

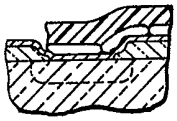


STEP 11.
DEPOSITE CONDUCTIVE METAL OVER ENTIRE SUBSTRATE.

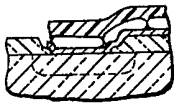


STEP 12.
APPLY NEW COMPOSITE RESIST.

STEP 13.
IRRADIATE IN AREA WHERE METAL IS NOT DESIRED.



STEP 14.
REMOVE IRRADIATED COMPOSITE RESIST, THUS EXPOSING UNDESIRED METAL, THE REMAINING COMPOSITE RESIST CONSTITUTING A POSITIVE MASK.



STEP 15.
REMOVE UNDESIRED METAL BY CHEMICAL ETCHING.

STEP 16.
IRRADIATE OVERALL AND THEN REMOVE REMAINING COMPOSITE RESIST TO OBTAIN ARTICLE OF FIG. 3

FIG. 4A

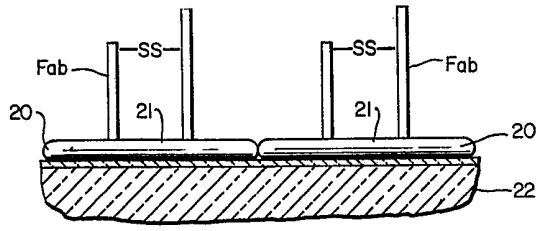


FIG. 7

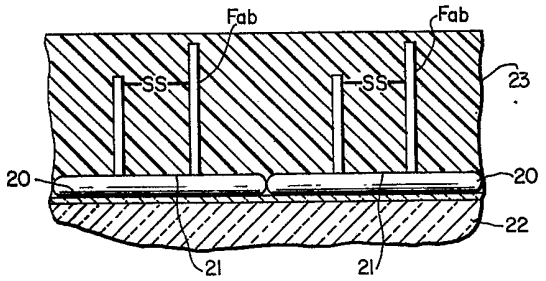


FIG. 8

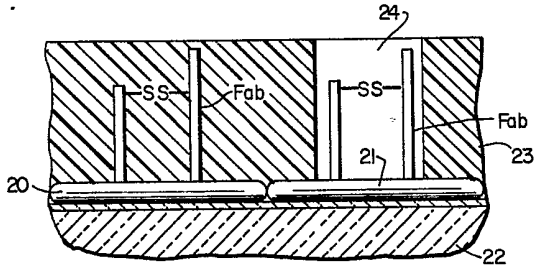


FIG. 9

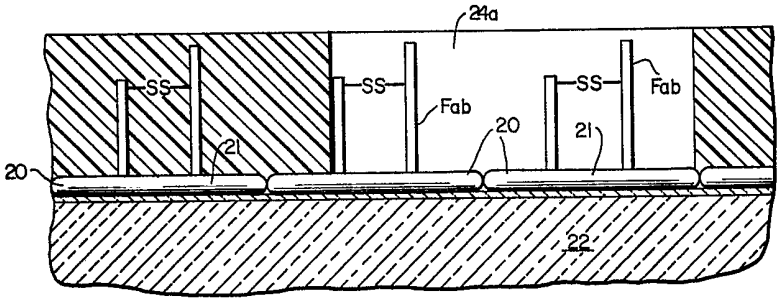


FIG. 9A