

**HIGHLY REFLECTIVE BIOGRATINGS**

This application is a continuation of application Ser. No. 07/963,249, filed Oct. 19, 1992, now abandoned which was a continuation of Ser. No. 07/525,828, filed May 17, 1990 also now abandoned.

**FIELD OF THE INVENTION**

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

**1. Background of the Invention**

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.

**2. Description of the Prior Art**

Reflective and transmissive biograting immunoassay systems and methods were disclosed in U.S. Pat. No. 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 reagent. The zones form a diffraction grating when the active binding reagent 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. Pat. No. 4,876,208 describes transmissive and reflective diffraction binding assays and biograting systems of the type described in U.S. Pat. No. 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.

**SUMMARY OF THE INVENTION**

This invention is directed to improved reflective diffraction biogratings suitable for use in the apparatus and methods of U.S. Pat. Nos. 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 reagent on the first surface, and a reflective metal layer having a thickness of at least about 1000 Å. The reflective layer can be supported on an optically flat surface of a 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 thin 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 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 reagent 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 reagent to form a diffraction grating pattern of alternating zones of active and deactivated binding reagent by exposing the surface to a deactivating amount of UV light through a transparent mask having a diffraction grating pattern of opaque zones thereon.