(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 September 2012 (27.09.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/129455 A4

(51) International Patent Classification:
G01N 35/08 (2006.01) *G01N 21/47* (2006.01)
G01N 21/64 (2006.01) *G01N 33/53* (2006.01)

(21) International Application Number:
PCT/US2012/030216

(22) International Filing Date:
22 March 2012 (22.03.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/465,688 22 March 2011 (22.03.2011) US
61/608,570 8 March 2012 (08.03.2012) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

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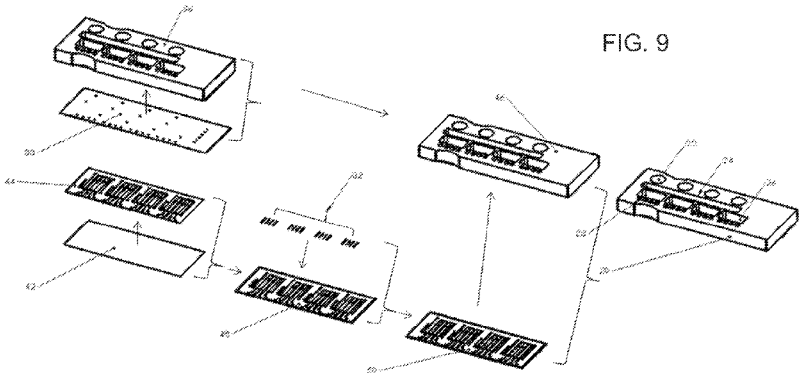
(54) Title: MICROFLUIDIC DEVICES AND METHODS OF MANUFACTURE AND USE

FIG. 9



(57) Abstract: Microfluidic devices are provided for conducting fluid assays, for example biological assays, that have the ability to move fluids through multiple channels and pathways in a compact, efficient, and low cost manner. Discrete flow detection elements, preferably extremely short hollow flow elements, with length preferably less than 700 micron, preferably less than 500 micron, and internal diameter preferably of between about 50 +/- 25 micron, are provided with capture agent, and are inserted into microfluidic channels by tweezer or vacuum pick-and-place motions at fixed positions in which they are efficiently exposed to fluids for conducting assays. Close-field electrostatic attraction is employed to define the position of the elements and enable ready withdrawal of the placing instruments. The microfluidic devices feature flow elements, channels, valves, and on-board pumps that are low cost to fabricate accurately, are minimally invasive to the fluid path and when implemented for the purpose, can produce multiplex assays on a single portable assay cartridge (chip) that have low coefficients of variation. Novel methods of construction, assembly and use of these features are presented, including co-valent bonding of selected regions of faces of surface-activatable bondable materials, such as PDMS to PDMS and PDMS to glass, while contiguous portions of one flexible sheet completes and seals flow channels, fixes the position of inserted analyte-detection elements in the channels, especially short hollow flow elements through which sample and reagent flow, and other portions form flexible valve membranes and diaphragms of pumps. A repeated make-and-break-contact manufacturing protocol prevents such bonding to interfere with moving the integral valve diaphragm portions from their valve seats defined by the opposed sheet member, which the flexible sheet material engages. Preparation of two subassemblies, each having a backing of relatively rigid material, followed by their assembly face-to-face in a permanent bond is shown. Hollow detection flow elements are shown fixed

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SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(88) Date of publication of the international search report: 29 November 2012

Published:

Date of publication of the amended claims: 31 January 2013

— *with international search report (Art. 21(3))*

— *with amended claims (Art. 19(1))*

in channels, that provide by-pass flow paths of at least 50% of the flow capacity through the elements; in preferred implementations, as much as 100% or more. Metallized polyester film is shown to have numerous configurations and advantages in non-permanently bonded constructions. A method of preparing detection elements for an assay comprises batch coating detection elements, or hollow flow elements by mixing and picking and placing the elements in flow channels of a microfluidic device, capturing the flow elements by bonding two opposed layers while sealing the flow channels.

AMENDED CLAIMS
received by the International Bureau on 10 December 2012 (10.12.2012)

1. A microfluidic device for conducting a fluid assay, for example a biological assay, having a flow channel in which is inserted at least one discrete detection element, in the form of a flow-receiving reaction vessel, in fixed position that is provided with capture agent, the element being positioned for exposure to fluid flows within the device for conducting an assay.

2. The device of claim 1 in which the element comprises an extremely short hollow flow element with length less than about 700 micron.

3. The device of claim 2 in which the length is less than about 500 micron.

4. The device of claim 2 or 3 in which the internal diameter of the hollow flow element is between about 75 +/- 50 micron.

5. The device of claim 2 or 3 in which the internal diameter of the hollow flow element is between 50 +/- 25 micron.

6. The device of any of the foregoing claims 1-5 in which the hollow flow element is inserted into its microfluidic channel by pick-and-place motion.

7. The device of claim 6 in which close-field electrostatic attraction has been employed to define the position of the hollow flow element and enable ready withdrawal of the placing instrument.

8. The device of any of the foregoing claims in which the element comprises a short hollow flow element of length less than 700 micron, having oppositely directed planar end surfaces and a cylindrical outer surface extending between those end surfaces.

9. The device of claim 8 in which the length is less than approximately 500 micron.

10. The device of claim 8 or 9 in which the hollow element is so located and fixed in the flow channel to permit flow through the element, and by-pass flow of at least equal volume along the outside of the fixed element.

11. The device of claims 6-10 in which the element is inserted into its microfluidic channel by pick-and-place motion effected by automated tweezer fingers engaging oppositely directed portions of the element.

12. The device of claim 11 in which the oppositely directed portions are parallel planar surfaces.

13. The device of 6-10 in which the element is inserted into its microfluidic channel by pick-and-place motion effected by automated vacuum pick up.

14. The device of claim 13 in which the vacuum pickup is effected by a device which engages an outer cylindrical surface of the element.

15. The device of any of the foregoing claims in which flow channel closure, flexible diaphragm for fluid-actuated valve or on-board pump diaphragm, preferably all three, are provided by respective portions of a flexible sheet that in other places of substantial area is joined by bonding to an opposed surface.

16. The device of claim 15 in which the flexible diaphragm sheet is comprised of a non-elastomeric, non-air-permeable flexible sheet, preferably a polyester film.

17. The device of claim 16 in which the flexible sheet is metallized, preferably with aluminum, to reflect incident or fluorescent light with respect to detector optics.

18. The device of claim 17 in which the detector is of epi-fluorescence type, and the metallized sheet is positioned to reflect incident excitation light and fluorescing light associated with the presence of a desired analyte.

19. The device of claim 16, 17or 18 in which the flexible non-air-permeable sheet is bonded face to face with an elastomeric film exposed for contact with the fluid sample.

20. The device of claim 15 in which the flexible sheet comprises elastomer, preferably PDMS.

21. The device of any of the foregoing claims in which the device is constructed to conduct multiplex assays within a single portable assay cartridge (chip).

22. The device of any of the foregoing claims in which at least some parts of the device are joined by co-valent bonding of activated surfaces of bondable material, a

contiguous portion of the same sheet fixing the position of a said detection element in its flow channel.

23. The device of any of the foregoing claims in which at least some parts of the device are joined by co-valent bonding of activated surfaces of bondable material, a

contiguous portion of the same sheet forming a flexible pump diaphragm.

24. The device of any of the foregoing claims in which at least some parts of the device are joined by co-valent bonding of activated surfaces of bondable material, a

contiguous portion of the same sheet forming a flexible valve diaphragm.

25. The device of claim 24 in which the flexible valve diaphragm portion engages a valve seat originally formed of surface-activated bondable material that has been subjected to a series of make-and break contacts that interrupt covalent bonding of the valve diaphragm portion with its opposed seat.

26. The device of any of the foregoing claims in which at least some parts of the device are joined by co-valent bonding of activated surfaces of bondable material, and respective contiguous portions of the same sheet seal an open side of a flow channel, fix the position of a

said detection element in its flow channel, form a flexible pump diaphragm or form a flexible valve diaphragm, preferably respective portions of the sheet performing all of these functions.

27. The device of any of the foregoing claims in which parts are permanently secured by co-valent bonding of selected regions of faces of surface-activated bondable materials.

28. The device of claim 27 in which the form of activation is oxidation.

29. The device of claim 27 or 28 in which at least one of the parts comprises surface activatable elastomer.

30. The device of claim 29 in which the elastomer is PDMS.

31. The device of claim 29 or 30 in which the bond is formed by opposed surfaces of surface-activated PDMS.

32. The device of claim 30 in which the bond is formed by one opposed surface of surface-activated PDMS and the other surface is surface-activated glass or polymer other than PDMS.

33. The device of any of the foregoing claims formed by preparation of two subassemblies, each having a backing of relatively rigid material and an oppositely directed face suitable for bonding to a mating face of the other subassembly, followed by bonding the assemblies face-to-face.

34. The device of claim 33 in which the bonding creates a permanent bond, preferably, in the case of like surfaces, such as of PDMS, a bond of surface-activated surfaces in which the original structure of mating surfaces is substantially eliminated by molecular diffusion.

35. The device of claim 33 in which the bond is separable such as for enabling re-use of the device.

36. The device of claim 35 in which the bonding is substantially formed by electrostatic attraction.

37. The device of any of the foregoing claims in which the detection element comprises a (preferably cylindrical) hollow flow element, the element being substantially uniformly coated on its inner surface with capture agent for a selected fluid assay.

38. The device of claim 37 in which the capture agent is antibody for conducting ELISA.

39. The device of claim 37 or 38 in which capture agent is substantially absent from all outer surfaces of the detection element, and the detection element is sized, relative to the channel in which it is inserted, to define a substantial flow path through the element and a substantial by-pass flow path along the exterior of the element.

40. The device of any of the foregoing claims in which the detection element is of depth greater than the depth of an open channel in which it is inserted, and a capturing layer closes and seals the channel, the capturing layer being elastically deformed by its contact with the detection element thereby applying forces thereto that fix the location of the element in the channel.

41. The device of claim 40 in which the capturing layer is co-valently bonded to the substance defining the open channel.

42. The device of claim 40 or 41 in which the capturing layer and the substance both comprise PDMS.

43. The device of claim 40, 41 or 42 in which a portion of the capturing layer forms a valve diaphragm adapted to engage a valve seat formed by opposed material, the portion having

been subjected to repeated make-and-break-seat-contact manufacturing protocol that interferes with co-valent bonding of the mating valve surfaces.

44. The device of any of the foregoing claims constructed to perform ELIS biological assay.

45. The device of any of the foregoing claims in which a series of between about 3 and 10 spaced-apart discrete detection elements of less than 700 micron length are fixed in a given channel.

46. The device of any of the foregoing claims in which the device contains a means of providing a fluorophor label to captured analyte, and the detection elements are exposed to a window transparent to outwardly proceeding fluorescent emission for detection.

47. The device of claim 46 in which the window is transparent to exterior–generated stimulating light emission to enable epi-fluorescent detection.

48. A microfluidic device for conducting a fluid assay, for example a biological assay, having a flow channel in which is inserted at least one discrete detection element that is provided with capture agent, the detection element being positioned for exposure to fluid flows within the device for conducting an assay, the device formed by preparation of two subassemblies, each having a backing of relatively rigid material and an oppositely directed face suitable for bonding to a mating face of the other subassembly, followed by bonding the assemblies face-to-face.

49. The device of claim 48 in which the bond is breakable, such as an electrostatic bond, to enable detachment of the two subassemblies.

50. The device of claim 48 in which the bond is permanent, formed by bonding together two surface-activated surfaces.

51. The device of claim 49 or 50 in which the member defining one of the surfaces has portions that fix the position of a said detection element in its flow channel, form a flexible pump diaphragm or form a flexible valve diaphragm, preferably respective portions of the sheet performing all of these functions.

52. The device of claim 51 in which a flexible valve diaphragm portion engages an opposed valve seat originally formed of surface-activated bondable material that has been subjected to a series of make-and break contacts that interrupt covalent bonding of the valve diaphragm portion with its opposed seat.

53. The device of claim 50, 51 or 52 in which the surfaces are both of PDMS.

54. A microfluidic device for conducting a fluid assay, for example a biological assay, having a flow channel in which is inserted at least one discrete detection element comprising an extremely short hollow flow element with length less than about 700 micron, and internal diameter of between about 50 +/- 25 micron, in fixed position, that is provided with capture agent, the flow element being positioned for exposure to fluid flows within the device for conducting an assay, the flow element being secured in fixed position by an overlaying layer of material that is surface-activated and bonded by molecular bonding to an opposing member in adjacent regions.

55. A microfluidic device for conducting a fluid assay, for example a biological assay, having a flow channel in which is inserted at least one discrete detection element (preferably an extremely short hollow flow element with length less than about 700 micron, and internal diameter of between about 75 +/- 50 micron)

in fixed position, that is provided with capture agent only on its interior, the flow element being positioned for exposure to fluid flows within the device for conducting an assay, the flow channel being of rectangular cross-section, the exterior of the element being of cylindrical cross-section, and by-pass flow paths are defined along the exterior of the element.

56. The microfluidic device of claim 55 in which the internal diameter of the hollow flow element is 50 +/- 25 micron.

57. The microfluidic device of claim 54, 55 or 56 in which the hollow flow element has length less than about 500 micron.

58. The microfluidic device of any of the foregoing claims comprising multiple substrates stacked together to create three primary layers: (a) Pneumatic/Fluidic Interface Layer, (b) Channel Closure layer, and (c) Fluidic/Reaction Vessel layer into which is inserted at least one reaction vessel (preferably a hollow flow element).

59. The microfluidic device of claim 58 containing microfluidic valves and pistons for driving, controlling, and manipulating the fluid flow.

60. The microfluidic device of claim 58 or 59 in which the Pneumatic/Fluidic Layer comprises a glass sheet upon which a flexible polymer film with through-cut channels is attached such that they form open channels which are closed on one side by the glass sheet and open on the other.

61. The microfluidic device of claim 58, 59 or 60 in which the Fluidic/Reaction vessel layer is comprised of a thin glass sheet, such as a sheet 200 micron thick, upon which a flexible polymer film with through-cut channels is attached, such they form open channels which are closed on one side by the glass sheet and open on the other.

62. The microfluidic device of claim 61 in which the channels provide a path for fluid, one or more channels to house reaction vessels (preferably inserted hollow flow elements), and provide features for on-board valves and pistons.

63. The microfluidic device of claim 62 in which reaction vessels in the form of hollow elements are inserted into the Fluidic/Reaction Vessel layer, and then it is attached to the

Channel Closure Layer, closing off the top of the channels in the Fluidic/Reaction Vessel layer and thereby forming closed channels.

64. The microfluidic device of any of claims 58-63 in which the Channel Closure Layer is permanently bonded to the Pneumatic/Fluidic Layer closing off the top of the channels in the Pneumatic/Fluidic Layer and thereby forming closed channels.

65. The microfluidic device of any of claims 58-64 in which the Channel Closure Layer is formed by attaching a mylar sheet (which may have a reflective coating and which may be attached to a sheet of flexible polymer).

66. The microfluidic device of any of claims 58-64 in which the Channel Closure Layer is formed by a sheet of flexible polymer.

67. The microfluidic device of claims 66 in which the Channel Closure Layer is comprised of PDMS.

68. The microfluidic device of any of claims 58-67 in which the Channel Closure Layer has through hole vias to allow the passage of fluids and pneumatics from the Pneumatic /Fluidic layer to the Fluidic/Reaction Vessel layer.

69. The microfluidic device of any of claims 58-68 in which the Channel Closure Layer is constructed to flex as part of valve actuation.

70. The microfluidic device of any of claims 58-69 in which the Channel Closure Layer is constructed to flex as part of pump actuation.

71. The microfluidic device of any of claims 58-70 within which a valve is actuated by applying negative pressure to Pneumatic channels contained in the Pneumatic/Fluidic Layer,

thereby flexing the Channel Closure Layer to lift it off a wall of the Fluidic/Reaction Vessel Layer, allowing fluid to flow.

72. The microfluidic device of any of claim 71 in which the negative pressure is about -8 psi.

73. The microfluidic device of any of claims 58-72 within which a valve is caused to maintain a tight seal on a wall of the Fluidic/Reaction Vessel Layer when negative pressure on the channel Closure Layer is released and the Closure Layer is allowed to relax, by applying positive pressure to Pneumatic channels contained in the Pneumatic/Fluidic Layer.

74. The microfluidic device of any of claim 73 in which the positive pressure is about +5 psi.

75. The microfluidic device of any of claims 58-74 within which a piston exposed to a Fluidic Channel is actuated by applying negative and positive pressure to Pneumatic channels contained in the Pneumatic/Fluidic Layer, thereby flexing the Channel Closure Layer and creating negative and positive pressure within the Fluidic Channel to which the piston is exposed.

76. The microfluidic device of claim 75 in which the negative pressure is about -8 psi and the positive pressure is about +5 psi.

77. The microfluidic device of claim 75 or 76 in which the piston is arranged with a microfluidic valve on either side which can be actuated in sequence to drive fluid in two directions in the channel.

78. The microfluidic device of claim 75, 76 or 77 having a fluidic channel along which is arranged a said piston and in which is inserted at least one of said elements, the piston arranged to cause the element to be exposed to flow.

79. The microfluidic device of claim 78 in which a plurality of said elements are in the channel for exposure to flow.

80. The microfluidic device of claim 78 or 79 in which the elements are hollow elements through the interior of which fluid flows.

81. The microfluidic device of claim 78, 79 or 80 in which the hollow detection elements are so located in the channel to permit flow through the element, and by-pass flow along the outside of the hollow element.

82. The microfluidic device of any of the claims 75 to 81 having a plurality of isolated channels along each of which is arranged a respective piston and in which is inserted at least one of said elements, preferably a respective series of spaced apart elements.

83. The microfluidic device of claim 82 in which the Fluidic/Reaction Vessel Layer is arranged with flow paths from a single sample supply through each of the plurality of isolated channels in response to pistons located in the respective isolated channels.

84. The microfluidic device of claim 82 or 83 in which the Fluidic/Reaction Vessel Layer is arranged with a plurality of inlet paths, for example buffer liquid and reagents, for flow to each of the plurality of isolated channels in response, respectively, to pistons located in respective isolated channels.

85. The microfluidic device of claim 82, 83 or 84 in which the Fluidic/Reaction Vessel Layer is arranged with a path from each of the plurality of isolated channels to waste in response, respectively, to pistons located in the respective isolation channels.

86. The microfluidic device of any of the claims 82 to 85 in which there is a common waste channel to which each of the pistons can propel liquid from its respective isolated channel.

87. The microfluidic device of any of the claims 58-86 comprising a reaction vessel channel exposed to a piston and containing at least one reaction vessel, the reaction vessel channel in communication through respective valves to inlet channels for sample, reagent and buffer liquid, and to an outlet channel to waste, pumps for the reaction vessel being effectively formed by cooperative action of each inlet valve with the piston and the outlet valve, the pumps being selectively operable to selectively cause flows through the at least one reaction vessel of sample, reagent and buffer liquids in performance of an assay, preferably, ELISA.

88. The microfluidic device of claim 87 in which there are a plurality of isolated reaction vessel channels, each exposed to a respective piston and containing at least one respective reaction vessel.

89. The microfluidic device of claim 87 or 88 in which each reaction vessel channel contains a plurality of reaction vessels, preferably the plurality comprising a series of spaced apart hollow flow elements.

90. A discrete detection element in the form of an extremely short hollow flow element with length less than about 700 micron, and internal diameter of between about 50 +/- 25 micron, the flow element provided with capture agent, the flow element being constructed to be fixed in position for exposure to fluid flows within a device for conducting an assay.

91. The discrete detection element of claim 90 in which the hollow flow element has length less than about 500 micron.

92. The discrete detection element of claim 90 or 91 in which capture agent resides only on the interior surface of the element.

93. A discrete hollow flow element carrying on its interior surface, but not its exterior surface, an assay capture agent, the element fixed in position in a fluid channel in manner that provides at least about 50 % by-pass flow capacity relative to the flow capacity through the element.

94. The hollow element of claim 93 in which the by-pass flow capacity is about 75% or more, relative to the flow capacity through the element.

95. The hollow element of claim 93 in which the by-pass flow capacity is about 100% or more, relative to the flow capacity through the element.

96. A method of manufacturing the device or element of any of the foregoing claims.

97. A method of use of the device or element of any of the foregoing claims 1-95.

98. A method of preparing detection elements for an assay comprising batch coating the detection elements, preferably hollow flow elements, by mixing in solution, and drying, and thereafter picking and placing the elements in flow channels of a microfluidic device, and preferably capturing the flow elements by bonding two opposed layers that capture the elements while sealing the flow channels.

99. A method of preparing hollow flow elements for an assay comprising batch coating the detection elements to coat the elements with capture agent, including eliminating or preventing the occurrence of active capture agent on outside surfaces of the hollow elements.

100. The method of claim 99 in which a suspension of the hollow elements in fluid with the capture agent is aggressively agitated (preferably by vortexing) to impart disrupting shear forces to the exterior surface of the elements, the shear force acting to prevent binding of the capture agent to the outside surface of the elements.

101. The method of claim 99 in which the exterior surface of the hollow elements is in treated condition that prevents formation of a coating on the exterior surface.

102. The method of claim 99 in which coated capture agent is removed or rendered inactive by selective exposure to a laser elimination process that removes or de-activates capture agent from a surface of a coated element.

103. A microfluidic valve comprising a flexible valve diaphragm portion arranged to engage a valve seat originally formed of surface-activated bondable material that, during bonding of adjacent surfaces, has been subjected to a series of make-and break contacts that interrupt covalent bonding of the valve diaphragm portion with its opposed valve seat.

104. The valve of claim 103 in which the flexible valve diaphragm and the valve seat are comprised of PDMS.