US007175980B2

(12) **United States Patent**
Qiu et al.

(10) **Patent No.: US 7,175,980 B2**
(45) **Date of Patent: Feb. 13, 2007**

(54) **METHOD OF MAKING A PLASTIC COLORIMETRIC RESONANT BIOSENSOR DEVICE WITH LIQUID HANDLING CAPABILITIES**

(75) Inventors: **Jean Qiu**, Andover, MA (US); **Peter Li**, Andover, MA (US); **Brian Cunningham**, Lexington, MA (US)

(73) Assignee: **SRU Biosystems, Inc.**, Woburn, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1009 days.

(21) Appl. No.: **10/201,878**

(22) Filed: **Jul. 23, 2002**

(65) **Prior Publication Data**

US 2003/0026891 A1 Feb. 6, 2003

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/196,058, filed on Jul. 15, 2002, and a continuation-in-part of application No. 10/180,647, filed on Jun. 26, 2002, and a continuation-in-part of application No. 10/180,374, filed on Jun. 26, 2002, which is a continuation-in-part of application No. 10/059,060, filed on Jan. 28, 2002, and a continuation-in-part of application No. 10/058,626, filed on Jan. 28, 2002, and a continuation-in-part of application No. 09/930,352, filed on Aug. 15, 2001.

(60) Provisional application No. 60/303,028, filed on Jul. 3, 2001, provisional application No. 60/283,314, filed on Apr. 12, 2001, provisional application No. 60/244,312, filed on Oct. 30, 2000.

(51) **Int. Cl.**
C12Q 1/00 (2006.01)

(52) **U.S. Cl.** **435/4**; 156/60; 156/288

(58) **Field of Classification Search** .... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,689,346	A	9/1972	Rowland	156/245
3,810,688	A	5/1974	Ballman et al.	350/96
3,856,404	A	12/1974	Hershler et al.	
3,856,604	A	12/1974	Hershler et al.	156/361
4,009,933	A	3/1977	Firester	350/152
4,050,895	A	9/1977	Hardy et al.	436/527
4,240,751	A	12/1980	Linnecke et al.	356/409

(Continued)

FOREIGN PATENT DOCUMENTS

CA 2394966 2/2001

(Continued)

OTHER PUBLICATIONS

Liu, et al., "Development of an optical fiber lactate sensor", Mikrochimica Acta, 1999, 131(1-2), pp. 129-135.

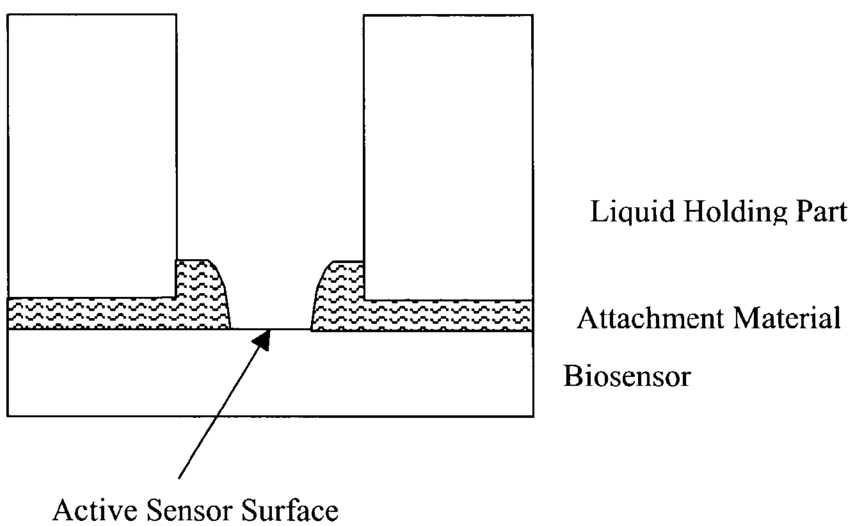
(Continued)

Primary Examiner—Christopher L. Chin
(74) *Attorney, Agent, or Firm*—McDonnell Boehnen Hulbert & Berghoff LLP

(57) **ABSTRACT**

Methods of producing liquid handling biosensor devices are provided. The liquid handling biosensor devices allow detection of biomolecular interactions in liquid. The use of labels is not required and the methods can be performed in a high-throughput manner.

3 Claims, 21 Drawing Sheets



U.S. PATENT DOCUMENTS

4,289,371	A	9/1981	Kramer	350/3.71
4,344,438	A	8/1982	Schultz	128/633
4,420,502	A	12/1983	Conley	427/54.1
4,536,608	A	8/1985	Sheng et al.	136/259
4,560,248	A	12/1985	Cramp et al.	385/12
4,576,850	A	3/1986	Martens	428/156
4,608,344	A	8/1986	Carter et al.	436/34
4,650,329	A	3/1987	Barrett et al.	356/481
4,652,290	A	3/1987	Cho et al.	65/31
4,668,558	A	5/1987	Barber	428/156
4,701,008	A	10/1987	Richard et al.	385/132
4,810,658	A	3/1989	Shanks et al.	436/172
4,815,843	A	3/1989	Tiefenthaler et al.	356/128
4,818,710	A	4/1989	Sutherland et al.	436/527
4,857,273	A	8/1989	Stewart et al.	436/82
RE33,064	E	9/1989	Carter	436/34
4,876,208	A	10/1989	Gustafson et al.	
4,882,288	A	11/1989	North et al.	436/525
4,888,260	A	12/1989	Cowen	430/1
4,931,384	A	6/1990	Layton et al.	435/7
4,952,056	A	8/1990	Tiefenthaler	356/73.1
4,958,895	A	9/1990	Wells et al.	350/96.12
4,992,385	A	2/1991	Godfrey	436/525
4,999,234	A	3/1991	Cowan	428/156
5,071,248	A	12/1991	Tiefenthaler et al.	356/128
5,118,608	A	6/1992	Layton et al.	435/7.1
5,155,785	A	10/1992	Holland et al.	385/89
5,156,785	A	10/1992	Zdrahala	264/108
5,170,448	A	12/1992	Ackley et al.	385/31
5,175,030	A	12/1992	Lu et al.	428/30
5,210,404	A	5/1993	Cush et al.	250/216
5,229,614	A	7/1993	Andersson et al.	250/370.12
5,242,828	A	9/1993	Bergström et al.	435/291
5,268,782	A	12/1993	Wenz et al.	359/81
5,310,686	A	5/1994	Sawyers et al.	
5,337,183	A	8/1994	Rosenblatt	359/248
5,413,884	A	5/1995	Koch et al.	430/5
5,442,169	A	8/1995	Kunz	250/227.21
5,455,178	A	10/1995	Fattinger	436/164
5,475,780	A	12/1995	Mizrahi	385/37
5,478,527	A	12/1995	Gustafson et al.	422/82
5,478,756	A	12/1995	Gizeli et al.	436/527
5,492,840	A	2/1996	Malmqvist et al.	
5,496,701	A	3/1996	Pollard-Knight	435/7.4
5,559,338	A	9/1996	Elliott et al.	250/492.1
5,598,267	A	1/1997	Sambles et al.	356/369
5,598,300	A	1/1997	Magnusson et al.	359/566
5,606,170	A	2/1997	Saaski et al.	250/458.1
5,615,052	A	3/1997	Doggett	359/811
5,629,214	A	5/1997	Crosby	436/518
5,631,171	A	5/1997	Sandstrom et al.	436/518
5,690,894	A	11/1997	Pinkel et al.	422/68.1
5,691,846	A	11/1997	Benson, Jr. et al.	359/530
5,732,173	A	3/1998	Bylander et al.	385/49
5,738,825	A	4/1998	Rudigier et al.	422/82
5,768,461	A	6/1998	Svetkoff et al.	385/116
5,771,328	A	6/1998	Wortman et al.	385/146
5,792,411	A	8/1998	Morris et al.	264/400
5,801,390	A	9/1998	Shiraishi	250/559.3
5,804,453	A	9/1998	Chen	436/518
5,814,516	A	9/1998	Vo-Dinh	435/287.2
5,814,524	A	9/1998	Walt et al.	436/514
5,821,343	A	10/1998	Keogh	
5,846,843	A	12/1998	Simon	436/527
5,864,641	A	1/1999	Murphy et al.	385/12
5,922,550	A	7/1999	Everhart et al.	435/7.21
5,925,878	A	7/1999	Challener	250/225
5,955,335	A	9/1999	Thust et al.	
5,955,378	A	9/1999	Challener	436/525
5,986,762	A	11/1999	Challener	356/375
5,991,480	A	11/1999	Kunz et al.	385/37
5,994,150	A	11/1999	Challener et al.	436/518
5,998,298	A	12/1999	Fleming et al.	438/692
6,035,089	A	3/2000	Grann et al.	385/129
6,042,998	A	3/2000	Brueck et al.	430/316
6,052,213	A	4/2000	Burt et al.	359/237
6,076,248	A	6/2000	Hoopman et al.	29/527.1
6,088,505	A	7/2000	Hobbs	385/147
6,100,991	A	8/2000	Challener	356/445
6,128,431	A	10/2000	Siminovitch	385/147
6,146,593	A	11/2000	Pinkel et al.	422/68.1
6,174,677	B1	1/2001	Vo-Dinh	435/6
6,185,019	B1	2/2001	Hobbs et al.	359/30
6,200,737	B1	3/2001	Walt et al.	430/320
6,215,928	B1	4/2001	Friesem et al.	385/37
6,218,194	B1	4/2001	Lyndin et al.	
6,235,488	B1	5/2001	Tom-Moy et al.	
6,303,179	B1	10/2001	Koulik et al.	
6,316,153	B1	11/2001	Goodman et al.	430/8
6,320,991	B1	11/2001	Challener et al.	356/445
RE37,473	E	12/2001	Challener	250/225
6,338,968	B1	1/2002	Hefti	436/518
6,340,598	B1	1/2002	Herron et al.	436/518
6,346,376	B1	2/2002	Sigrist et al.	435/5
6,377,721	B1	4/2002	Walt et al.	385/12
6,404,554	B1	6/2002	Lee et al.	359/576
6,449,097	B1	9/2002	Zhu et al.	359/576
6,558,957	B1	5/2003	Roinestad et al.	436/164
6,570,657	B1	5/2003	Hoppe et al.	356/445
6,579,673	B2	6/2003	McGrath et al.	435/5
6,587,276	B2	7/2003	Daniell	359/622
6,661,952	B2	12/2003	Simpson et al.	385/37
6,707,561	B1	3/2004	Budach et al.	356/521
6,748,138	B2	6/2004	Wang et al.	385/37
6,902,703	B2	6/2005	Marquiss et al.	
2002/0018610	A1	2/2002	Challener et al.	385/12
2002/0123050	A1	9/2002	Poponin	
2002/0127565	A1	9/2002	Cunningham et al.	
2002/0135752	A1	9/2002	Sokolov et al.	
2002/0168295	A1	11/2002	Cunningham et al.	
2002/0171045	A1	11/2002	Perraut	
2003/0003599	A1	1/2003	Wagner et al.	
2003/0017580	A1	1/2003	Cunningham et al.	
2003/0017581	A1	1/2003	Li et al.	
2003/0026891	A1	2/2003	Qiu et al.	
2003/0027327	A1	2/2003	Cunningham et al.	
2003/0027328	A1	2/2003	Cunningham et al.	
2003/0032039	A1	2/2003	Cunningham et al.	
2003/0059855	A1	3/2003	Cunningham et al.	
2003/0068657	A1	4/2003	Lin et al.	
2003/0077660	A1	4/2003	Pien et al.	
2003/0092075	A1	5/2003	Pepper	
2003/0113766	A1	6/2003	Pepper et al.	
2003/0148542	A1	8/2003	Pawlak	
2003/0210396	A1	11/2003	Hobbs et al.	356/416
2004/0011965	A1	1/2004	Hodgkinson	
2004/0132172	A1	7/2004	Cunningham et al.	
2004/0132214	A1	7/2004	Lin et al.	
2004/0151626	A1	8/2004	Cunningham et al.	

FOREIGN PATENT DOCUMENTS

CA	2394966		8/2001
CA	2395318		8/2001
CH	669050	A5	2/1989
CH	670521	A5	6/1989
EP	0112721		7/1987
EP	0326219		1/1989
EP	0326291		8/1989
EP	0517777		12/1992
EP	0660924		7/1995
FR	2801977		12/1999
GB	2156970	A	10/1985
GB	2227089		7/1990

WO	8100912	2/1981
WO	8100912	4/1981
WO	0075353	3/1983
WO	8402578	7/1984
WO	8607149	12/1986
WO	9008318	7/1990
WO	9113339	9/1991
WO	9204653	3/1992
WO	9221768	12/1992
WO	9314392	7/1993
WO	9503538	2/1995
WO	9857200	12/1998
WO	9909392	2/1999
WO	9909396	2/1999
WO	9954714	10/1999
WO	9966330	12/1999
WO	0023793	4/2000
WO	0029830	5/2000
WO	0104697	1/2001
WO	WO 02/061429	8/2002

OTHER PUBLICATIONS

Coming Inc. v. *SRU Biosystems, Inc.*, Memorandum Opinion dated Nov. 15, 2005 in the United States District Court for the district of Delaware.

Haidner, "*Zero-Order Gratings Used as an Artificial Distributed Index Medium*", Optik, Wissenschaftlich Verlag GmbH, Stuttgart, DE, vol. 89, No. 3, pp. 107-112, 1992.

Peng, et al., "*Experimental Demonstration of Resonant Anomalies in Diffraction from two-Dimensional Gratings*", Optics Letters, Optical Society of America, vol. 21, No. 9, pp. 549-551, 1996.

Wilson, et al., "*The Optical Properties 1-19 of 'Moth Eye' Antireflection Surfaces*", Optical ACTA, vol. 29, No. 7, pp. 993-1009, 1982.

Bagnich, et al., "*Tunable Optical Filter*", SU 1465855, Derwent Publications, English Translation, Abstract Only, Derwent Publications Ltd.

Magnusson, et al., "*New Principal for Optical Filters*", Appl. Phys. Letters, 61(9) p. 1022-1024 (1992).

International Search Report for foreign counterpart application PCT/US01/50723, Sep. 17, 2002.

Nellen, et al., "Integrated Optical Input Grating Couplers as Biochemical Sensors", *Sensors and Actuators*, 15 (1988) 285-295.

W. Lukosz and K. Tiefenthaler, "Embossing technique for fabricating integrated optical components in hard inorganic waveguiding materials," *Optics Letters*, vol. 8, pp. 537-539 (1983).

K. Tiefenthaler and W. Lukosz, "Integrated optical switches and gas sensors," *Optics Letters*, vol. 10, pp. 137-139 (1984).

Chabay, "Optical Waveguides," *Analytical Chemistry*, vol. 54, pp. 1071A-1080A (1982).

Sutherland et al., "Optical Detection of Antibody-Antigen Reactions at a Glass-Liquid Interface," *Clin. Chem.*, vol. 30, pp. 1533-1538 (1984).

Ronald T. Holm and Edward D. Palik, "Internal-reflection spectroscopy," *Laser Focus*, vol. 15, pp. 60-65 (Aug. 1979).

N.J. Harrick and George I. Loeb, "Multiple Internal Reflection Fluorescence Spectrometry," *Analytical Chemistry*, vol. 45, pp. 687-691 (1973).

P.K. Tien, "Light Waves in Thin Films and Integrated Optics," *Applied Optics*, vol. 10, pp. 2395-2413 (1971).

Dakss, et. al., "Grating Coupler for Efficient Excitation of Optical Guided Waves in Thin Films," *Applied Physics Letters*, vol. 16, pp. 523-525 (1970).

Sutherland et al., "Immunoassays at a Quartz-Liquid Interface: Theory, Instrumentation and Preliminary Application to the Fluorescent Immunoassay of Human Immunoglobulin G," *Journal of Immunological Methods*, vol. 74, pp. 253-265 (1984).

English translation of CH 670 521 A5.

English translation of CH 669 050 A5.

Neuschafer et al., Evanescent resonator chips: a universal platform with superior sensitivity for fluorescence-based microarrays. Biosensors & Bioelectronics, 18 (2003) 489-497.

Budach et al., Generation of transducers for fluorescence-based microarrays with enhanced sensitivity and their application for gene expression profiling. Analytical Chemistry. Jun. 1, 2003; 75(11):2571-7.

Anderson, et al., "Proteomics: applications in basic and applied biology", *Current Opinion in Biotechnology*, 2000, 11:408-412.

MacBeath, et al., "Printing Proteins as Microarrays for High-Throughput Function Determination", *Science*, vol. 289, pp. 1760-1763, 2000.

deWildt, et a., "Antibody arrays for high-throughput screening of antibody-antigen interactions", *Nature Biotechnology*, vol. 18, pp. 989-994, 2000.

Cunningham, et al., "A plastic colorimetric resonant optical biosensor for multiparallel detection of label-free biochemical interactions", *Sensors and Actuators B*, 85 (2002) 219-226.

Caruso, et al., "Quartz Crystal Microbalance Study of DNA Immobilization and Hybridization for Nucleic Acid Sensor Development", *Analytical Chemistry*, vol. 69, No. 11, pp. 2043-2049, 1997.

Hefti, et al, "Sensitive detection method of dielectric dispersions in aqueous-based, surface-bound macromolecular structures using microwave spectroscopy", *Applied Physics Letters*, vol. 75, No. 12, pp. 1802-1804, 1999.

Wu, et al., "Bioassay of prostate-specific antigen (PSA) using microcantilevers", *Nature Biotechnology*, vol. 19, pp. 856-860, 2001.

Wasserman, et al., "Structure and Reactivity of Alkylsiloxane Monolayers Formed by Reaction of Alkyltrichlorosilanes on Silicon Substrates", *Langmuir*, 5, 1074-1087, 1989.

Kallury, et al., "X-ray Photoelectron Spectroscopy of Silica Surfaces Treated with Polyfunctional Silanes", *Anal. Chem.*, 60, 169-172, 1988.

Cunningham, et al., "Colorimetric resonant reflection as a direct biochemical assay technique", *Sensors and Actuators B*, 81 (2002) 316-328.

Mullaney, et al., "Epitope Mapping of Neutralizing Botulinum Neurotoxin A Antibodies by Phage Display", *Infection and Immunity*, vol. 69, No. 10, pp. 6511-6514, 2001.

U.S. Appl. No. 09/929,957, filed Aug. 15, 2001.

U.S. Appl. No. 09/930,352, filed Aug. 15, 2001.

U.S. Appl. No. 10/415,037, filed Oct. 23, 2001.

U.S. Appl. No. 10/399,940, filed Oct. 23, 2001.

U.S. Appl. No. 10/059,060, filed Jan. 28, 2002.

U.S. Appl. No. 10/201,818, filed Jul. 23, 2002.

U.S. Appl. No. 10/237,641, filed Sep. 9, 2002.

U.S. Appl. No. 10/277,908, filed Aug. 26, 2002.

U.S. Appl. No. 10/233,730, filed Sep. 3, 2002.

U.S. Appl. No. 10/180,374, filed Jun. 26, 2002.

U.S. Appl. No. 10/180,647, filed Jun. 26, 2002.

U.S. Appl. No. 10/253,846, filed Sep. 25, 2002.

U.S. Appl. No. 10/196,058, filed Jul. 15, 2002.

U.S. Appl. No. 10/667,696, filed Sep. 22, 2003.

U.S. Appl. No. 10/058,626, filed Jan. 28, 2002.

Bertoni, et al., "Frequency-Selective Reflection and Transmission by a Periodic Dielectric Layer", *IEEE Transactions on Antennas and Propagation*, vol. 37, No. 1, pp. 78-83 (1989).

Brundrett, et al., "Normal-incidence guided-mode resonant grating filters: design and experimental demonstration", *Optics Letters*, vol. 23, No. 9, pp. 700-702 (1998).

Peng, "*Polarization-control Components and Narrow-band Filters Based on Subwavelength Grating Structures*" 1996.

Statement of Applicants dated Jun. 10, 2004.

Lenau, Torben; *Material, Silicon Nitride*, 1996, 97, 98.

Cerac, Technical publications: *Tantalum Oxide, Ta_2O_5 for Optical Coating*, 2000, Cerac, Inc.

Cunningham et al., *Sensors and Actuators* B 85; pp. 219-226 (2002).

Cunningham et al., *Sensors and Actuators* B 81; pp. 316-328 (2002).

Pandey, A. and Mann, M., *Nature*. 405(6788):837-46 (2000).

Patterson, S.D., *Current Opinions in Biotechnology*, 11(4):413-8 (2000).

International Search Report for foreign counterpart application PCT/US03/01175.

Cowan, "The Recording and Large Scale Replication of Crossed Holographic Grating Arrays using Multiple Beam Interferometry", SPIE, vol. 503, *Application, Theory, and Fabrication of Periodic Structures*, pp. 120-129 (1984).
Cowan, "Holographic honeycomb microlens", vol. 24, No. 5, *Optical Engineering*, pp. 796-802 (1985).
Cowan, et al., "The Recording and Replication of Holographic Micropatterns for the Ordering of Photographic Emulsion Grains in Film Systems", vol. 31, No. 3, *J. Imaging Sci.*, pp. 100-107 (1987).
Wang, et al., "Guided-mdoe Resonances in Planar Dielectric-Layer Diffraction Gratings", vol. 7, No. 8, *J. Opt. Soc. Am.*, pp. 1470-1474 (1990).
Cowan, "Aztec Surface-Relief Volume Diffractive Strucutre", vol. 7, No. 8, *J. Opt. Soc. Am.*, pp. 1529-1544 (1990).
Patel, et al., "Multiwavelength Tunable Liquid-Crystal Etalon Filter", vol. 3, No. 7, *IEEE Photonics Technology Letters*, pp. 643+-644 (1991).
Patel et al., "Electrically Tunable and Polarization Insensitive Fabry-Perot etalon with a Liquid-Crystal Film", vol. 58, No. 22, *American Institute of Physics*, pp. 2491-2493 (1991).
Magnusson, et al., "Direct Optical Immunosensing (Sensitivity and Selectivity)", *Sensor and Actuators B*, 6, pp. 122-126 (1992).
Wang, et al., "Theory and Applications of Guided-Mode Resonance Filters", vol. 32, No. 14, *Applied Optics*, pp. 2606-2613 (1993).
Wang, et al., "Design of Waveguide-Grating Filters with Symmerical Line Shapes and Low Sidebands", vol. 19, No. 12, *Optical Society of America*, pp. 919-921 (1994).
Jin, et al., "A Biosensor Concept Based on Imaging Ellipsometry for Visualization of Biomolecular Interactions", 232, *Analytical Biochemistry*, p. 69-72 (1995).
Brecht, et al., "Optical Probes and Transducers", vol. 10, *Biosensors & Bioelectronics*, pp. 923-936 (1995).
Magnusson, et al., "Transmission Bandpass Guided-Mode Resonance Filters", vol. 34, No. 35, *Applied Optics*, pp. 8106-8109 (1995).
Peng, et al., "Experimental Demonstration of Resonant Anomalies in Diffraction from Two-Dimensional Gratings", vol. 21, No. 8, *Optics Letters*, pp. 549-551 (1996).
Sigal, et al., "A Self-Assembled Monolayer for the Binding and Study of Histidine-Tagged Proteins by Surface Plasmon Resonance", vol. 68, No. 3, *Analytical Chemistry*, pp. 490-497 (1996).
Peng, et al., "Resonant Scattering from Two-Dimensional Gratings", vol. 13, No. 5, *J. Opt. Soc. Am. A.*, pp. 993-1005 (1996).
Jordan, et al., "Surface Plasmon Resonance Imaging Measurements of Electrostatic Biopolymer Adsorption onto Chemically Modified Gold Surfaces", vol. 69, No. 7, *Analytical Chemistry*, pp. 1449-1456 (1997).
Raguin, et al., "Structured Surfaces Mimic Coating Performance", *Laser Focus World*, pp. 113-117 (1997).
Lin, et al., "A Porous Silicon-Based Optical Interferometric Biosensor", vol. 278, *Science*, pp. 840-843 (1997).
Morhard, et al., Immobilization of Antibodies in Micropatterns for Cell Detection by Optical Diffraction, *Sensors and Actuators B 70*, pp. 232-242 (2000).
Jenison, et al., "Interference-Based Detection of Nucleic Acid Targets on Optically Coated Silicon", vol. 19, *Nature Biotechnology*, pp. 62-64 (2001).
Cunningham, et al., U.S. Provisional Patent Application, "*Resonant Reflection Microarray*", U.S. Appl. No. 60/244,312, filed Oct. 30, 2000.
Cunningham, et al., U.S. Provisional Patent Application, "*Resonant Reflection Microarray*", U.S. Appl. No. 60/283,314, filed Apr. 12, 2001.
Cunningham, et al., U.S. Provisional Patent Application, "*Resonant Reflection Microarray*", U.S. Appl. No. 60,303,028, filed Jul. 3, 2001.
Hobbs, et al., "Automated Interference Lithography Systems for Generation of Sub-Micron Feature Size Patterns", *SPIE*, vol. 3879, pp. 124-135, Sep. 1999.
Cunningham, "Introduction to Bioanalytical Sensors", *Techniques in Analytical Chemistry*, pp. 260-291.
Challener, et al., "A Multiplayer Grating-Based Evanescent Wave Sensing Technique", *Elsevier Science B.B.*, pp. 42-46 (2000).
Huber, et al., "Direct Optical Immunosensing (Sensitivity and Selectivity)", *Sensors and Actuators B*, 6, pp. 122-126 (1992).
Invitation to Pay Additional Fees in foreign counterpart application PCT/US01/50723.

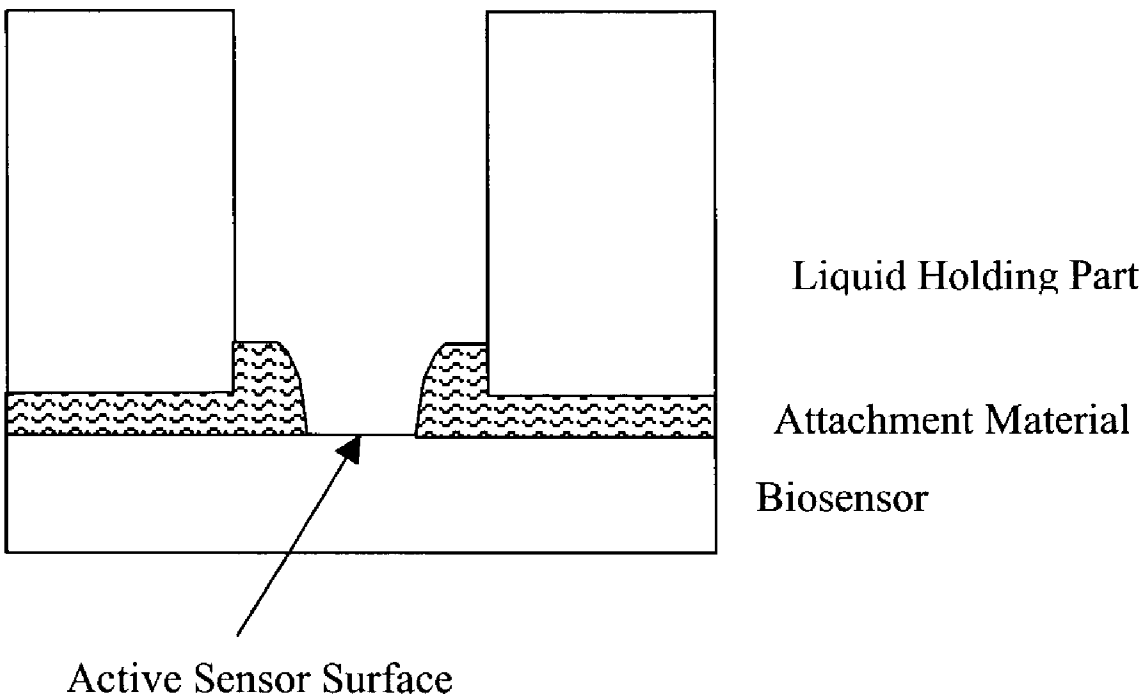

Liquid Holding Part
Attachment Material
Biosensor
Active Sensor Surface

Figure 1

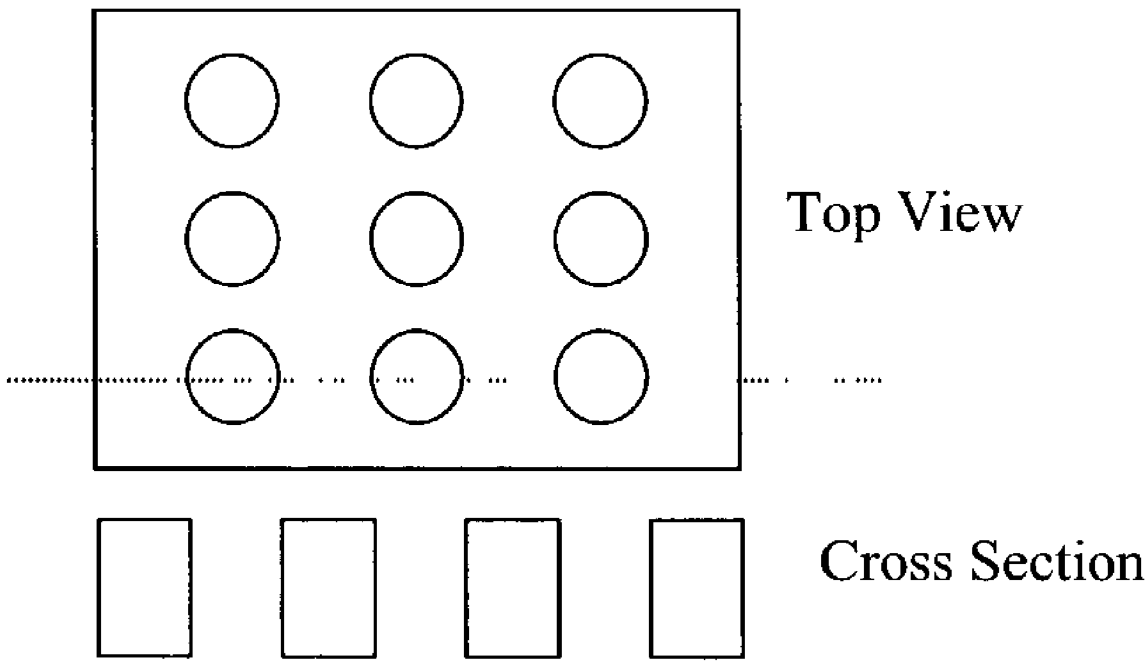


Figure 2

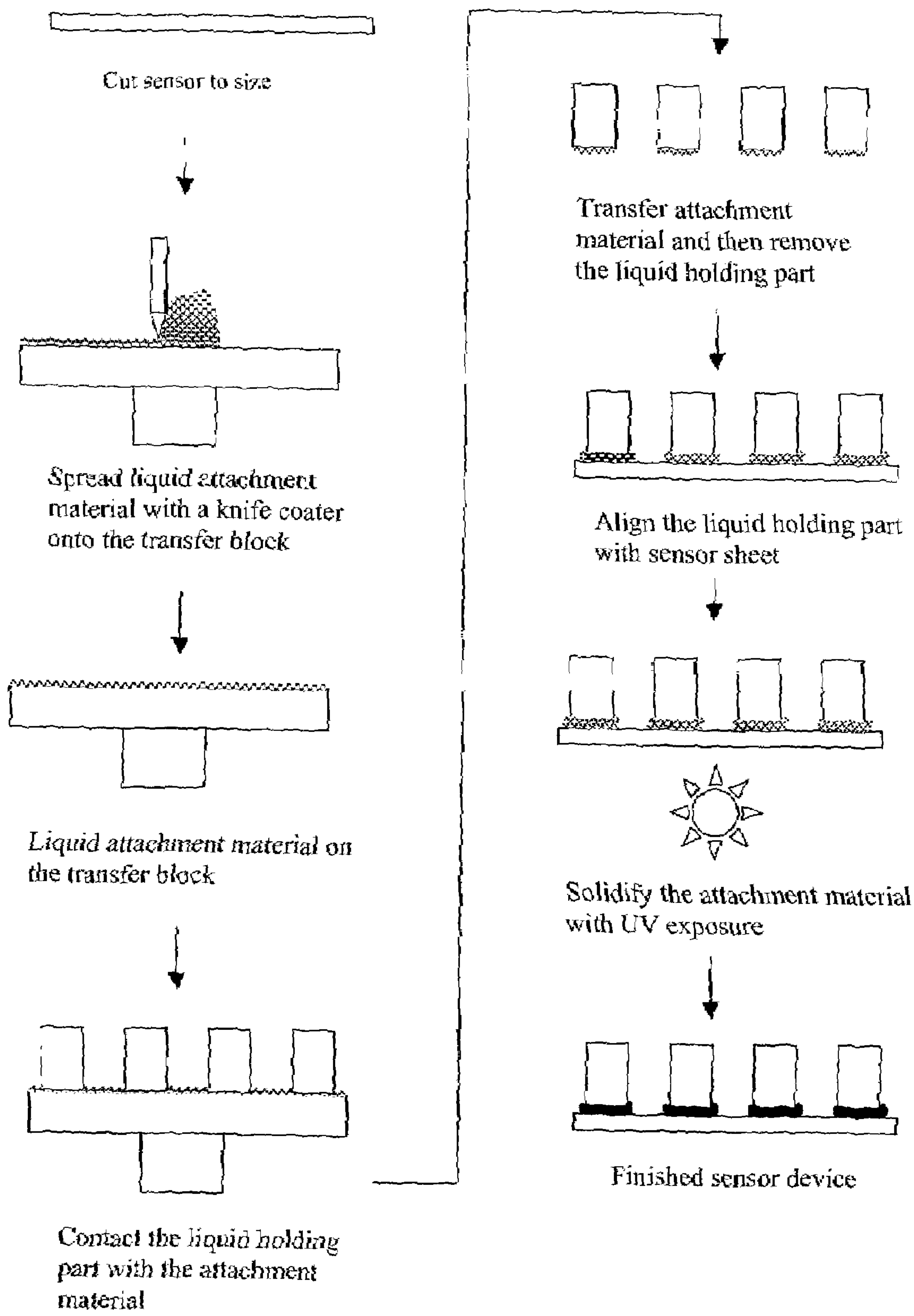

Cut sensor to size
Spread liquid attachment material with a knife coater onto the transfer block
Liquid attachment material on the transfer block
Contact the liquid holding part with the attachment material
Transfer attachment material and then remove the liquid holding part
Align the liquid holding part with sensor sheet
Solidify the attachment material with UV exposure
Finished sensor device

Figure 3

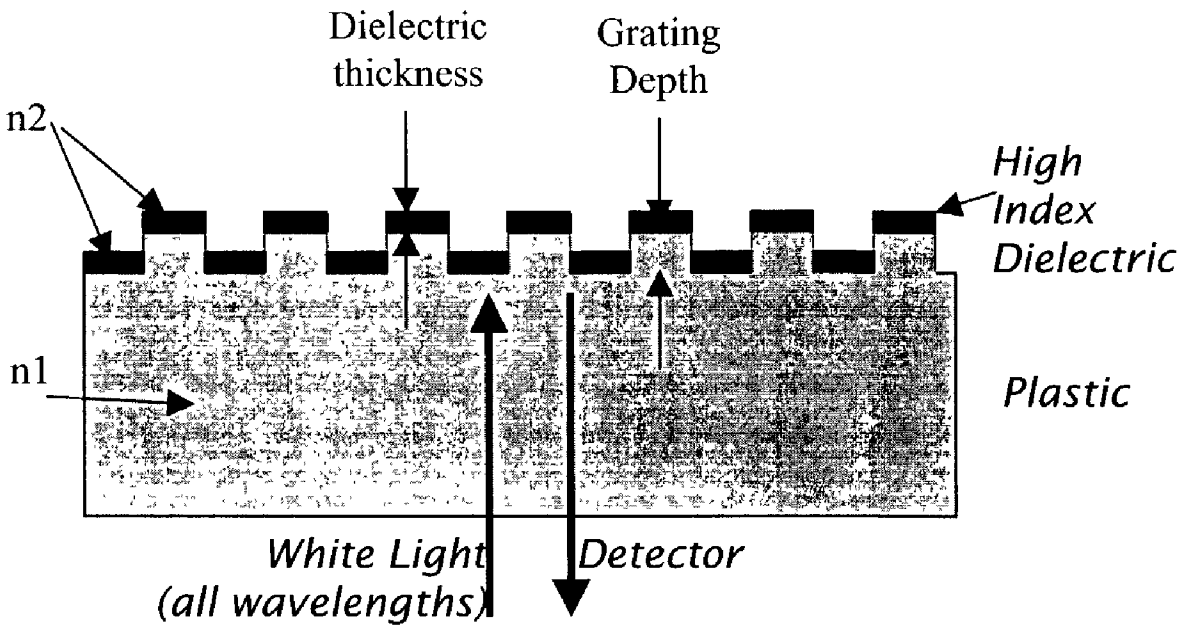

Dielectric
thickness
Grating
Depth
n2
High
Index
Dielectric
n1
Plastic
White Light
(all wavelengths)
Detector

Figure 4

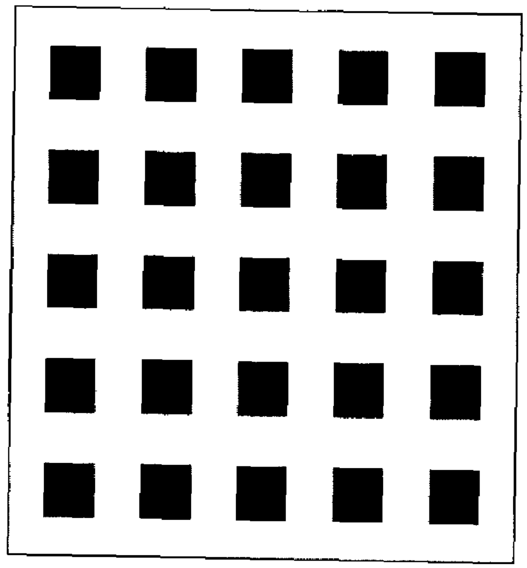


Figure 5A

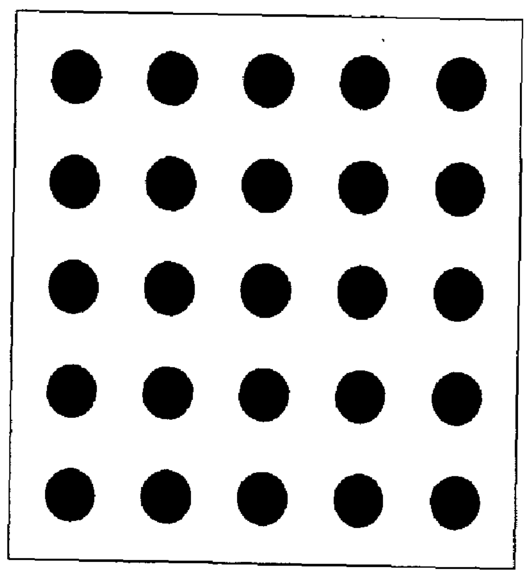


Figure 5B

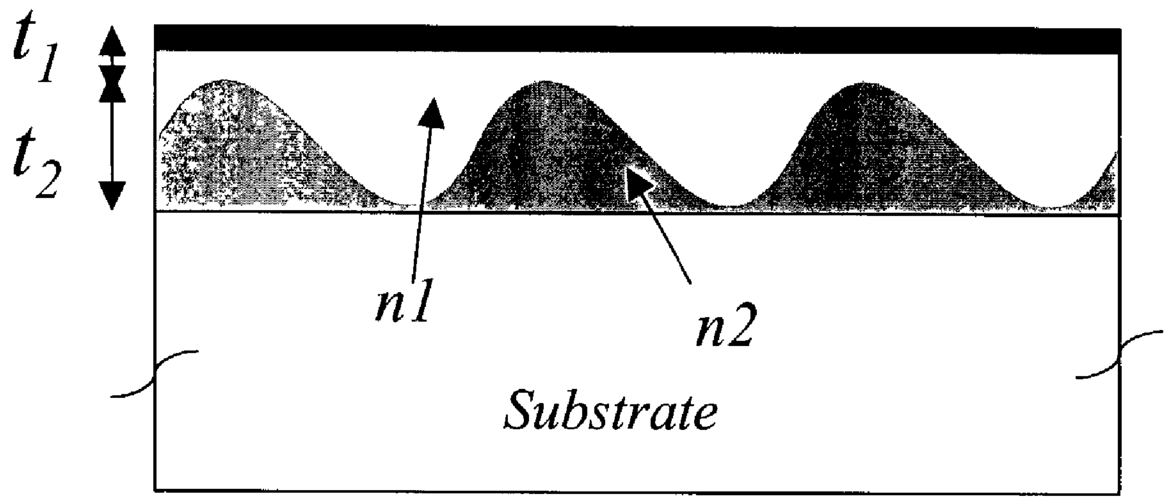


Figure 6

Amine

- ➢Sulfo-succinimidyl-6-(biotinamido)hexanoate (Sulfo-NHS-LC-Biotin)
  •Streptavidin / avidin then biotinylated molecule
- ➢N,N'-disuccinimidyl carbonate (DSC); • $-NH_2$, non-cleavable
- ➢Dimethyl pimelimidate (DMP); • $-NH_2$, non-cleavable
- ➢Dimethyl 3,3'-dithiobispropionimidate (DTBP); • $-NH_2$, cleavable
- ➢1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide Hydrochloride (EDC) and N-Hydroxysulfosuccinimide (Sulfo-NHS); • -COOH
- ➢Sulfo-succinimidyl 6-[a-methyl-a-(2-pyridyl-dithio)toluamido] hexanoate (Sulfo-LC-SMPT); • -SH, cleavable
- ➢N-(B-Maleimidopropyloxy)succinimide ester (BMPS)
  • $-SH_2$, non-cleavable
- ➢Sulfo-succinimidyl 4-[N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC); • -SH, non-cleavable

Aldehyde

- ➢ Directly with aldehyde or first amino then aldehyde
  • $-NH_2$

Ni(II)

- ➢Using Nitrilotriacetic acid (NTA) group, which forms a chelate with Ni(II)
  • His-tagged molecules

Figure 7

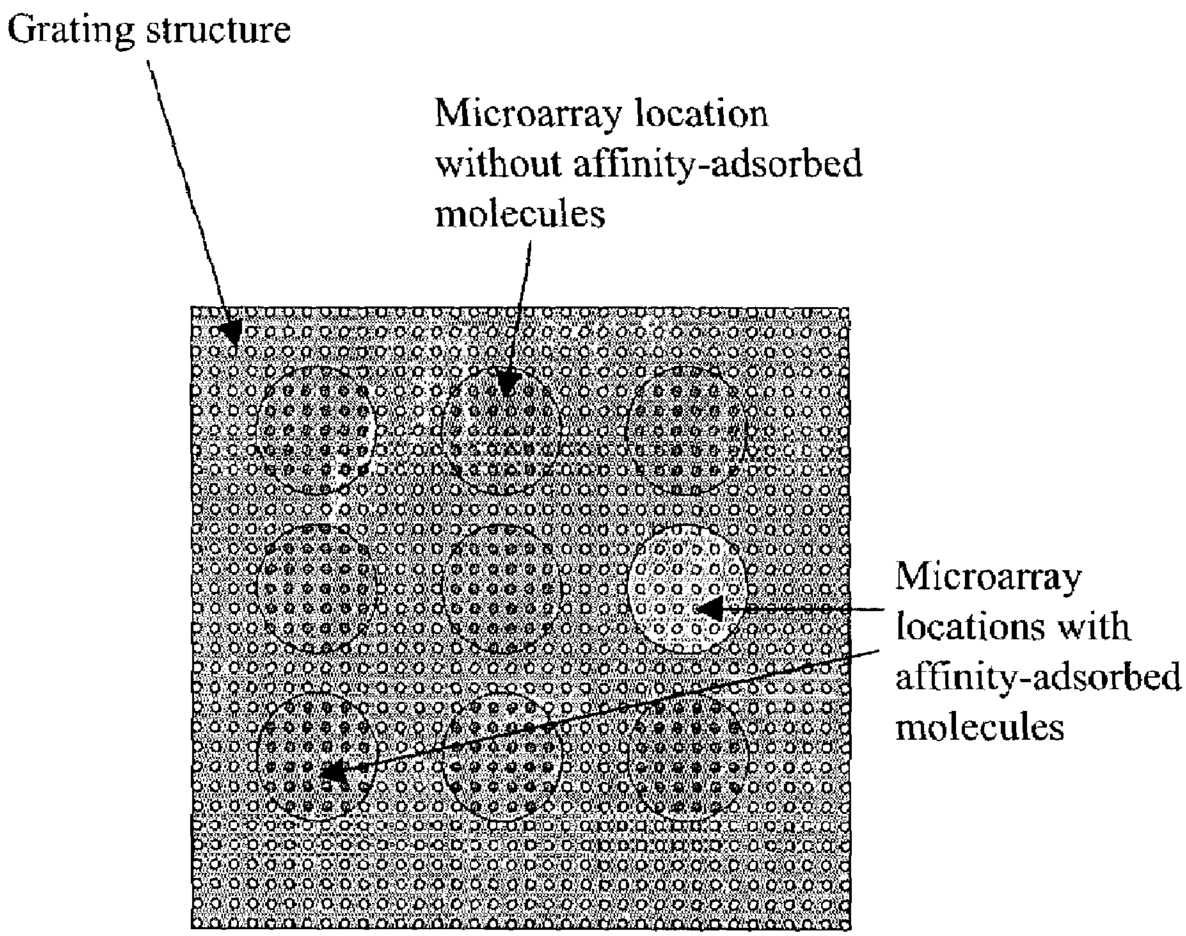

Grating structure
Microarray location without affinity-adsorbed molecules
Microarray locations with affinity-adsorbed molecules

Figure 8

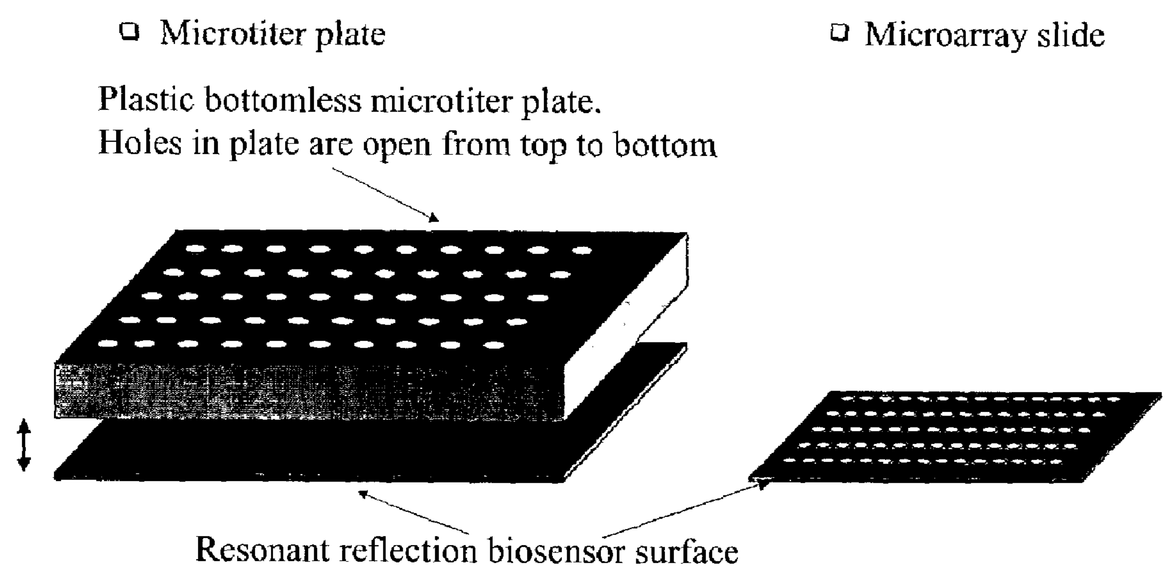


Figure 9A

Figure 9B

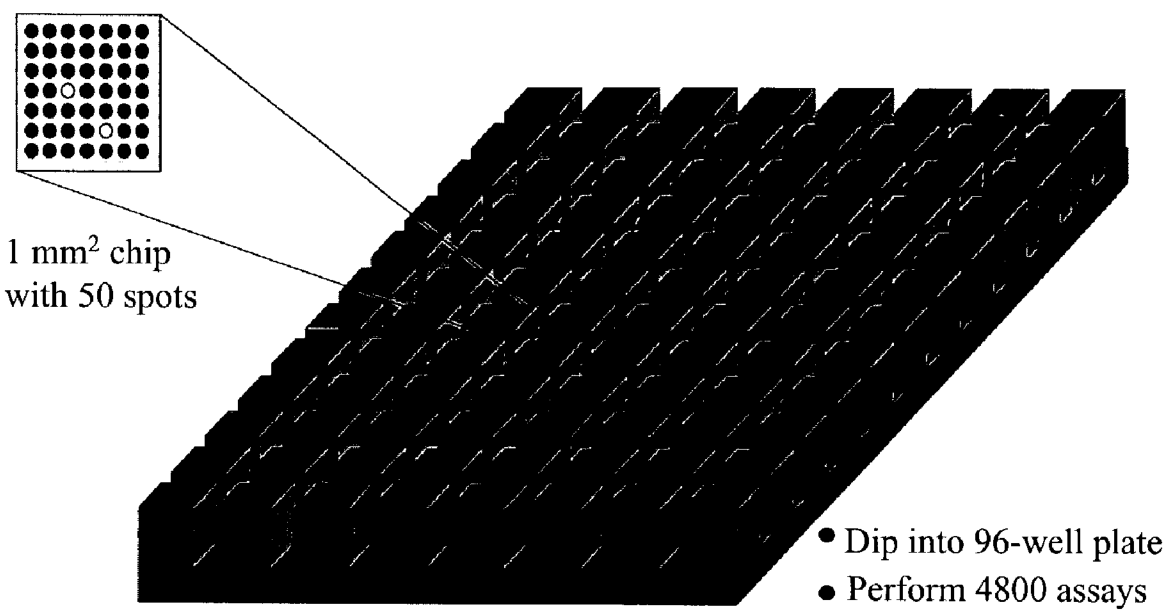

1 mm² chip
with 50 spots
• Dip into 96-well plate
• Perform 4800 assays

Figure 10

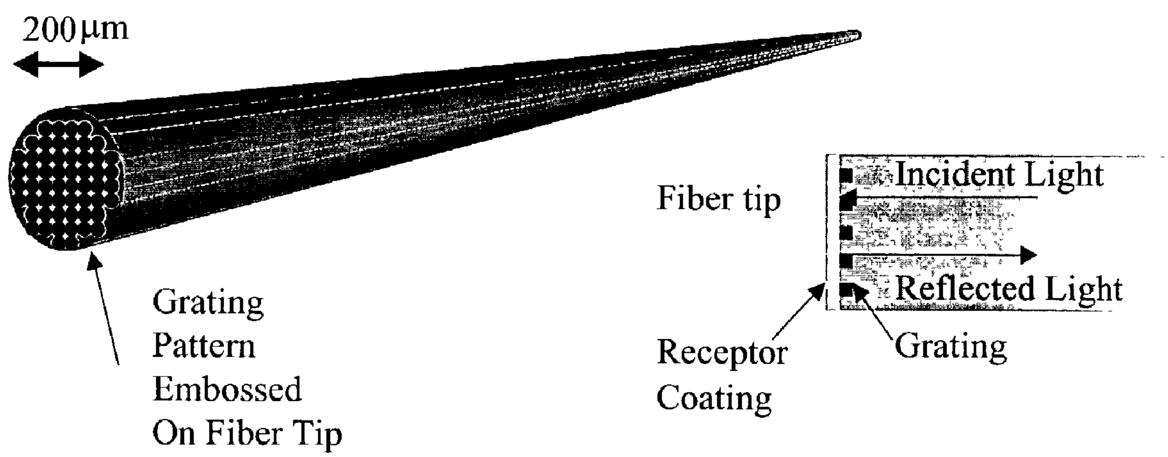

200µm
Grating
Pattern
Embossed
On Fiber Tip
Fiber tip
Incident Light
Reflected Light
Receptor
Coating
Grating

Figure 11

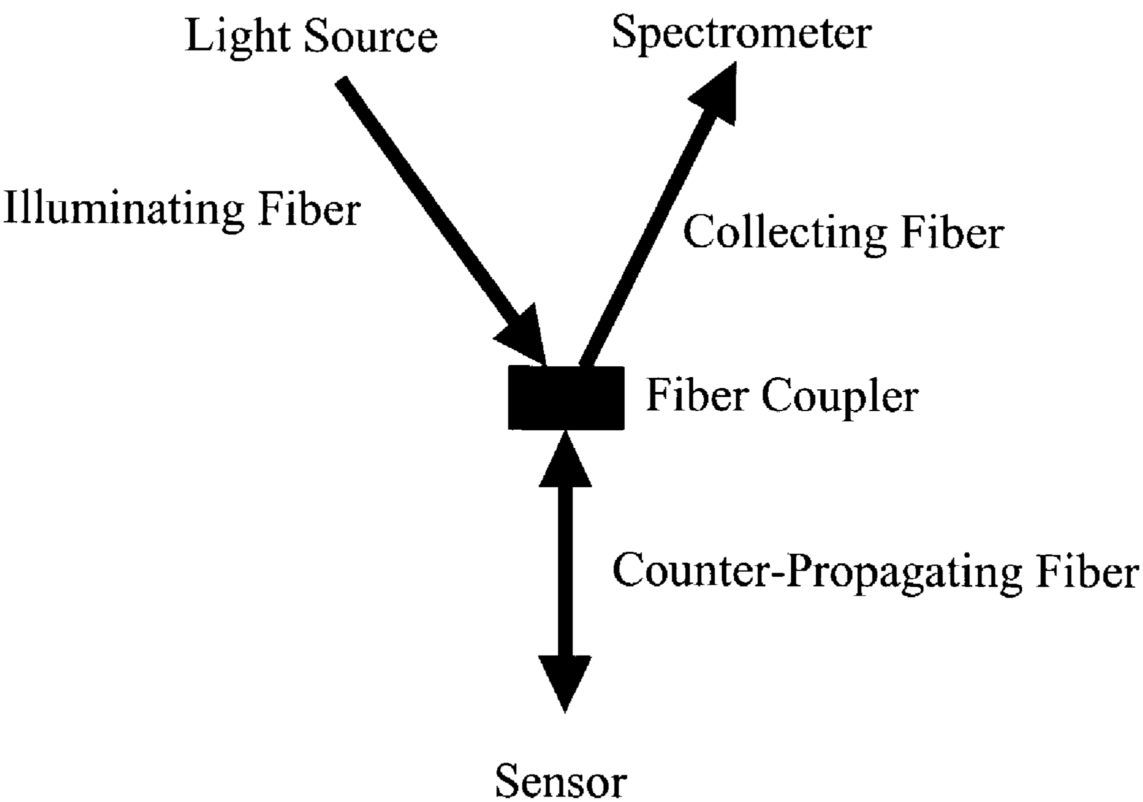

Light Source
Spectrometer
Illuminating Fiber
Collecting Fiber
Fiber Coupler
Counter-Propagating Fiber
Sensor

Figure 12

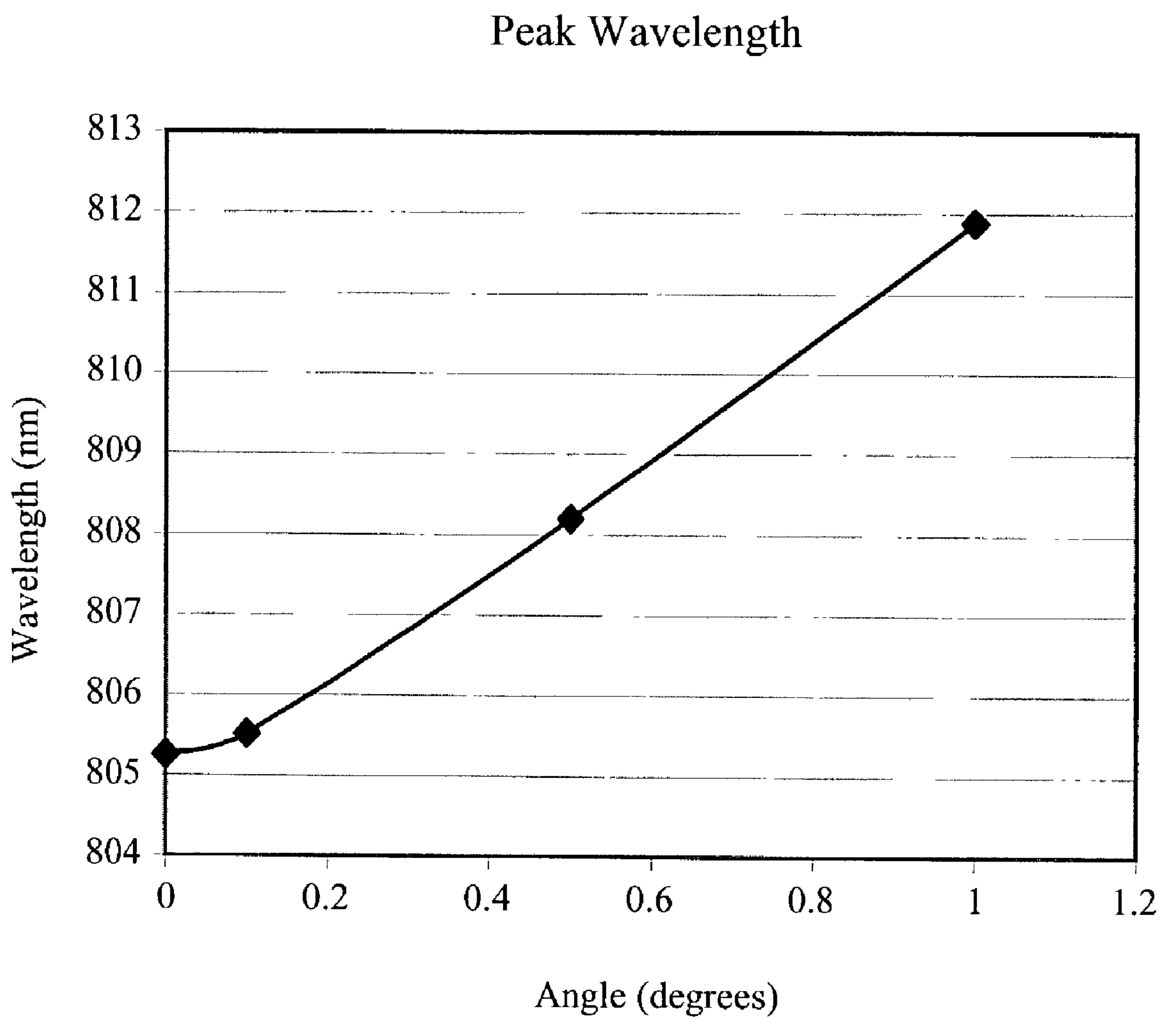

Peak Wavelength
813
812
811
810
809
808
807
806
805
804
Wavelength (nm)
0
0.2
0.4
0.6
0.8
1
1.2
Angle (degrees)

Figure 13

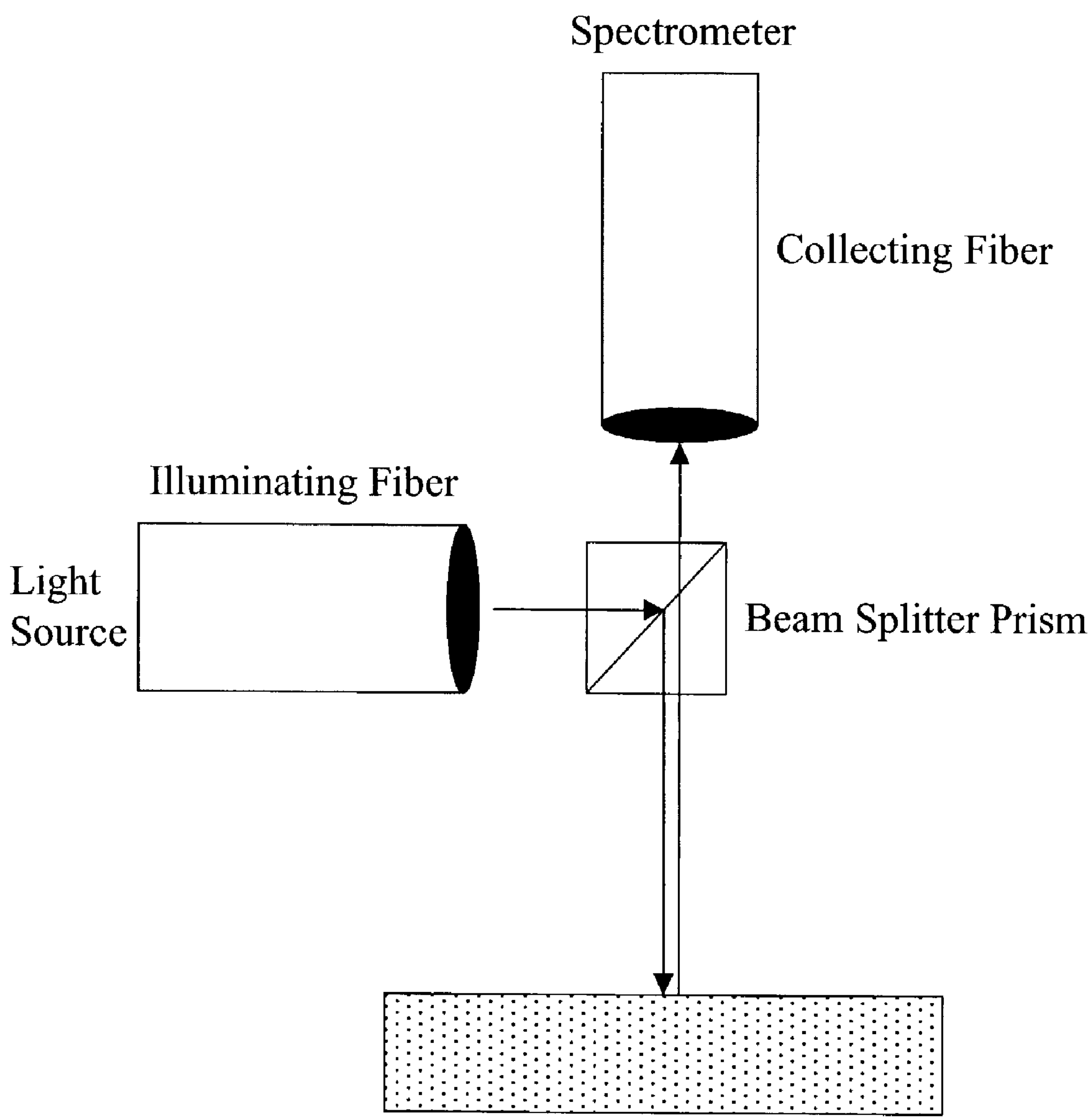

Spectrometer
Collecting Fiber
Illuminating Fiber
Light
Source
Beam Splitter Prism

Figure 14

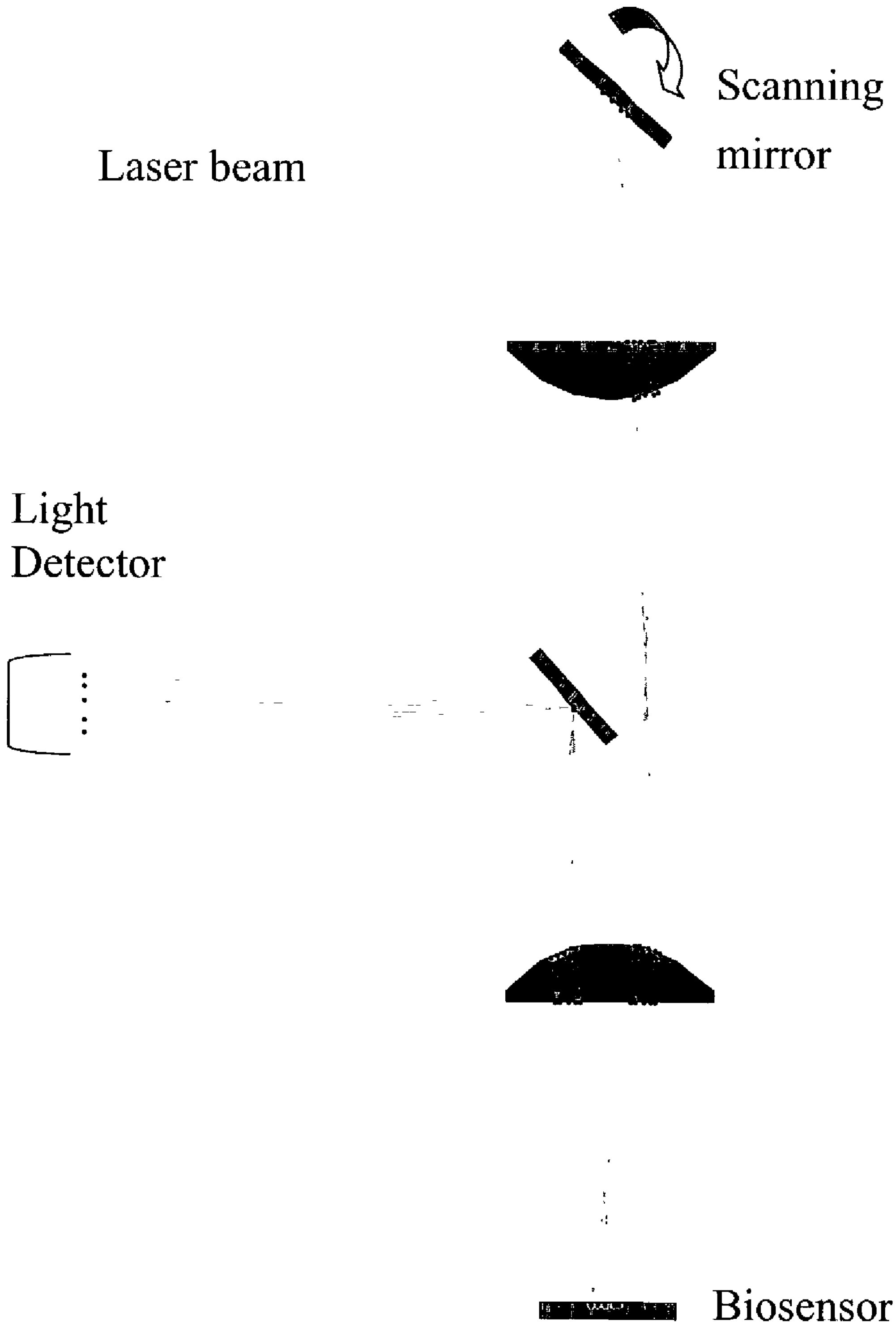


Figure 15

Concentric Circle Design

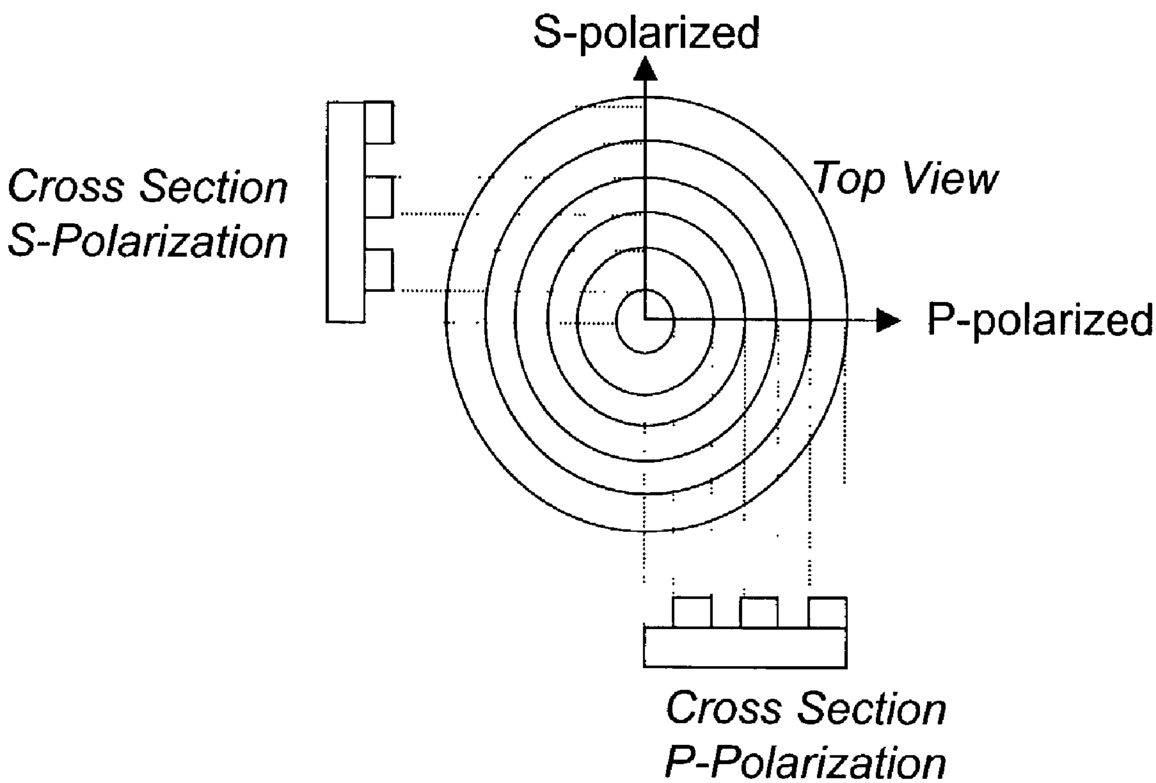


Figure 16

Hexagonal Grating Design

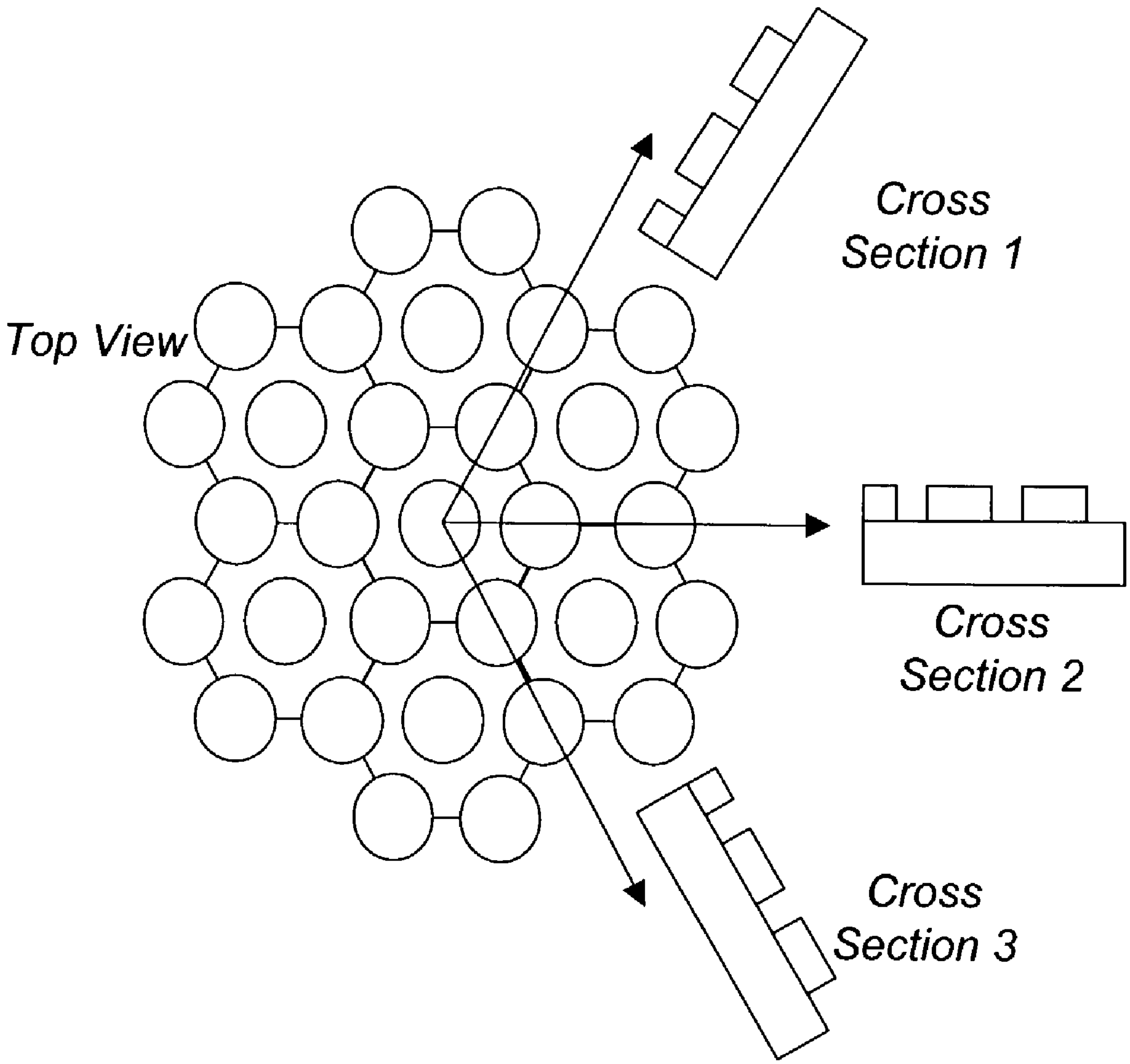


Figure 17

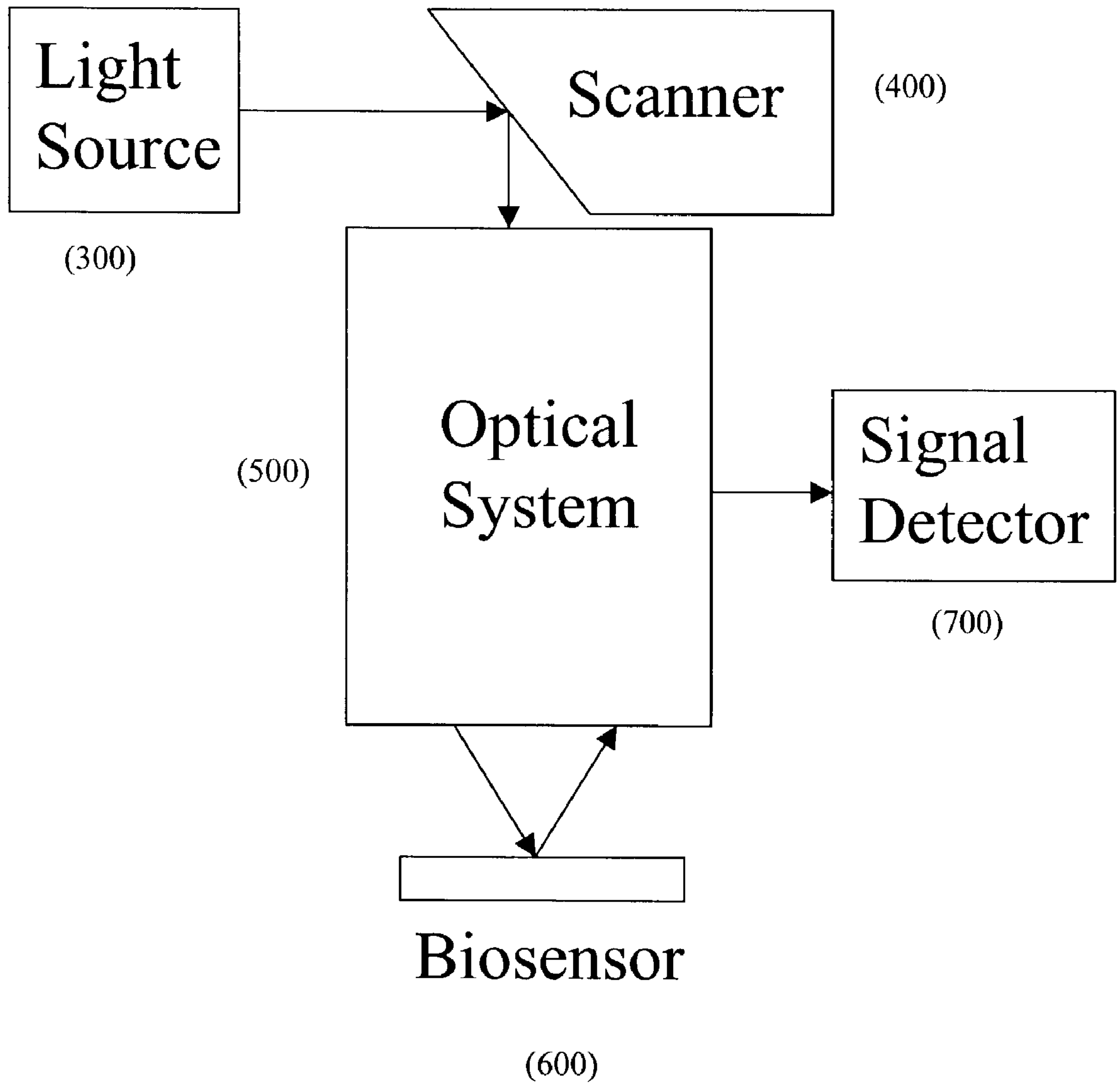

Light Source
(300)
Scanner
(400)
Optical System
(500)
Signal Detector
(700)
Biosensor
(600)

Figure 18

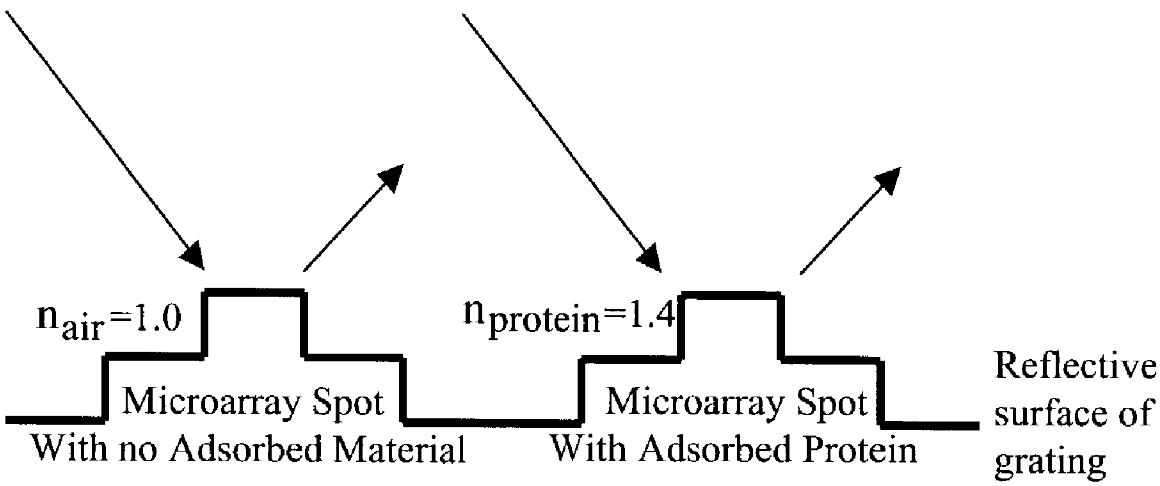

n_{air}=1.0
$n_{protein}$=1.4
Microarray Spot
With no Adsorbed Material
Microarray Spot
With Adsorbed Protein
Reflective
surface of
grating

Figure 19

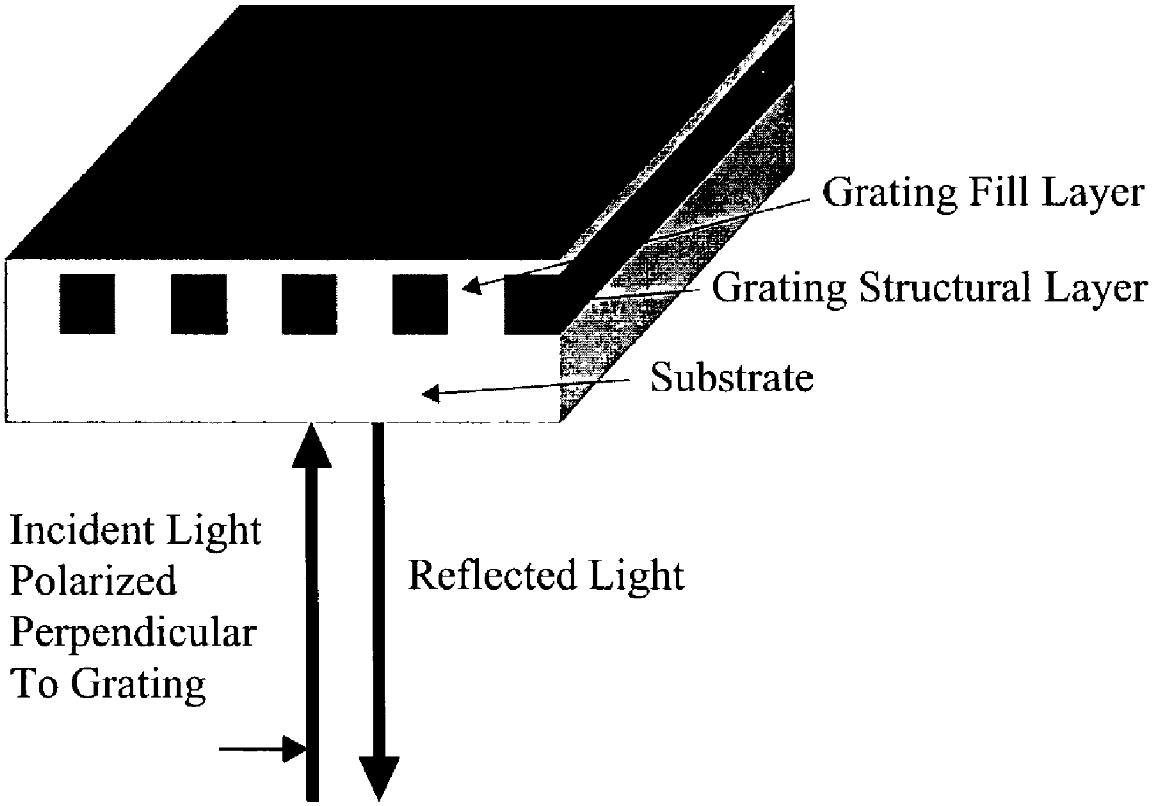
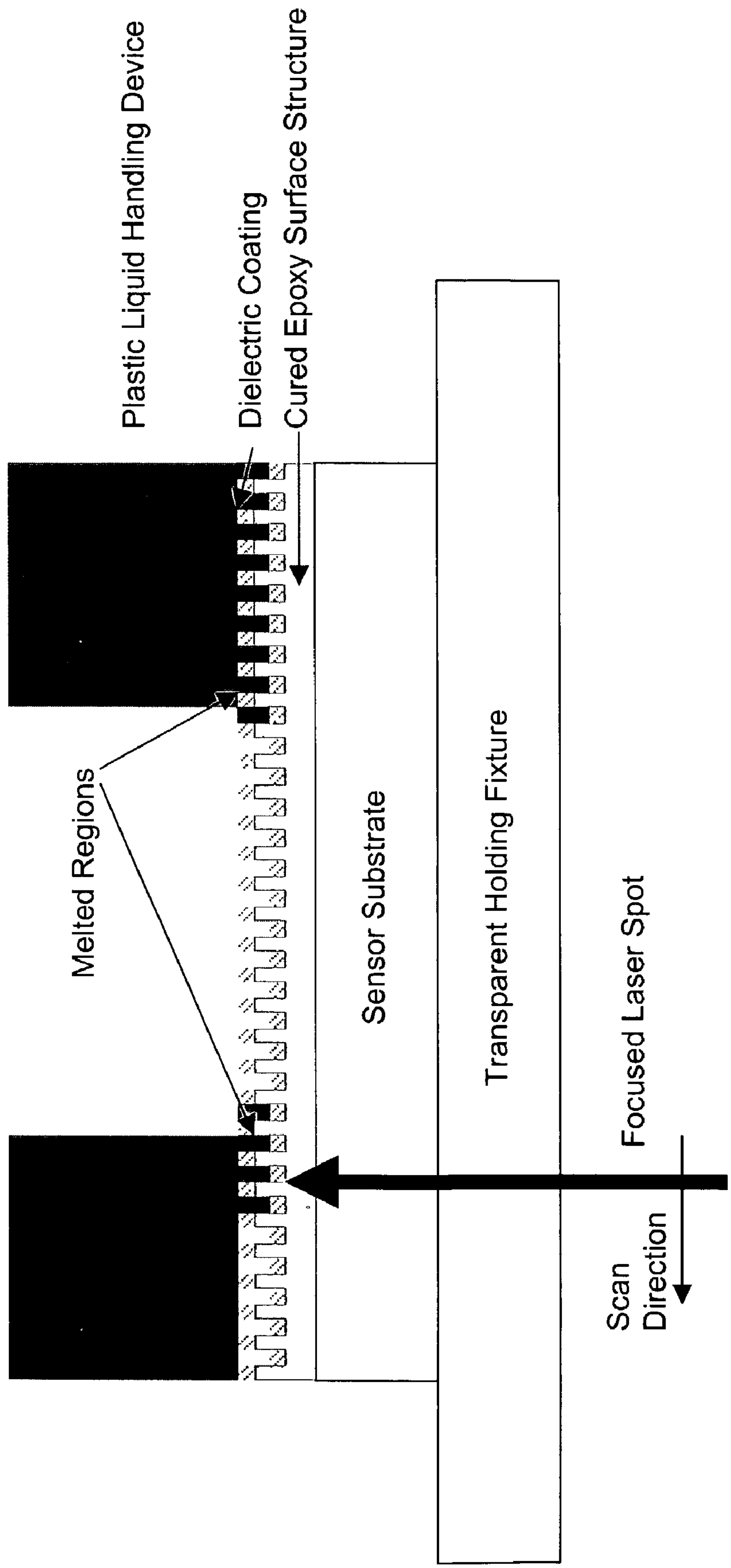

Grating Fill Layer
Grating Structural Layer
Substrate
Incident Light Polarized Perpendicular To Grating
Reflected Light

Figure 20

Figure 21


Plastic Liquid Handling Device
Dielectric Coating
Cured Epoxy Surface Structure
Melted Regions
Sensor Substrate
Transparent Holding Fixture
Focused Laser Spot
Scan Direction

METHOD OF MAKING A PLASTIC COLORIMETRIC RESONANT BIOSENSOR DEVICE WITH LIQUID HANDLING CAPABILITIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 10/180,374, filed Jun. 26, 2002, U.S. application Ser. No. 10/180,647, filed Jun. 26, 2002, and U.S. application Ser. No. 10/196,058 entitled "A Method for Producing a Colorimetric Resonant Reflection Biosensor on Rigid Surfaces" filed Jul. 15, 2002, which are continuations-in-part of U.S. application Ser. No. 10/059,060, filed Jan. 28, 2002 and U.S. application Ser. No. 10/058,626, filed Jan. 28, 2002, which are continuations-in-part of U.S. application Ser. No. 09,930,352, filed Aug. 15, 2001, which claims the benefit of U.S. provisional application 60/244,312 filed Oct. 30, 2000; U.S. provisional application 60/283,314 filed Apr. 12, 2001; and U.S. provisional application 60/303,028 filed Jul. 3, 2001, all of which are incorporated herein in their entirety.

FIELD OF THE INVENTION

The invention relates to methods for making liquid handling biosensor devices for detecting biomolecular interactions. Specifically, the invention relates to a biosensor device with liquid handling capability that allows for detection of biomolecular interactions in liquid. The detection can occur without the use of labels and can be done in a high-throughput manner.

BACKGROUND OF THE INVENTION

With the completion of the sequencing of the human genome, one of the next grand challenges of molecular biology will be to understand how the many protein targets encoded by DNA interact with other proteins, small molecule pharmaceutical candidates, and a large host of enzymes and inhibitors. See e.g., Pandey & Mann, "Proteomics to study genes and genomes," *Nature,* 405, p. 837–846, 2000; Leigh Anderson et al., "Proteomics: applications in basic and applied biology," *Current Opinion in Biotechnology,* 11, p. 408–412, 2000; Patterson, "Proteomics: the industrialization of protein chemistry," *Current Opinion in Biotechnology,* 11, p. 413–418, 2000; MacBeath & Schreiber, "Printing Proteins as Microarrays for High-Throughput Function Determination," *Science,* 289, p. 1760–1763, 2000; De Wildt et al., "Antibody arrays for high-throughput screening of antibody-antigen interactions," *Nature Biotechnology,* 18, p. 989–994, 2000. To this end, tools that have the ability to simultaneously quantify many different biomolecular interactions with high sensitivity will find application in pharmaceutical discovery, proteomics, and diagnostics. Further, for these tools to find widespread use, they must be simple to use, inexpensive to own and operate, and applicable to a wide range of analytes that can include, for example, polynucleotides, peptides, small proteins, antibodies, and even entire cells.

Biosensors have been developed to detect a variety of biomolecular complexes including oligonucleotides, antibody-antigen interactions, hormone-receptor interactions, and enzyme-substrate interactions. In general, biosensors consist of two components: a highly specific recognition element and a transducer that converts the molecular recognition event into a quantifiable signal. Signal transduction has been accomplished by many methods, including fluorescence, interferometry (Jenison et al., "Interference-based detection of nucleic acid targets on optically coated silicon," *Nature Biotechnology,* 19, p. 62–65; Lin et al., "A porous silicon-based optical interferometric biosensor," *Science,* 278, p. 840–843, 1997), and gravimetry (A. Cunningham, Bioanalytical Sensors, John Wiley & Sons (1998)).

Of the optically-based transduction methods, direct methods that do not require labeling of analytes with fluorescent compounds are of interest due to the relative assay simplicity and ability to study the interaction of small molecules and proteins that are not readily labeled. Direct optical methods include surface plasmon resonance (SPR) (Jordan & Corn, "Surface Plasmon Resonance Imaging Measurements of Electrostatic Biopolymer Adsorption onto Chemically Modified Gold Surfaces," *Anal. Chem.,* 69:1449–1456 (1997), (grating couplers (Morhard et al., "Immobilization of antibodies in micropatterns for cell detection by optical diffraction," *Sensors and Actuators B,* 70, p. 232–242, 2000), ellipsometry (Jin et al., "A biosensor concept based on imaging ellipsometry for visualization of biomolecular interactions," *Analytical Biochemistry,* 232, p. 69–72, 1995), evanascent wave devices (Huber et al., "Direct optical immunosensing (sensitivity and selectivity)," *Sensors and Actuators B,* 6, p. 122–126, 1992), and reflectometry (Brecht & Gauglitz, "Optical probes and transducers," *Biosensors and Bioelectronics,* 10, p. 923–936, 1995). Theoretically predicted detection limits of these detection methods have been determined and experimentally confirmed to be feasible down to diagnostically relevant concentration ranges. However, to date, these methods have yet to yield commercially available high-throughput instruments that can perform high sensitivity assays without any type of label in a format that is readily compatible with the microtiter plate-based or microarray-based infrastructure that is most often used for high-throughput biomolecular interaction analysis. Therefore, there is a need in the art for compositions and methods that can achieve these goals.

SUMMARY OF THE INVENTION

The invention relates to compositions and methods for detecting binding of one or more specific binding substances to their respective binding partners. The invention provides a method of producing a liquid handling colorimetric resonant reflection biosensor device that allows detection of molecular interactions in liquid. The method of the invention involves securing a liquid holding part comprising, for example, multiple holes onto a biosensor with an attachment material, such as a liquid adhesive, without allowing any attachment material to enter the holes of the liquid holding part. Using securing methods known in the art, adhesive typically escapes from the bottom surface of liquid holding parts, such as bottomless microtiter plates, into the area within the walls of liquid holding chambers. The invention provides a method of ensuring no attachment material enters the holes of a liquid holding part during the securing process.

One embodiment of the invention provides a method of making a liquid handling calorimetric resonant reflection biosensor device. The method comprises applying an attachment material to a surface of a transfer block, contacting a liquid holding part with the attachment material on the surface of the transfer block, removing the liquid holding part and any attachment material present on the liquid holding part from the transfer block, contacting a biosensor with the liquid holding part so that the attachment material is between the biosensor and the liquid holding part, and exposing the liquid holding part and biosensor to ultraviolet light to solidify the attachment material, wherein the liquid holding part is immobilized onto the biosensor.

The biosensor can comprise a grating comprised of or coated with a dielectric material having a high refractive index or a reflective material, wherein when the biosensor is illuminated a resonant grating effect is produced on the reflected radiation spectrum.

The attachment material can be an ultraviolet (UV) curable polymer. The liquid holding part can be a bottomless microtiter plate or a fluid flow channel. The attachment material is applied by a knife coating technique or a rod coating technique.

The step of contacting further can further comprise placing the biosensor in a first part of a holding fixture and placing the liquid handling part in a second part of the holding fixture, wherein the first and second parts of the holding fixture are brought together so that the biosensor and the liquid holding part are in contact with each other.

The thickness of the attachment material on the surface of the transfer block can be about 0.025 mm to about 5 mm thick.

Another embodiment of the invention provides a method of making a liquid handling calorimetric resonant reflection biosensor device. The method comprises die cutting a transfer pressure sensitive adhesive into a pattern of a liquid holding part to create a patterned adhesive, transferring the patterned adhesive onto the liquid holding part, contacting a biosensor with the patterned adhesive of the liquid holding part, and applying pressure to the biosensor or liquid holding part.

Still another embodiment of the invention provides a method of making a liquid handling colorimetric resonant reflection biosensor device comprising laser welding or ultrasonic welding a liquid holding part to a colorimetric resonant reflection biosensor.

Specific preferred embodiments of the invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** represents a cross-section of a biosensor of the invention bonded to a liquid holding part.

FIG. **2** shows a top view and cross-section of a liquid holding part.

FIG. **3** depicts a systematic illustration of a process for making a biosensor device with liquid holding capacity.

FIG. **4** shows a cross-sectional view of one embodiment of a biosensor wherein light is shown as illuminating the bottom of the biosensor; however, light can illuminate the biosensor from either the top or the bottom.

FIGS. **5**A–B shows a grating comprising a rectangular grid of squares (FIG. **5**A) or holes (FIG. **5**B);

FIG. **6** shows a biosensor cross-section profile utilizing a sinusoidally varying grating profile;

FIG. **7** shows three types of surface activation chemistry (Amine, Aldehyde, and Nickel) with corresponding chemical linker molecules that can be used to covalently attach various types of biomolecule receptors to a biosensor;

FIG. **8** shows an example of a biosensor used as a microarray;

FIGS. **9**A–B shows two biosensor formats that can incorporate a colorimetric resonant reflectance biosensor. FIG. **9**A shows a biosensor that is incorporated into a microtitre plate. FIG. **9**B shows a biosensor in a microarray slide format;

FIG. **10** shows an array of arrays concept for using a biosensor platform to perform assays with higher density and throughput;

FIG. **11** demonstrates an example of a biosensor that occurs on the tip of a fiber probe for in vivo detection of biochemical substances;

FIG. **12** shows an example of the use of two coupled fibers to illuminate and collect reflected light from a biosensor;

FIG. **13** shows resonance wavelength of a biosensor as a function of incident angle of detection beam;

FIG. **14** shows an example of the use of a beam splitter to enable illuminating and reflected light to share a common collimated optical path to a biosensor;

FIG. **15** shows an example of a system for angular scanning of a biosensor;

FIG. **16** shows a resonant reflection or transmission filter structure consisting of a set of concentric rings;

FIG. **17** shows a grid structure comprising a hexagonal grid of holes (or a hexagonal grid of posts) that closely approximates the concentric circle structure of FIG. **16** without requiring the illumination beam to be centered upon any particular location of the grid.

FIG. **18** shows a schematic diagram of a detection system.

FIG. **19** shows a graphic representation of how adsorbed material, such as a protein monolayer, will increase the reflected wavelength of a biosensor that comprises a three-dimensional grating.

FIG. **20** shows one embodiment colorimetric resonant reflection biosensor comprising a one-dimensional grating.

FIG. **21** demonstrates laser bonding of a biosensor sheet to a plastic fluid handling device containing an open area. Focused laser light directed through the transparent holding fixture and sensor sheet locally melts the plastic fluid handling device material, creating a watertight bond to the biosensor sheet.

DETAILED DESCRIPTION OF THE INVENTION

Liquid-Containing Vessels

A biosensor or optical device of the invention can comprise an inner surface, for example, a bottom surface of a liquid-containing vessel. Such a device is liquid handling biosensor or optical device. A liquid-containing vessel can be, for example, a microtiter plate well, a test tube, a petri dish, or a fluid flow channel such as a microfluidic channel. One embodiment of this invention is a biosensor that is incorporated into any type of microtiter plate. For example, a biosensor can be incorporated into the bottom surface of a microtiter plate by assembling the walls of the reaction vessels over the resonant reflection surface, as shown in FIG. **9**A and B, so that each reaction "spot" can be exposed to a distinct test sample. Therefore, each individual microtiter plate well can act as a separate reaction vessel. Separate chemical reactions can, therefore, occur within adjacent wells without intermixing reaction fluids and chemically distinct test solutions can be applied to individual wells.

Several methods for attaching a biosensor or optical device to the bottom surface of bottomless microtiter plates can be used, including, for example, adhesive attachment, ultrasonic welding, and laser welding.

The present invention provides a method for making a liquid handling biosensor device. The device comprises a liquid holding part, a biosensor or optical device as described herein, and an attachment material. A detailed cross-section of one embodiment of a liquid handling biosensor device is shown in FIG. **1**. A liquid holding part can comprise multiple holes, with surface areas surrounding the openings of the holes, such as a bottomless microtiter plate. A top view and a cross-section of a liquid holding part are shown in FIG. **2**. The attachment material can be a liquid, such as Lens Bond Optical Cement Type J-91 (Summers Optical, Fort Washington, Pa.), or a pressure sensitive adhesive, such as 3M™ Adhesive Transfer Tape 9458 (3M Co., St. Paul, Minn.). Preferably, curable polymer.

A liquid handling biosensor device allows detection of biomolecular binding in liquid. Liquid comprising potential binding partners can be contained over the biosensor surface within the liquid handling device. A signal can be produced and detected resulting from interaction between a binding partner and a binding substance immobilized on the biosensor surface. The design facilitates reading of the biosensor by a detection system, such as those described herein.

In one embodiment of the invention, the method involves placing a biosensor or optical device into a cutting fixture and cutting it to a predetermined size. The predetermined size can be, for example, the size of a standard microtiter plate (such as, 1"×3" or 3"×3"). Standards for microplates have been proposed by the Society for Biomolecular Screening (Danbury, Conn.). A biosensor or optical device is secured, for example, by vacuum, and a template comprising open channels arranged in a particular pattern is placed over the biosensor or optical device. A cutting means, such as a knife, is used to cut the biosensor or optical device according to the cutting pattern.

In another embodiment, an attachment material is applied to a transfer block with a particular, uniform thickness. The transfer block can be made of, for example, polished glass, metal, or plastic. Knife coating techniques can be used to control the thickness and uniformity of the attachment material. For example, a bead of adhesive material is placed on an edge of the transfer block. A knife, perpendicularly arranged at a particular distance above the surface of the transfer block, is moved in a controlled fashion across the surface of the block. Excess adhesive is pushed off the transfer block, leaving a uniform layer of adhesive material over the surface of the transfer block. Preferably, the thickness of the adhesive material is about 0.025 mm to about 5 mm. The thickness and uniformity of the attachment material can also controlled, for example, by rod coating techniques. Rod coating is performed using a wire-wound rod to remove excess fluid from the surface of the block.

A liquid holding part is placed on the transfer block comprising the attachment material. The liquid holding part is then lifted from the transfer block. The surface areas surrounding the openings comprise the attachment material. Preferably, the openings are free from any attachment material. Thus, the attachment material is confined to the surface areas of the holding part.

The biosensor or optical device is then attached to the liquid holding part. The biosensor or optical device can be aligned with the liquid holding part such that a plurality of wells are available for detecting binding reactions as described herein. The optical properties of the biosensor or optical device can be altered if attachment material escapes from the liquid holding part onto the exposed surface of the biosensor or optical device. Attachment material is prevented from escaping by ensuring a controlled thickness of attachment material is applied to the transfer block. Attachment material is also prevented from escaping by ensuring the liquid holding part and the biosensor or optical device are properly aligned. A holding fixture with two parts can be used to ensure proper alignment. The liquid holding part is placed in a first part of the holding fixture and the biosensor or optical device is placed in a second part of the holding fixture. The two parts of the holding fixture are then brought together to ensure that no shifting occurs once the holding part and biosensor or optical device contact each other.

When the holding part and biosensor or optical device are properly aligned and contacted with one another, the attachment material is solidified. For example, the attachment material can be solidified by exposure to ultraviolet (UV) light. Such bonding techniques using UV light are known in the art. For example, bonding can be achieved by exposure under a Xenon RC-600 UV lamp (Xenon Corp., Woburn, Mass.) for 95 seconds. The process for making a device of the invention is illustrated in FIG. **3**.

Another embodiment of the invention provides a method of making a liquid handling calorimetric resonant reflection biosensor device. The method comprises die cutting a transfer pressure sensitive adhesive into a pattern of a liquid holding part to create a patterned adhesive, transferring the patterned adhesive onto the liquid holding part, contacting a biosensor with the patterned adhesive of the liquid holding part, and applying pressure to the biosensor or liquid holding part. A holding fixture can also be used to bring the liquid holding part in contact with the biosensor.

Another embodiment of the invention provides a method of making a liquid handling colorimetric resonant reflection biosensor device comprising laser welding or ultrasonic welding a liquid holding part to a colorimetric resonant reflection biosensor. A holding fixture can also be used to bring the liquid holding part in contact with the biosensor.

In order to attach a biosensor sheet to a plastic fluid handling device such as a bottomless microtiter plate by laser welding, the biosensor sheet is first brought into physical contact with the fluid handling device. See, e.g., FIG. **21**. Next, laser illumination is applied through the biosensor sheet, so the plastic of the fluid handling device in contact with the biosensor sheet absorbs laser energy, and becomes locally heated above its melting point. The "active" biosensor regions (regions not in contact with the plastic fluid handling device) are not exposed to the laser energy, and are not heated above the melting point of the biosensor material. In the laser-exposed regions, where the plastic fluid-handling device is heated above its melting point, the plastic flows over the surface of the biosensor sheet, thus forming a bond between the fluid-handling device and the biosensor. The laser exposure can be applied in any pattern, in order to define bonded regions which correspond to the wells of a microtiter plate, or to fluid flow channels. In a preferred embodiment, the material comprising the plastic fluid handling device is capable of absorbing the applied laser energy. If the material comprising the plastic fluid handling device does not strongly absorb the laser energy (i.e. it is "white" or transparent), a material can be applied to the fluid handling device which is energy-absorbing before laser exposure occurs.

For example, a plastic laser welding system (Leister Technologies, Schaumburg, Ill.) is capable of supplying directed laser energy with a wavelength of 800–980 nm at energy densities ranging from 10^3 to 10^5 W/cm^2. The laser energy can be focused to a spot size as small as 0.6 mm, and is delivered by an optical fiber with a collimating lens at the tip. While plastic within the illuminated region is locally heated above its melting temperature (polyester melts at approximately 250° C.), regions 0.1 mm away from the illuminated region are not melted. Before welding, the biosensor is brought into contact with the plastic fluid handling device by pressing the biosensor against the fluid handling device with an optically transparent fixture using a force of ~60 psi. The laser illumination is applied through the transparent fixture and the biosensor in order to locally melt the plastic fluid handling device. The illumination spot is scanned over the assembly to define the bonded areas. For "black" colored fluid handling devices, the plastic absorbs the laser energy efficiently, and is melted easily. For "white" or "clear" fluid handling devices, a thin (<1 mm thick) coating of, for example, Gentex Clearweld (Carbondale, Pa.) is applied to the bonded surface of the fluid handling device before it is brought into contact with the biosensor.

The most common assay formats for pharmaceutical high-throughput screening laboratories, molecular biology research laboratories, and diagnostic assay laboratories are microtiter plates. The plates are, for example, standard-sized plastic cartridges that can contain 96, 384, or 1536 individual reaction vessels arranged in a grid. Due to the standard mechanical configuration of these plates, liquid dispensing, robotic plate handling, and detection systems are designed to work with this common format. A biosensor of the invention can be incorporated into the bottom surface of a standard microtiter plate. See, e g., FIG. **9**. Because the biosensor surface can be fabricated in large areas, and because the readout system does not make physical contact with the biosensor surface, an arbitrary number of individual biosensor areas can be defined that are only limited by the focus resolution of the illumination optics and the x-y stage that scans the illumination/detection probe across the biosensor surface.

Colorimetric Resonant Reflection Biosensors

A colorimetric resonant biosensor comprises a subwavelength structured surface (SWS), which is used to create a sharp optical resonant reflection at a particular wavelength that can be used to, for example, track with high sensitivity the interaction of biological materials, such as specific binding substances or binding partners or both. See e.g., U.S. application Ser. Nos. 09/930,352; 10/059,060, and 10/058,626, all of which are incorporated herein in their entirety.

The microreplicating methods of the invention can be used to make a subwavelength structured surface (SWS), such as a colorimetric resonant reflection diffractive grating surface. See U.S. application Ser. Nos. 10/058,626 and 10/059,060. Such a grating surface can be used to create a sharp optical resonant reflection at a particular wavelength that can be used to track with high sensitivity the interaction of biological materials, such as specific binding substances or binding partners or both. The colorimetric resonant reflection diffractive grating surface acts as a surface binding platform for specific binding substances.

Subwavelength structured surfaces are an unconventional type of diffractive optic that can mimic the effect of thin-film coatings. (Peng & Morris, "Resonant scattering from two-dimensional gratings," *J. Opt. Soc. Am. A,* Vol. 13, No. 5, p. 993, May; Magnusson, & Wang, "New principle for optical filters," *Appl. Phys. Lett.,* 61, No. 9, p. 1022, August, 1992; Peng & Morris, "Experimental demonstration of resonant anomalies in diffraction from two-dimensional gratings," *Optics Letters,* Vol. 21, No. 8, p. 549, April, 1996). A SWS structure contains a one-, two- or three-dimensional grating in which the grating period is small compared to the wavelength of incident light so that no diffractive orders other than the reflected and transmitted zeroth orders are allowed to propagate. A SWS structure can comprise an optical grating sandwiched between a substrate layer and a cover layer that fills the grating. Optionally, a cover layer is not used. When the effective index of refraction of the grating region is greater than the substrate or the cover layer, a waveguide is created. When a filter is designed properly, incident light passes into the waveguide region and propagates as a leaky mode. An optical grating structure selectively couples light at a narrow band of wavelengths into the waveguide. The light propagates only a very short distance (on the order of 10–100 micrometers), undergoes scattering, and couples with the forward- and backward-propagating zeroth-order light. This highly sensitive coupling condition can produce a resonant grating effect on the reflected radiation spectrum, resulting in a narrow band of reflected or transmitted wavelengths. The depth and period of the one-, two- or three-dimensional grating are less than the wavelength of the resonant grating effect.

The reflected or transmitted wavelengths produced by an optical grating structure can be modulated by the addition of molecules such as specific binding substances or binding partners or both to the upper surface the grating surface or cover layer. This structure is a biosensor. The added molecules increase the optical path length of incident radiation through the structure, and thus modify the wavelength at which maximum reflectance or transmittance will occur.

In one embodiment, a biosensor, when illuminated with white light, is designed to reflect only a single wavelength or a narrow band of wavelengths. When specific binding substances are attached to the surface of the biosensor, the reflected wavelength is shifted due to the change of the optical path of light that is coupled into the grating. By linking specific binding substances to a biosensor surface, complementary binding partner molecules can be detected without the use of any kind of fluorescent probe or particle label. The detection technique is capable of resolving changes of, for example, ~0.1 nm thickness of protein binding, and can be performed with the biosensor surface either immersed in fluid or dried.

A detection system can include, for example, a light source that illuminates a small spot of a biosensor at normal incidence through, for example, a fiber optic probe, and a spectrometer that collects the reflected light through, for example, a second fiber optic probe also at normal incidence. Because no physical contact occurs between the excitation/detection system and the biosensor surface, no special coupling prisms are required and the biosensor can be easily adapted to any commonly used assay platform including, for example, microtiter plates and microarray slides. A single spectrometer reading can be performed in several milliseconds; it is thus possible to quickly measure a large number of molecular interactions taking place in parallel upon a biosensor surface, and to monitor reaction kinetics in real time.

This technology is useful in applications where large numbers of biomolecular interactions are measured in parallel, particularly when molecular labels would alter or inhibit the functionality of the molecules under study. High-throughput screening of pharmaceutical compound libraries with protein targets, and microarray screening of protein-protein interactions for proteomics are examples of applications that require the sensitivity and throughput afforded by the compositions and methods of the invention.

FIG. **4** is a diagram of an example of a colorimetric resonant reflection diffractive grating biosensor. In FIG. **4**, n_1 represents the refractive index of an optical grating. n_2 represents the refractive index of a high refractive index material. Layer thicknesses (i.e. cover layer, one or more specific binding substances, or an optical grating) are selected to achieve resonant wavelength sensitivity to additional molecules on the top surface. The grating period is selected to achieve resonance at a desired wavelength.

A SWS biosensor comprises an optical grating, a substrate layer that supports the grating, and one or more specific binding substances immobilized on the surface of the grating opposite of the substrate layer. Optionally, a cover layer covers the grating surface. An optical grating made according to the invention is coated with a high refractive index dielectric film which can be comprised of a material that includes, for example, zinc sulfide, titanium dioxide, tantalum oxide, and silicon nitride. A cross-sectional profile of a grating with optical features can comprise any periodically repeating function, for example, a "square-wave." An optical grating can also comprise a repeating pattern of shapes selected from the group consisting of lines, squares, circles, ellipses, triangles, trapezoids, sinusoidal waves, ovals, rectangles, and hexagons.

Sensor Characteristics

Linear gratings (i.e., one dimensional gratings) have resonant characteristics where the illuminating light polarization is oriented perpendicular to the grating period. A schematic diagram of a linear grating structure is shown in FIG. **20**. A calorimetric resonant reflection biosensor can also comprise, for example, a two-dimensional grating, e.g., a hexagonal array of holes (see FIG. **5**B) or squares (see FIG. **5**A). Other shapes can be used as well. A linear grating has the same pitch (i.e. distance between regions of high and low refractive index), period, layer thicknesses, and material properties as a hexagonal array grating. However, light must be polarized perpendicular to the grating lines in order to be resonantly coupled into the optical structure. Therefore, a polarizing filter oriented with its polarization axis perpendicular to the linear grating must be inserted between the illumination source and the biosensor surface. Because only a small portion of the illuminating light source is correctly polarized, a longer integration time is required to collect an equivalent amount of resonantly reflected light compared to a hexagonal grating.

An optical grating can also comprise, for example, a "stepped" profile, in which high refractive index regions of a single, fixed height are embedded within a lower refractive index cover layer. The alternating regions of high and low refractive index provide an optical waveguide parallel to the top surface of the biosensor.

It is also possible to make a resonant biosensor in which the high refractive index material is not stepped, but which varies with lateral position. FIG. **6** shows a profile in which the high refractive index material of the two-dimensional grating, n_2, is sinusoidally varying in height. To produce a resonant reflection at a particular wavelength, the period of the sinusoid is identical to the period of an equivalent stepped structure. The resonant operation of the sinusoidally varying structure and its functionality as a biosensor has been verified using GSOLVER (Grating Solver Development Company, Allen, Tex., USA) computer models.

A biosensor of the invention can further comprise a cover layer on the surface of an optical grating opposite of a substrate layer. Where a cover layer is present, the one or more specific binding substances are immobilized on the surface of the cover layer opposite of the grating. Preferably, a cover layer comprises a material that has a lower refractive index than a material that comprises the grating. A cover layer can be comprised of, for example, glass (including spin-on glass (SOG)), epoxy, or plastic.

For example, various polymers that meet the refractive index requirement of a biosensor can be used for a cover layer. SOG can be used due to its favorable refractive index, ease of handling, and readiness of being activated with specific binding substances using the wealth of glass surface activation techniques. When the flatness of the biosensor surface is not an issue for a particular system setup, a grating structure of SiN/glass can directly be used as the sensing surface, the activation of which can be done using the same means as on a glass surface.

Resonant reflection can also be obtained without a planarizing cover layer over an optical grating. For example, a biosensor can contain only a substrate coated with a structured thin film layer of high refractive index material. Without the use of a planarizing cover layer, the surrounding medium (such as air or water) fills the grating. Therefore, specific binding substances are immobilized to the biosensor on all surfaces of an optical grating exposed to the specific binding substances, rather than only on an upper surface.

In general, a biosensor of the invention will be illuminated with white light that will contain light of every polarization angle. The orientation of the polarization angle with respect to repeating features in a biosensor grating will determine the resonance wavelength. For example, a "linear grating" (i.e., a one-dimensional grating) biosensor structure consisting of a set of repeating lines and spaces will have two optical polarizations that can generate separate resonant reflections. Light that is polarized perpendicularly to the lines is called "s-polarized," while light that is polarized parallel to the lines is called "p-polarized." Both the s and p components of incident light exist simultaneously in an unfiltered illumination beam, and each generates a separate resonant signal. A biosensor structure can generally be designed to optimize the properties of only one polarization (the s-polarization), and the non-optimized polarization is easily removed by a polarizing filter.

In order to remove the polarization dependence, so that every polarization angle generates the same resonant reflection spectra, an alternate biosensor structure can be used that consists of a set of concentric rings. In this structure, the difference between the inside diameter and the outside diameter of each concentric ring is equal to about one-half of a grating period. Each successive ring has an inside diameter that is about one grating period greater than the inside diameter of the previous ring. The concentric ring pattern extends to cover a single sensor location such as a microarray spot or a microtiter plate well. Each separate microarray spot or microtiter plate well has a separate concentric ring pattern centered within it. See, e.g., FIG. **16**. All polarization directions of such a structure have the same cross-sectional profile. The concentric ring structure must be illuminated precisely on-center to preserve polarization independence. The grating period of a concentric ring structure is less than the wavelength of the resonantly reflected light. The grating period is about 0.01 micron to about 1 micron. The grating depth is about 0.01 to about 1 micron.

In another embodiment, an array of holes or posts are arranged to closely approximate the concentric circle structure described above without requiring the illumination beam to be centered upon any particular location of the grid. See e.g. FIG. **17**. Such an array pattern is automatically generated by the optical interference of three laser beams incident on a surface from three directions at equal angles. In this pattern, the holes (or posts) are centered upon the corners of an array of closely packed hexagons as shown in FIG. **17**. The holes or posts also occur in the center of each hexagon. Such a hexagonal grid of holes or posts has three polarization directions that "see" the same cross-sectional profile. The hexagonal grid structure, therefore, provides equivalent resonant reflection spectra using light of any polarization angle. Thus, no polarizing filter is required to remove unwanted reflected signal components. The period of the holes or posts can be about 0.01 microns to about 1 micron and the depth or height can be about 0.01 microns to about 1 micron.

Another grating that can be produced using the methods of the invention is a volume surface-relief volume diffractive grating (a SRVD grating), also referred to as a three-dimensional grating. SRVD gratings have a surface that reflects predominantly at a particular narrow band of optical wavelengths when illuminated with a broad band of optical wavelengths. Where specific binding substances and/or binding partners are immobilized on a SRVD grating, producing a SRVD biosensor, the reflected narrow band of wavelengths of light is shifted. One-dimensional surfaces, such as thin film interference filters and Bragg reflectors, can select a narrow range of reflected or transmitted wavelengths from a broadband excitation source, however, the deposition of additional material, such as specific binding substances and/or binding partners onto their upper surface results only in a change in the resonance linewidth, rather than the resonance wavelength. In contrast, SRVD biosensors have the ability to alter the reflected wavelength with the addition of material, such as specific binding substances and/or binding partners to the surface. The depth and period of relief volume diffraction structures are less than the resonance wavelength of light reflected from a biosensor.

A three-dimensional surface-relief volume diffractive grating can be, for example, a three-dimensional phase-quantized terraced surface relief pattern whose groove pattern resembles a stepped pyramid. When such a grating is illuminated by a beam of broadband radiation, light will be coherently reflected from the equally spaced terraces at a wavelength given by twice the step spacing times the index of refraction of the surrounding medium. Light of a given wavelength is resonantly diffracted or reflected from the steps that are a half-wavelength apart, and with a bandwidth that is inversely proportional to the number of steps. The reflected or diffracted color can be controlled by the deposition of a reflective material so that a new wavelength is selected, depending on the index of refraction of the coating.

An example of a three-dimensional phase-quantized terraced surface relief pattern is a pattern that resembles a stepped pyramid. Each inverted pyramid is approximately 1 micron in diameter, preferably, each inverted pyramid can be about 0.5 to about 5 microns diameter, including for example, about 1 micron. The pyramid structures can be close-packed so that a typical microarray spot with a diameter of about 150–200 microns can incorporate several hundred stepped pyramid structures. The relief volume diffraction structures have a period of about 0.1 to about 1 micron and a depth of about 0.1 to about 1 micron. FIG. **19** demonstrates how individual microarray locations (with an entire microarray spot incorporating hundreds of pyramids now represented by a single pyramid for one microarray spot) can be optically queried to determine if specific binding substances or binding partners are adsorbed onto the surface. When the structure is illuminated with white light, structures without significant bound material will reflect wavelengths determined by the step height of the structure. When higher refractive index material, such as binding partners or specific binding substances, are incorporated over the reflective metal surface, the reflected wavelength is modified to shift toward longer wavelengths. The color that is reflected from the terraced step structure is theoretically given as twice the step height times the index of refraction of a reflective material that is coated onto the first surface of a sheet material of a SRVD biosensor. A reflective material can be, for example silver, aluminum, or gold.

One or more specific binding substances, as described above, are immobilized on the reflective material of a SRVD biosensor. One or more specific binding substances can be arranged in microarray of distinct locations, as described above, on the reflective material.

Because the reflected wavelength of light from a SRVD biosensor is confined to a narrow bandwidth, very small changes in the optical characteristics of the surface manifest themselves in easily observed changes in reflected wavelength spectra. The narrow reflection bandwidth provides a surface adsorption sensitivity advantage compared to reflectance spectrometry on a flat surface.

A SRVD biosensor reflects light predominantly at a first single optical wavelength when illuminated with a broad band of optical wavelengths, and reflects light at a second single optical wavelength when one or more specific binding substances are immobilized on the reflective surface. The reflection at the second optical wavelength results from optical interference. A SRVD biosensor also reflects light at a third single optical wavelength when the one or more specific binding substances are bound to their respective binding partners, due to optical interference.

Readout of the reflected color can be performed serially by focusing a microscope objective onto individual microarray spots and reading the reflected spectrum, or in parallel by, for example, projecting the reflected image of the microarray onto a high resolution color CCD camera.

In one embodiment of the invention, an optical device is provided. An optical device comprises a structure similar to a biosensor of the invention; however, an optical device does not comprise one of more binding substances immobilized on the grating. An optical device can be used as, for example, a narrow band optical filter.

Specific Binding Substances and Binding Partners

One or more specific binding substances can be immobilized on colorimetric resonant reflectance gratings produced by the methods of the invention by for example, physical adsorption or by chemical binding where a specific binding substance is bound to a colorimetric resonant reflectance grating, to produce a biosensor. A specific binding substance can be, for example, a nucleic acid, polypeptide, antigen, polyclonal antibody, monoclonal antibody, single chain antibody (scFv), F(ab) fragment, $F(ab')_2$ fragment, Fv fragment, small organic molecule, cell, virus, bacteria, or biological sample. A biological sample can be for example, blood, plasma, serum, gastrointestinal secretions, homogenates of tissues or tumors, synovial fluid, feces, saliva, sputum, cyst fluid, amniotic fluid, cerebrospinal fluid, peritoneal fluid, lung lavage fluid, semen, lymphatic fluid, tears, or prostatitc fluid.

Preferably, one or more specific binding substances are arranged in a microarray of distinct locations on a biosensor. A microarray of specific binding substances comprises one or more specific binding substances on a surface of a biosensor such that a surface contains many distinct locations, each with a different specific binding substance or with a different amount of a specific binding substance. For example, an array can comprise 1, 10, 100, 1,000, 10,000, or 100,000 distinct locations. Such a biosensor surface is called a microarray because one or more specific binding substances are typically laid out in a regular grid pattern in x-y coordinates. However, a microarray of the invention can comprise one or more specific binding substances laid out in any type of regular or irregular pattern. For example, distinct locations can define a microarray of spots of one or more specific binding substances. A microarray spot can be about 50 to about 500 microns in diameter. A microarray spot can also be about 150 to about 200 microns in diameter. One or more specific binding substances can be bound to their specific binding partners.

A microarray on a biosensor of the invention can be created by placing microdroplets of one or more specific binding substances onto, for example, an x-y grid of locations on an optical grating or cover layer surface. When the biosensor is exposed to a test sample comprising one or more binding partners, the binding partners will be preferentially attracted to distinct locations on the microarray that comprise specific binding substances that have high affinity for the binding partners. Some of the distinct locations will gather binding partners onto their surface, while other locations will not.

A specific binding substance specifically binds to a binding partner that is added to the surface of a biosensor of the invention. A specific binding substance specifically binds to its binding partner, but does not substantially bind other binding partners added to the surface of a biosensor. For example, where the specific binding substance is an antibody and its binding partner is a particular antigen, the antibody specifically binds to the particular antigen, but does not substantially bind other antigens. A binding partner can be, for example, a nucleic acid, polypeptide, antigen, polyclonal antibody, monoclonal antibody, single chain antibody (scFv), F(ab) fragment, $F(ab')_2$ fragment, Fv fragment, small organic molecule, cell, virus, bacteria, and biological sample. A biological sample can be, for example, blood, plasma, serum, gastrointestinal secretions, homogenates of tissues or tumors, synovial fluid, feces, saliva, sputum, cyst fluid, amniotic fluid, cerebrospinal fluid, peritoneal fluid, lung lavage fluid, semen, lymphatic fluid, tears, and prostatitc fluid.

One example of a microarray of the invention is a nucleic acid microarray, in which each distinct location within the array contains a different nucleic acid molecule. In this embodiment, the spots within the nucleic acid microarray detect complementary chemical binding with an opposing strand of a nucleic acid in a test sample.

While microtiter plates are the most common format used for biochemical assays, microarrays are increasingly seen as a means for maximizing the number of biochemical interactions that can be measured at one time while minimizing the volume of precious reagents. By application of specific binding substances with a microarray spotter onto a biosensor of the invention, specific binding substance densities of 10,000 specific binding substances/in^2 can be obtained. By focusing an illumination beam to interrogate a single microarray location, a biosensor can be used as a label-free microarray readout system.

Immobilization of One or More Specific Binding Substances

Immobilization of one or more binding substances onto a biosensor is performed so that a specific binding substance will not be washed away by rinsing procedures, and so that its binding to binding partners in a test sample is unimpeded by the biosensor surface. Several different types of surface chemistry strategies have been implemented for covalent attachment of specific binding substances to, for example, glass for use in various types of microarrays and biosensors. See, e.g., FIG. **7**. These same methods can be readily adapted to a biosensor of the invention. Surface preparation of a biosensor so that it contains the correct functional groups for binding one or more specific binding substances is an integral part of the biosensor manufacturing process.

One or more specific binding substances can be attached to a biosensor surface by physical adsorption (i.e., without the use of chemical linkers) or by chemical binding (i.e., with the use of chemical linkers). Chemical binding can generate stronger attachment of specific binding substances on a biosensor surface and provide defined orientation and conformation of the surface-bound molecules.

Liquid-Containing Vessels

A grating of the invention can comprise an inner surface, for example, a bottom surface of a liquid-containing vessel. A liquid-containing vessel can be, for example, a microtiter plate well, a test tube, a petri dish, or a microfluidic channel. One embodiment of this invention is a biosensor that is incorporated into any type of microtiter plate. For example, a biosensor can be incorporated into the bottom surface of a microtiter plate by assembling the walls of the reaction vessels over the resonant reflection surface, as shown in FIGS. **9**A and **9**B, so that each reaction "spot" can be exposed to a distinct test sample. Therefore, each individual microtiter plate well can act as a separate reaction vessel. Separate chemical reactions can, therefore, occur within adjacent wells without intermixing reaction fluids and chemically distinct test solutions can be applied to individual wells.

Several methods for attaching a biosensor or grating of the invention to the bottom surface of bottomless microtiter plates can be used, including, for example, adhesive attachment, ultrasonic welding, and laser welding.

The most common assay formats for pharmaceutical high-throughput screening laboratories, molecular biology research laboratories, and diagnostic assay laboratories are microtiter plates. The plates are standard-sized plastic cartridges that can contain 96, 384, or 1536 individual reaction vessels arranged in a grid. Due to the standard mechanical configuration of these plates, liquid dispensing, robotic plate handling, and detection systems are designed to work with this common format. A biosensor of the invention can be incorporated into the bottom surface of a standard microtiter plate. See, e g., FIG. **9**A. Because the biosensor surface can be fabricated in large areas, and because the readout system does not make physical contact with the biosensor surface, an arbitrary number of individual biosensor areas can be defined that are only limited by the focus resolution of the illumination optics and the x-y stage that scans the illumination/detection probe across the biosensor surface.

Methods of using Biosensors

Biosensors can be used to study one or a number of specific binding substance/binding partner interactions in parallel. Binding of one or more specific binding substances to their respective binding partners can be detected, without the use of labels, by applying one or more binding partners to a biosensor that have one or more specific binding substances immobilized on their surfaces. A biosensor is illuminated with light and a maxima in reflected wavelength, or a minima in transmitted wavelength of light is detected from the biosensor. If one or more specific binding substances have bound to their respective binding partners, then the reflected wavelength of light is shifted as compared to a situation where one or more specific binding substances have not bound to their respective binding partners. Where a biosensor is coated with an array of distinct locations containing the one or more specific binding substances, then a maxima in reflected wavelength or minima in transmitted wavelength of light is detected from each distinct location of the biosensor.

In one embodiment of the invention, a variety of specific binding substances, for example, antibodies, can be immobilized in an array format onto a biosensor of the invention. See, e.g., FIG. **8**. The biosensor is then contacted with a test sample of interest comprising binding partners, such as proteins. Only the proteins that specifically bind to the antibodies immobilized on the biosensor remain bound to the biosensor. Such an approach is essentially a large-scale version of an enzyme-linked immunosorbent assay; however, the use of an enzyme or fluorescent label is not required. For high-throughput applications, biosensors can be arranged in an array of arrays, wherein several biosensors comprising an array of specific binding substances are arranged in an array. See, e.g., FIG. **10**. Such an array of arrays can be, for example, dipped into microtiter plate to perform many assays at one time. In another embodiment, a biosensor can occur on the tip of a fiber probe for in vivo detection of biochemical substance. See, FIG. **11**.

The activity of an enzyme can be detected by applying one or more enzymes to a biosensor to which one or more specific binding substances have been immobilized. The biosensor is washed and illuminated with light. The reflected wavelength of light is detected from the biosensor. Where the one or more enzymes have altered the one or more specific binding substances of the biosensor by enzymatic activity, the reflected wavelength of light is shifted.

Additionally, a test sample, for example, cell lysates containing binding partners can be applied to a biosensor of the invention, followed by washing to remove unbound material. The binding partners that bind to a biosensor can be eluted from the biosensor and identified by, for example, mass spectrometry. Optionally, a phage DNA display library can be applied to a biosensor of the invention followed by washing to remove unbound material. Individual phage particles bound to the biosensor can be isolated and the inserts in these phage particles can then be sequenced to determine the identity of the binding partner.

For the above applications, and in particular proteomics applications, the ability to selectively bind material, such as binding partners from a test sample onto a biosensor of the invention, followed by the ability to selectively remove bound material from a distinct location of the biosensor for further analysis is advantageous. Biosensors of the invention are also capable of detecting and quantifying the amount of a binding partner from a sample that is bound to a biosensor array distinct location by measuring the shift in reflected wavelength of light. For example, the wavelength shift at one distinct biosensor location can be compared to positive and negative controls at other distinct biosensor locations to determine the amount of a binding partner that is bound to a biosensor array distinct location.

Detection Systems

A detection system can comprise a biosensor a light source that directs light to the biosensor, and a detector that detects light reflected from the biosensor. In one embodiment, it is possible to simplify the readout instrumentation by the application of a filter so that only positive results over a determined threshold trigger a detection.

A light source can illuminate a biosensor from its top surface, i.e., the surface to which one or more specific binding substances are immobilized or from its bottom surface. By measuring the shift in resonant wavelength at each distinct location of a biosensor of the invention, it is possible to determine which distinct locations have binding partners bound to them. The extent of the shift can be used to determine the amount of binding partners in a test sample and the chemical affinity between one or more specific binding substances and the binding partners of the test sample.

A biosensor can be illuminated twice. The first measurement determines the reflectance spectra of one or more distinct locations of a biosensor array with one or more specific binding substances immobilized on the biosensor. The second measurement determines the reflectance spectra after one or more binding partners are applied to a biosensor. The difference in peak wavelength between these two measurements is a measurement of the amount of binding partners that have specifically bound to a biosensor or one or more distinct locations of a biosensor. This method of illumination can control for small nonuniformities in a surface of a biosensor that can result in regions with slight variations in the peak resonant wavelength. This method can also control for varying concentrations or molecular weights of specific binding substances immobilized on a biosensor

Computer simulation can be used to determine the expected dependence between a peak resonance wavelength and an angle of incident illumination. For example, the substrate chosen was glass ($n_{substrate}$=1.50). The grating is an optical pattern of silicon nitride squares (t_2=180 nm, n_2=2.01 (n=refractive index), k_2=0.001 (k=absorption coefficient)) with a period of 510 nm, and a filling factor of 56.2% (i.e., 56.2% of the surface is covered with silicon nitride squares while the rest is the area between the squares). The areas between silicon nitride squares are filled with a lower refractive index material. The same material also covers the squares and provides a uniformly flat upper surface. For this simulation, a glass layer was selected (n_1=1.40) that covers the silicon nitride squares by t_2=100 nm.

The reflected intensity as a function of wavelength was modeled using GSOLVER software, which utilizes full 3-dimensional vector code using hybrid Rigorous Coupled Wave Analysis and Modal analysis. GSOLVER calculates diffracted fields and diffraction efficiencies from plane wave illumination of arbitrarily complex grating structures. The illumination can be from any incidence and any polarization.

FIG. **13** plots the dependence of the peak resonant wavelength upon the incident illumination angle. The simulation shows that there is a strong correlation between the angle of incident light, and the peak wavelength that is measured. This result implies that the collimation of the illuminating beam, and the alignment between the illuminating beam and the reflected beam will directly affect the resonant peak linewidth that is measured. If the collimation of the illuminating beam is poor, a range illuminating angles will be incident on the biosensor surface, and a wider resonant peak will be measured than if purely collimated light were incident.

Because the lower sensitivity limit of a biosensor is related to the ability to determine the peak maxima, it is important to measure a narrow resonant peak. Therefore, the use of a collimating illumination system with the biosensor provides for the highest possible sensitivity.

One type of detection system for illuminating the biosensor surface and for collecting the reflected light is a probe containing, for example, six illuminating optical fibers that are connected to a light source, and a single collecting optical fiber connected to a spectrometer. The number of fibers is not critical, any number of illuminating or collecting fibers are possible. The fibers are arranged in a bundle so that the collecting fiber is in the center of the bundle, and is surrounded by the six illuminating fibers. The tip of the fiber bundle is connected to a collimating lens that focuses the illumination onto the surface of the biosensor.

In this probe arrangement, the illuminating and collecting fibers are side-by-side. Therefore, when the collimating lens is correctly adjusted to focus light onto the biosensor surface, one observes six clearly defined circular regions of illumination, and a central dark region. Because the biosensor does not scatter light, but rather reflects a collimated beam, no light is incident upon the collecting fiber, and no resonant signal is observed. Only by defocusing the collimating lens until the six illumination regions overlap into the central region is any light reflected into the collecting fiber. Because only defocused, slightly uncollimated light can produce a signal, the biosensor is not illuminated with a single angle of incidence, but with a range of incident angles. The range of incident angles results in a mixture of resonant wavelengths due to the dependence shown in FIG. **13**. Thus, wider resonant peaks are measured than might otherwise be possible.

Therefore, it is desirable for the illuminating and collecting fiber probes to spatially share the same optical path. Several methods can be used to co-locate the illuminating and collecting optical paths. For example, a single illuminating fiber, which is connected at its first end to a light source that directs light at the biosensor, and a single collecting fiber, which is connected at its first end to a detector that detects light reflected from the biosensor, can each be connected at their second ends to a third fiber probe that can act as both an illuminator and a collector. The third fiber probe is oriented at a normal angle of incidence to the biosensor and supports counter-propagating illuminating and reflecting optical signals. An example of such a detection system is shown in FIG. **12**.

Another method of detection involves the use of a beam splitter that enables a single illuminating fiber, which is connected to a light source, to be oriented at a 90 degree angle to a collecting fiber, which is connected to a detector. Light is directed through the illuminating fiber probe into the beam splitter, which directs light at the biosensor. The reflected light is directed back into the beam splitter, which directs light into the collecting fiber probe. An example of such a detection device is shown in FIG. **14**. A beam splitter allows the illuminating light and the reflected light to share a common optical path between the beam splitter and the biosensor, so perfectly collimated light can be used without defocusing.

Angular Scanning

Detection systems of the invention are based on collimated white light illumination of a biosensor surface and optical spectroscopy measurement of the resonance peak of the reflected beam. Molecular binding on the surface of a biosensor is indicated by a shift in the peak wavelength value, while an increase in the wavelength corresponds to an increase in molecular absorption.

As shown in theoretical modeling and experimental data, the resonance peak wavelength is strongly dependent on the incident angle of the detection light beam. FIG. **13** depicts this dependence as modeled for a biosensor of the invention. Because of the angular dependence of the resonance peak wavelength, the incident white light needs to be well collimated. Angular dispersion of the light beam broadens the resonance peak, and reduces biosensor detection sensitivity. In addition, the signal quality from the spectroscopic measurement depends on the power of the light source and the sensitivity of the detector. In order to obtain a high signal-to-noise ratio, an excessively long integration time for each detection location can be required, thus lengthening overall time to readout a biosensor plate. A tunable laser source can be used for detection of grating resonance, but is expensive.

In one embodiment of the invention, these disadvantages are addressed by using a laser beam for illumination of a biosensor, and a light detector for measurement of reflected beam power. A scanning mirror device can be used for varying the incident angle of the laser beam, and an optical system is used for maintaining collimation of the incident laser beam. See, e.g., "Optical Scanning" (Gerald F. Marchall ed., Marcel Dekker (1991). Any type of laser scanning can be used. For example, a scanning device that can generate scan lines at a rate of about 2 lines to about 1,000 lines per second is useful in the invention. In one embodiment of the invention, a scanning device scans from about 50 lines to about 300 lines per second.

In one embodiment, the reflected light beam passes through part of the laser scanning optical system, and is measured by a single light detector. The laser source can be a diode laser with a wavelength of, for example, 780 nm, 785 nm, 810 nm, or 830 nm. Laser diodes such as these are readily available at power levels up to 150 mW, and their wavelengths correspond to high sensitivity of Si photodiodes. The detector thus can be based on photodiode biosensors. An example of such a detection system is shown in FIG. **18**. A light source (**300**) provides light to a scanner device (**400**), which directs the light into an optical system (**500**). The optical system (**500**) directs light to a biosensor (**600**). Light is reflected from the biosensor (**600**) to the optical system (**500**), which then directs the light into a light signal detector (**700**). One embodiment of a detection system is shown in FIG. **15**, which demonstrates that while the scanning mirror changes its angular position, the incident angle of the laser beam on the surface changes by nominally twice the mirror angular displacement. The scanning mirror device can be a linear galvanometer, operating at a frequency of about 2 Hz up to about 120 Hz, and mechanical scan angle of about 10 degrees to about 20 degrees. In this example, a single scan can be completed within about 10 msec. A resonant galvanometer or a polygon scanner can also be used. The example shown in FIG. **15** includes a simple optical system for angular scanning. It consists of a pair of lenses with a common focal point between them. The optical system can be designed to achieve optimized performance for laser collimation and collection of reflected light beam.

The angular resolution depends on the galvanometer specification, and reflected light sampling frequency. Assuming galvanometer resolution of 30 arcsec mechanical, corresponding resolution for biosensor angular scan is 60 arcsec, i.e. 0.017 degree. In addition, assume a sampling rate of 100 ksamples/sec, and 20 degrees scan within 10 msec. As a result, the quantization step is 20 degrees for 1000 samples, i.e. 0.02 degree per sample. In this example, a resonance peak width of 0.2 degree, as shown by Peng and Morris (Experimental demonstration of resonant anomalies in diffraction from two-dimensional gratings, *Optics Lett.,* 21:549 (1996)), will be covered by 10 data points, each of which corresponds to resolution of the detection system.

The advantages of such a detection system includes: excellent collimation of incident light by a laser beam, high signal-to-noise ratio due to high beam power of a laser diode, low cost due to a single element light detector instead of a spectrometer, and high resolution of resonance peak due to angular scanning.

All references cited in this application are incorporated herein in their entirety.

We claim:

1. A method of making a liquid handling colorimetric resonant reflection biosensor device, comprising:

(a) die cutting a transfer pressure sensitive adhesive into a pattern of a liquid holding part to create a patterned adhesive;

(b) transferring the patterned adhesive onto the liquid holding part;

(c) contacting a biosensor with the patterned adhesive of the liquid holding part, and

(d) applying pressure to the biosensor or liquid holding part; whereby a liquid handling colorimetric resonant reflection biosensor device is made.

2. The method of claim **1**, wherein the biosensor comprises a grating comprised of or coated with a dielectric material having a high refractive index or a reflective material, wherein when the biosensor is illuminated light is resonantly reflected.

3. The method of claim **1**, wherein the liquid holding part is a bottomless microliter plate or a fluid flow channel.

* * * * *