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(54) Title: PRINTED MULTI-ZONE MICROZONE PLATES

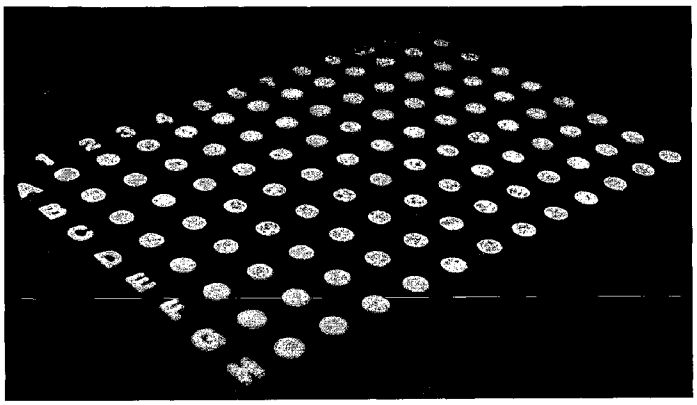

Figure 1

(57) Abstract: A multi-zone microzone plate for use in multi-assay analyses including a substrate having a hydrophobic surface, and a microzone pattern printed on the substrate surface, wherein the microzone pattern includes hydrophilic material therein, and a method of producing thereof.

PRINTED MULTI-ZONE MICROZONE PLATES

FIELD OF THE INVENTION

The present invention is generally directed to multi-assay analyses which are widely used in chemical and biological analysis, drug evaluation, ELISA and large scale disease screening, and in particular to a multi-zone microzone plate, and method of producing thereof.

BACKGROUND TO THE INVENTION

Many analytical systems in healthcare, health screening, drug screening/evaluation, pathological analyses require multi-assay work. The multi-assay work is essential, as it provides (a) rapid evaluation of certain chemical and biochemical reactions under many different conditions; (b) rapid results to determine anti-body and antigen in human samples; and (c) rapid evaluation. As multi-assay systems can perform a large number of experiments in a short time, it has become a reliable tool in medical tests, biomedical research and drug research.

Multi-assay experiments are primarily done by using 96-well plates, which is the most widely used consumable item in those tests. The 96-well plates are typically made by polymer moulding, are expensive and require a significant amount of samples (10-500 micro litres). The current market price of the 96-well plates are around AU$10 and are therefore not cheap enough to be used for underdeveloped countries/regions for human healthcare and disease screening.

Carrilho *et al* [Emanuel Carrilho, Scott T. Phillips, Sarah J. Vella, Andrew W. Martinez and George M. Whitesides, Anal. Chem., 2009, 81 (15), pp 5990-5998] have proposed a fabrication method for paper-based 96- and 384- zone plate. Their method is to apply photolithographic photo resist to a paper sheet to form hydrophobic barriers which encircle hydrophilic paper zones. Those paper zones are capable of absorbing liquid samples. Paper, which is made of cellulose fibres, is an excellent material for colorimetric analysis. However, paper can deform when experiencing repeated wetting and rewetting cycles. Also, it is not always easy to chemically functionalize cellulose fibre in the paper without deforming the sheet. Carrilho *et al* reported that microzone plate made of paper requires 2-60 micro litres of liquid sample to fill up one zone; such a sample quantity is quite large. It is also likely that some barriers will break and sample

leakage from one zone to the next is likely to happen. Another disadvantage of the method reported by Carrilho *et al* is that it is difficult to use their method to mass produce paper-based microzone plates at high speed. Since microzone plates made of low cost materials are designed to be disposable items, mass production needs to be made possible.

It is therefore an object of the present invention to provide a multi-zone microzone plate that overcomes at least one of the disadvantages associated with known systems.

It is another object of the present invention to provide a method for producing a multi-zone microzone plate using different materials and a printing process to facilitate mass production of the microzone plates.

BRIEF DESCRIPTION OF THE INVENTION

According to one aspect of the present invention, there is provided a multi-zone microzone plate for use in multi-assay analyses including a substrate having a hydrophobic surface, and a microzone pattern printed on the substrate surface, wherein the microzone pattern includes hydrophilic material therein.

According to another aspect of the present invention, there is provided a method of producing a multi-zone microzone plate for use in multi-assay analyses, including printing a microzone pattern on a hydrophobic surface of a substrate, the microzone pattern including hydrophilic material therein.

The present invention provides a novel method to make low-cost, disposable multi-zone microzone plates by means of printing. The multi-zone microzone plate made using this method is capable of conducting multi-sample assay for chemical and biological analysis and ELISA-type analysis.

The present invention preferably uses polymer films of thickness of more than 10 microns as a substrate. Alternatively, water repellent paper such as photographic paper, wax paper, strongly sized paper, or non-woven non-cellulosic paper as used for example for strong envelopes, may be used as the substrate. The films or paper can be transparent, opaque or having any background colour for purpose of sample colour or fluorescent detections.

The present invention can also use laminated polymer films of thickness of more than 10 microns as a substrate. The laminated polymer films may have any background colour for purpose of sample colour or fluorescent detections.

Similarly the water-resistant paper may have a thickness of more than 10 microns, translucent, opaque or have a background colour.

One preferred method is to use UV-curable or any other types of energy curable fluids or varnish as the printing ink; these fluids and varnish include IR curable, electron beam curable, microwave curing, and moisture curable fluids.

This method may use contact (or non-contact printing *i.e.* digital inkjet printing) to print the above mentioned ink onto the substrate. Flexographic, gravure, lithographic, xerographic, wax printing, ink jet printing processes, etc. can therefore be used to print a microzone pattern of ink onto the substrate.

This method preferably uses cellulose powder (or any polymer powder or mineral powder, or any synthetic or natural inorganic or organic powder) to form a porous pattern over the printed UV-curable ink. The powder sticks to the printed but uncured ink film and therefore forms porous microzone pattern. The powder can be single component, or a mixture of multi-component powder, or surface modified powder. One or more different hydrophilic powders may be laid in layers to the ink or varnish.

This method may use a UV ink dryer, or any other type of ink curing device that matches the drying mechanism of the ink, including an IR dryer and electron beam dryer, microwave dryer, to cure the ink and therefore fix the porous cellulose microzone pattern on the film.

Another preferred method is to use a suitable aqueous or non-aqueous, organic or non-organic binder system and cellulose (or other, polymer or mineral) powder to form printable paste "inks". Contact printing and patterned coating methods are used to print or coat the paste ink onto polymer film to form the required microzone pattern. The evaporation of the solvent or water (either naturally or aided by thermal and other forms of energy) from the paste ink will allow the ink to cure on the film and form a porous microzone pattern. The binder in the ink promotes adhesion of between the dried ink and the film.

In the microzone plate according to the present invention, the microzone pattern created by printing may be porous and able to absorb small quantity of liquid. The microzone pattern created by printing according to the present invention can be wetted and rewetted without deforming.

Chemical, biological reactions can be performed in the microzones of the plate and the colour developed in these microzones can be measured using a camera or a scanner and any suitable software such as PhotoShop (Registered Trademark of Adobe Systems).

According to the present invention, chemical, biological reactions can be performed in the microzones and the colour developed in the microzones can be measured using suitable reflective or transmission optical densitometers. Alternatively, chemical, biological reactions can be performed in the microzones and fluorescent signal developed in the microzones can be measured using a plate reader or any other suitable type of fluorescence measurement apparatus. Furthermore, chemical, biological reactions can be performed in the microzones and detection reaction can be monitored by means or electrochemical detection.

BRIEF DESCRIPTION OF THE DRAWINGS

It will be convenient to further describe the present invention with respect to the accompanying drawings which illustrate possible examples according to the present invention. Other examples of the invention are possible, and consequently, the particularity of the accompanying drawings is not to be understood as superseding the generality of the preceding description of the invention.

In the drawings:

Figure 1 is a photo showing a 96-zone microzone plate fabricated on polypropylene overhead transparency film according to the present invention;

Figure 2 is photo showing a porous microzone pattern printed using a paste made of cellulose powder and starch solution according to the present invention;

Figure 3 is a photo of a printed 96-zone microzone plate according to the present invention showing the colorimetric differences of liquids added to the plate.

Figure 4 is a graph sowing the reflective colour density measurement of colour density of the inked zones of the microzone plate of Figure 3;

Figure 5 is a plan of using a printed microzone plate according to the present invention to determine the chemical interference of UA to NO_2^-;

Figure 6 is a photo of the interference analysis of a microzone plate according to the present invention; and

Figure 7 is a graph showing four calibration curves of NO_2^- in the presence of different concentrations of UA.

DETAILED DESCRIPTION OF THE INVENTION

The following description describes a number of different examples showing the multi-zone microzone plate and the method of producing the microzone plate according to the present invention, as well as applications of the microzone plate.

Example 1 – Printing of microzone plate using polymer film, UV curable ink based and cellulose powder

A 96-zone printing master was made using rubber. The size of the dots on the master is 3 mm in diameter. A UV curable post print varnish (UV 412, Flint Inks) was used as the ink. The rubber printing master was inked with the varnish and printed onto an overhead transparency by contact. The printed microzone pattern on the transparency film was then exposed to cellulose powder (Microgranullar cellulose, Aldrich). Cellulose powder stuck to the printed varnish microzone pattern, forming a pattern of cellulose powder. The microzone plate was then exposed to UV radiation (UV ink dryer), the printed varnish was cured. Curing of the varnish allows cellulose powder to strongly stick onto the microzone plate, forming a porous surface of the microzone pattern. Figure 1 shows the printed microzone plate on an overhead transparency.

Example 2 – Printing of cellulose-binder paste ink on the polymer film

A cellulose powder pattern were printed using a cellulose-binder paste "ink" onto an overhead transparency film using contact printing. A starch solution (0.5%, w/w) was cooked at 95°C until the solution became transparent; the solution was left to cool down to the room temperature. One gram of cellulose powder was mixed with 30 ml of starch solution aided by strong stirring to form a printable paste cellulose "ink". A rubber master was used for contact printing and the dot size on the master was 2 mm diameter. The printed pattern was let dry. A blue aqueous liquid was then introduced from the centre of the pattern to demonstrate the wettability of the pattern. The pattern was rapidly and fully wetted by the blue liquid. Figure 2 shows the printed pattern fully wetted by the

blue liquid. (The printed pattern can also handle organic solvent, and can use oil to match refractive index for transmission analysis)

Example 3 – Liquid sample receptive ability and colorimetric development ability of the printed 96-zone pattern

Three ink jet printing inks were used to test the microzone plate according to the present invention having 96 zones. The inks were diluted in a two-fold sequent – row A 100% (undiluted), row B 50%, row C 25%, row D 12.5% and row E 6.25%. One microliter of ink was added to each zone. Figure 3 shows the photo colorimetric development of all liquids in the 96-zone pattern showing the colorimetric differences. Figure 4 shows reflective density measurements of the colour density of the inked zones.

Example 4 – Reaction interference study using the printed 96-zone plate

Uric acid (IA) and NO_2^- are two important biomarkers of several human health conditions. They present in human body fluid such as blood and saliva. Although detection methods for UA and NO_2^- are available in the literature, their detection interference must be investigated. This is because that these two biological species co-exist in human samples.

The printed 96-zone plate was used to investigate the detection interference of UA to NO_2^-. Figure 5 shows the division of the printed 96-zone plate into four regions, region 1, 2, 3 and 4. Each region tests a series of NO_2^- solution in the presence of UA of a fixed concentration. Therefore the four regions test a series of NO_2^- samples with the UA concentration ranging from 0-1000 µM. Figure 6 shows the photo of the printed 96-zone plate filled with analyte (NO_2^-) and interferent (UA). Figure 7 shows the colour density measurement of the tests. Colour density measurements of NO_2^- were plotted against NO_2^- concentrations, with the interference (UA) concentration in the legend box to provide four calibration curves. Results show that UA does not have significant interference to NO_2^- measurement, and the present of UA in NO_2^- samples does not cause significant error of measurement. This result is in agreement with the tests published by the Harvard Medical School [Blicharz TM, Rissin DM, Bowden M, Hayman RB, DiCesare C, Bhatia JS, Grand-Pierre N, Siqueira WL, Helmerhorst EJ, Loscalzo J, Oppenheim FG, Walt DR (2008) Clin Chem 54:1473-1480].

This example shows the capability of the printed plates to sort out chemical interference issues under many different conditions very quickly. The printed plate is therefore able to conduct multi-chemical reactions in a short time.

Modifications and variations as would be deemed obvious to the person skilled in the art are included within the ambit of the present invention as claimed in the appended claims.

CLAIMS:

1. A multi-zone microzone plate for use in multi-assay analyses including a substrate having a hydrophobic surface, and a microzone pattern printed on the substrate surface, wherein the microzone pattern includes hydrophilic material therein.

2. A multi-zone microzone plate according to claim 1 wherein the substrate is formed from a water resistant paper.

3. A multi-zone microzone plate according to claim 1, wherein the substrate is formed from a polymer film.

4. A multi-zone microzone plate according to claim 3, wherein the polymer film is a laminate.

5. A multi-zone microzone plate according to any one of claims 2 to 4, wherein the substrate has a thickness greater than 10 microns.

6. A multi-zone microzone plate according to any one of the preceding claims, wherein the substrate is transparent or translucent.

7. A multi-zone microzone plate according to any one of claims 1 to 5, wherein the substrate is opaque.

8. A multi-zone microzone plate according to any one of the preceding claims, wherein the substrate is coloured.

9. A multi-zone microzone plate according to any one of the preceding claims, wherein the microzone pattern is printed using an ink or varnish, a hydrophilic powder being adhered to the ink or varnish prior to curing to form a porous said microzone pattern.

10. A multi-zone microzone plate according to claim 9, wherein the ink or varnish include UV curable, IR curable, electron beam curable, microwave curable, and other energy curable fluids, and moisture curable fluids.

11. A multi-zone microzone plate according to claim 8 or 9, wherein the hydrophilic powder is formed from materials including cellulose, polymer, glass bead or fibre, or mineral.

12. A multi-zone microzone plate according to claim 11, wherein one or more different hydrophilic powders are applied in layers to the ink or varnish.

13. A multi-zone microzone plate according to any one of claims 1 to 8, wherein the microzone pattern is printed using a mixture of an aqueous or non-aqueous, organic or non-organic binder and a hydrophilic powder which when dry forms a porous said microzone pattern.

14. A multi-zone microzone plate according to claim 13, wherein the hydrophilic powder is formed from materials including cellulose, polymer, glass bead or fibre, or mineral.

15. A method of producing a multi-zone microzone plate for use in multi-assay analyses, including printing a microzone pattern on a hydrophobic surface of a substrate, the microzone pattern including hydrophilic material therein.

16. A method according to claim 15, wherein the substrate is formed from a water resistant paper.

17. A method according to claim 15, wherein the substrate is formed from a polymer film.

18. A method according to claim 17, wherein the polymer film is a laminate.

19. A method according to any one of claims 16 to 18, wherein the substrate has a thickness greater than 10 microns.

20. A method according to any one of claims 17 to 19, wherein the substrate is transparent or translucent.

21. A method according to any one of claims 17 to 19, wherein the substrate is opaque.

22. A method according to any one of claims 17 to 21, wherein the substrate is coloured.

23. A method according to any one of claims 15 to 22, including printing the microzone pattern using an ink or varnish, a hydrophilic powder being adhered to the ink or varnish prior to curing to form a porous said microzone pattern.

24. A method according to claim , wherein the ink or varnish include UV curable, IR curable, electron beam curable, microwave curable, and other energy curable fluids, and moisture curable fluids.

25. A method according to claim 19 or 20, wherein the hydrophilic powder is formed from materials including cellulose, polymer, glass beads or mineral.

26. A method according to claim 25, wherein one or more different hydrophilic powders are applied in layers to the ink or varnish.

27. A method according to any one of claims 15 to 22, including printing the microzone pattern using a mixture of an aqueous or non-aqueous or organic or non-organic binder and a hydrophilic powder which when dry forms a porous said microzone pattern.

28. A method according to claim 28, wherein the hydrophilic powder is formed from materials including cellulose, polymer, glass bead or fibre, or mineral.

29. A method according to any one of claims 15 to 28, including printing the microzone pattern using a contact printing process including flexographic, gravure and lithographic processes.

30. A method according to any one of claims 15 to 28, including printing the microzone pattern using a digital printing process including xerographic, wax printing and ink jet printing.

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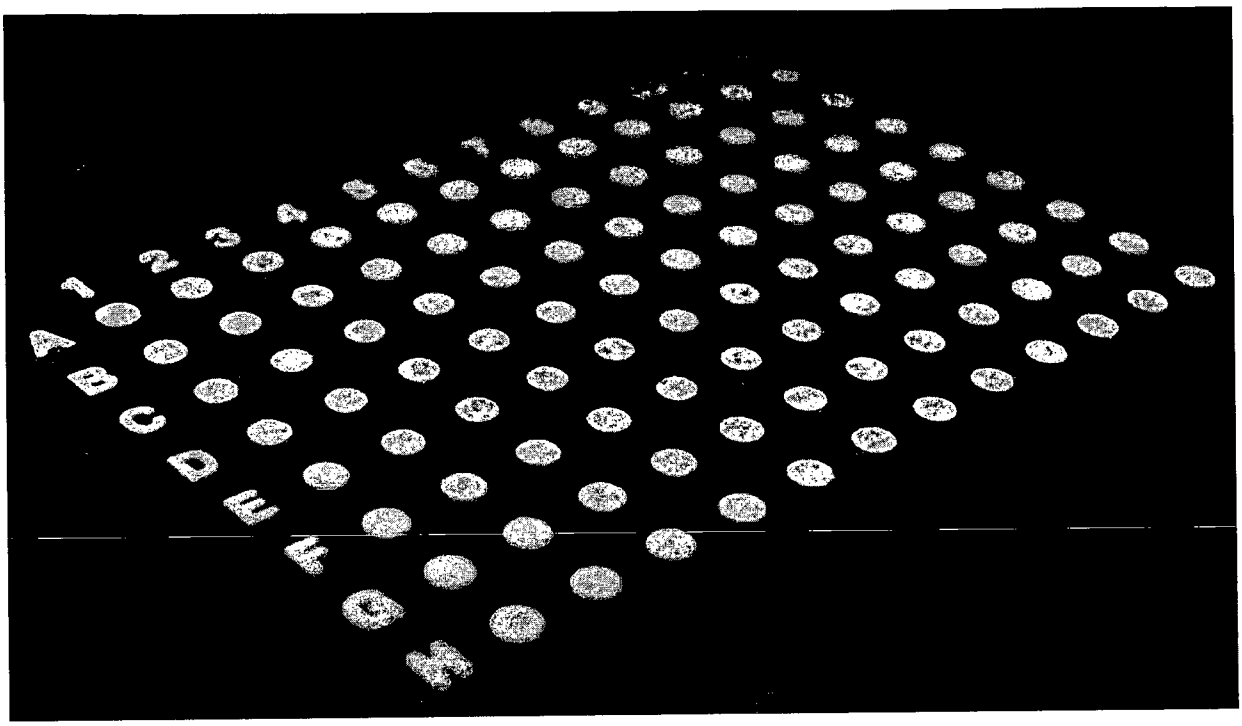

Figure 1

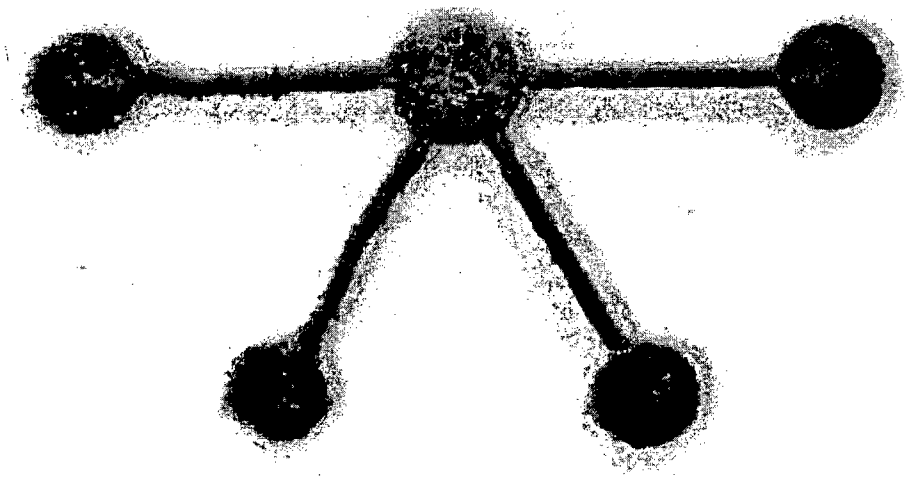

Figure 2

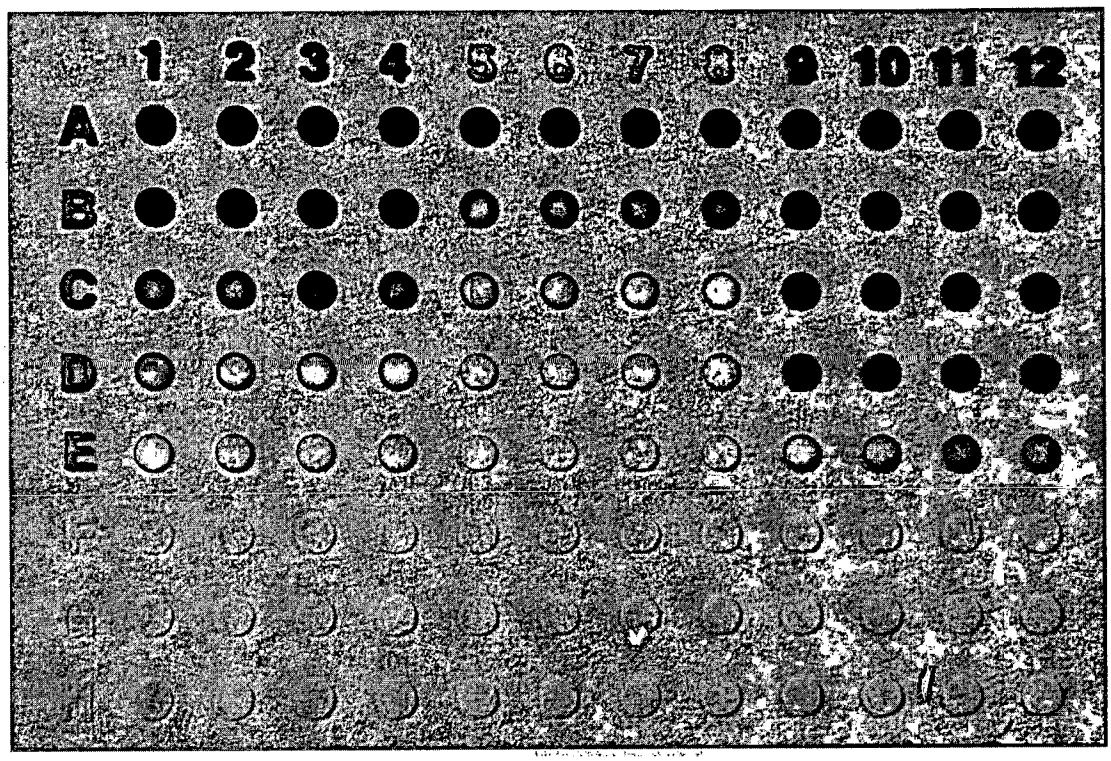

Figure 3

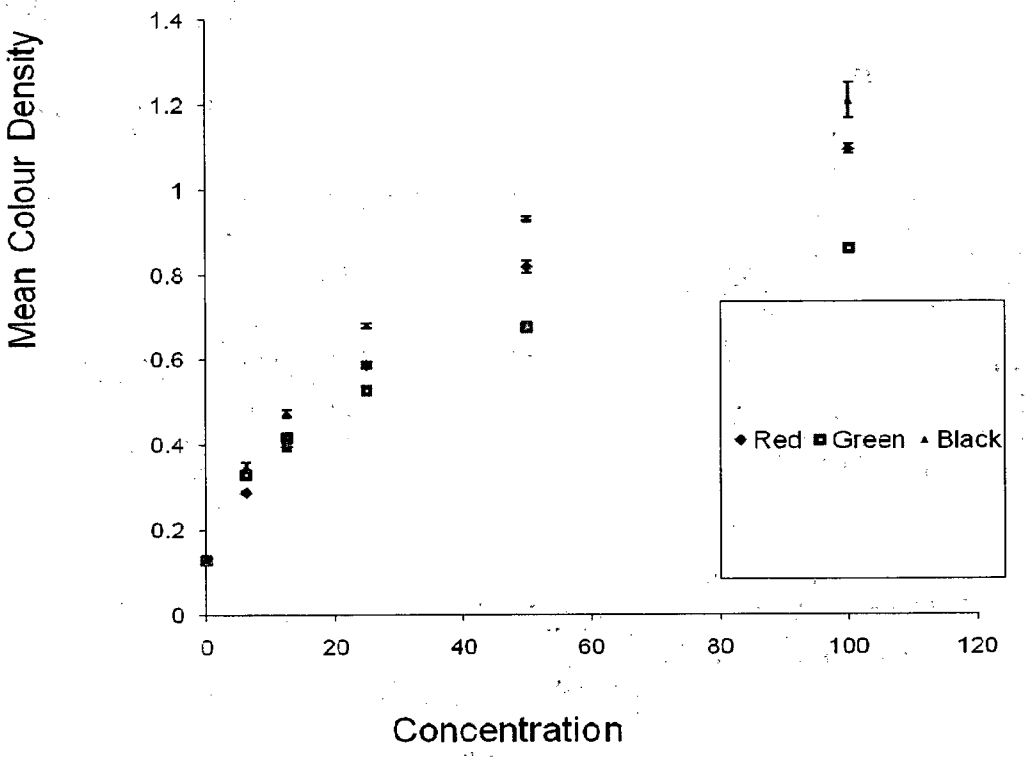

Figure 4

	1 2 3 4 5 6	7 8 9 10 11 12
A B C D	Region 1 NO_2^- : 0, 62.5, 125, 250, 500, 1000 µM UA: 0 µM in all samples	Region 2 NO_2^- : 0, 62.5, 125, 250, 500, 1000 µM UA: 100 µM in all samples
E F G H	Region 3 NO_2^- : 0, 62.5, 125, 250, 500, 1000 µM UA: 500 µM in all samples	Region 4 NO_2^- : 0, 62.5, 125, 250, 500, 1000 µM UA: 1000 µM in all samples

Figure 5

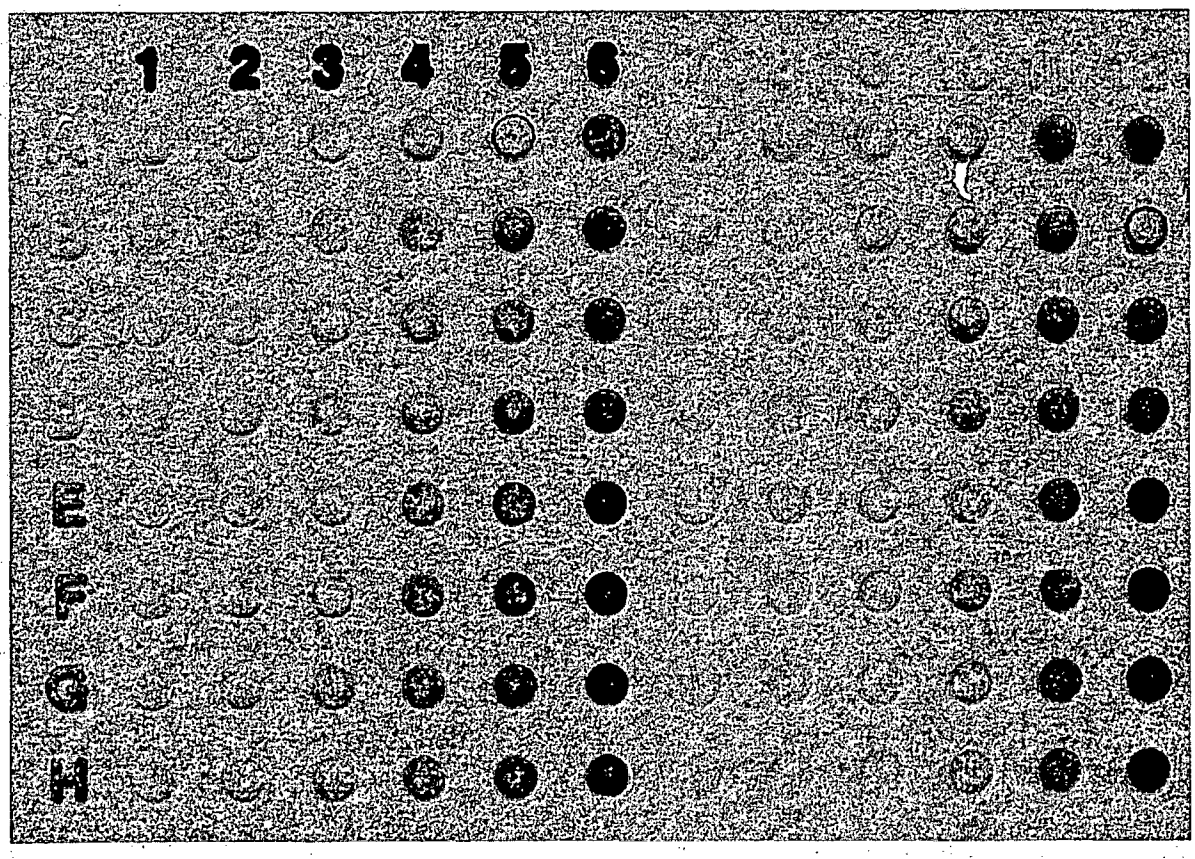

Figure 6

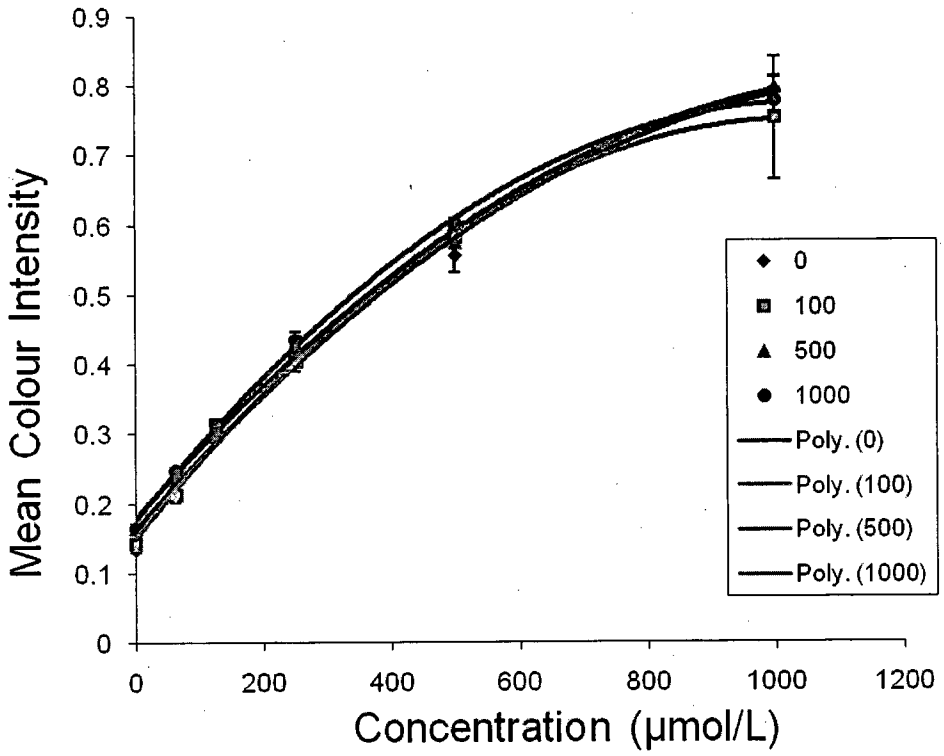
Mean Colour Intensity
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
Concentration (µmol/L)
0
100
500
1000
Poly. (0)
Poly. (100)
Poly. (500)
Poly. (1000)

Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2011/000138

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

G03F 7/038 (2006.01) ***C12M 1/00*** (2006.01) ***G03F 7/00*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPODOC, WPI, CA, GOOGLE: ink, microtiter, microarray, bioassay, hydrophobic, hydrophilic, lithography, print, stamp, inkjet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/039286 A2 (PRIMORIGEN BIOSCIENCES, LLC) 26 March 2009 See [0016], [0080], [0088], [0089], [0129], p. 19	1-5, 7-17, 19, 21-25, 29
X	WO 2003/031054 A2 (SURMODICS, INC.) 17 April 2003 See example 1 and 2, p. 6 lines 25-34	1-5, 7-17, 19, 21-30
X	US 6,287,872 B1 (SCHÜRENBERG ET AL.) 11 September 2001 See abstract, col. 6 line 10	1-3, 5, 7, 8, 15-17, 19, 21, 22, 30
X	US 2002/0045270 A1 (SCHURENBERG ET AL.) 18 April 2002 See [0048], [0049]	1-3, 5, 7, 8, 15-17, 19, 21, 22, 30

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 March 2011	22 MAR 2011
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **MATTHEW FISHER** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2082

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2011/000138

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 284 495 A2 (MICROMASS LIMITED) 19 February 2003 See abstract, [0004]	1-3, 5, 7, 8, 15-17, 19, 21, 22, 29
X	WO 1998/045406 A1 (MINNESOTA MINING AND MANUFACTURING COMPANY) 15 October 1998 See abstract, figure 4 and 5A, p. 15 lines 21-32, p. 16 line 25, p. 17 lines 3 and 20-36	1-8, 11, 15-22, 25, 29
X	WO 1998/041323 A1 (MOXTEK, INC.) 24 September 1998 See p. 7, p. 13 line 27 – p. 14 line 26	1-5, 7, 8, 11, 15-19, 21, 22, 25, 29
A	US 2002/0034616 A1 (VANMAELE ET AL.) 21 March 2002 See whole document	
A	WO 2009/120963 A2 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 1 October 2009 See whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2011/000138

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2009039286	CN	101883865	EP	2207898	US	2009075837
		US	2009111709	WO	2009058867		
WO	03031054	CA	2462868	EP	1444034	MX	PA04003232
		US	2003099949				
US	6287872	DE	19754978	GB	2332273	US	2002051738
US	2002045270	DE	10043042	GB	2370114	US	2004197921
		US	7399640				
EP	1284495	CA	2398680	GB	2381068	GB	2413892
		US	2003116707	US	6952011	US	2005274885
		US	7294831	US	2008093548	US	7888637
		US	2006016984	WO	2004072616		
WO	9845406	AU	38299/97	AU	71471/98	BR	9714569
		BR	9814334	CA	2286195	CA	2315634
		EP	0973863	EP	1040181	US	2001024805
		US	6391578	WO	9932601		
WO	9841323	AU	64556/98	US	5958345		
US	2002034616	EP	1188481	EP	1245278	US	6783735
		US	2002057995	US	2004197236		
WO	2009120963	CN	101978272	EP	2265959	WO	2009121038

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX