Abstract: The present invention provides novel microfluidic substrates and methods that are useful for performing biological, chemical and diagnostic assays. The substrates can include a plurality of electrically addressable, channel bearing fluidic modules integrally arranged such that a continuous channel is provided for flow of immiscible fluids.

Title: MICROFLUIDIC DEVICES AND METHODS OF USE THEREOF
Filed on 7 July 2006 (07.07.2006) (71) Applicant (for all designated States except US): RAIN-
US 60/833,151 (CIP) DANCE TECHNOLOGIES, INC. [US/US]: 530 Whitfield Street, Guilford, CT 06437 (US).

Filed on 24 July 2006 (24.07.2006) (88) Date of publication of the international search report:
US 60/837,871 (CIP)
Filed on 14 August 2006 (14.08.2006) (71) Inventors; and
US 60/837,695 (CIP)
Filed on 14 August 2006 (14.08.2006) (75) Inventors/Applicants (for US only): LINK, Darren, R.
US 60/841,716 (CIP) [US/US]; 11 Stony Hill Road, Guilford, CT 06437 (US).
Filed on 1 September 2006 (01.09.2006) WEINER, Michael [US/US]; 55 Leighton Trail, Guilford,
Filed on 8 September 2006 (08.09.2006) (74) Agent: ERLIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris,
US 60/843,327 (CIP) Glovsky And Popeo PC, One Financial Center, Boston, MA 02111 (US).
Filed on 8 September 2006 (08.09.2006) (81) Designated States (unless otherwise indicated, for every
US 60/856,540 (CIP) kind of national protection available): AE, AG, AL, AM,
Filed on 3 November 2006 (03.11.2006) AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH,
US 60/856,440 (CIP) CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES,
Filed on 3 November 2006 (03.11.2006) FT, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN,
US 60/858,278 (CIP) IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR,
Filed on 8 November 2006 (08.11.2006) LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX,
US 60/858,279 (CIP) MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO,
Filed on 8 November 2006 (08.11.2006) RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM,
US 60/860,665 (CIP) TN, TR, TT, TZ, UA, UG, US (patent), UZ, VC, VN, ZA,
Filed on 22 November 2006 (22.11.2006) ZM, ZW
US 60/873,766 (CIP)
Filed on 8 December 2006 (08.12.2006) (84) Designated States (unless otherwise indicated, for every
US 60/874,561 (CIP) kind of regional protection available): ARIPA (BW, GH,
Filed on 12 December 2006 (12.12.2006) GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
US 60/874,640 (CIP) ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
Filed on 12 December 2006 (12.12.2006) European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, PT,
US 60/876,209 (CIP) FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL,
Filed on 20 December 2006 (20.12.2006) PT, RO, SE, SI, SK, TR), OAPI (BF, BZ, CF, CG, CI, CM,
US 60/899,258 (CIP) GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
Filed on 2 February 2007 (02.02.2007) Published:
US 60/903,153 (CIP) — with international search report
Filed on 23 February 2007 (23.02.2007) — before the expiration of the time limit for amending the
US 60/904,293 (CIP) claims and to be republished in the event of receipt of amendments
Filed on 28 February 2007 (28.02.2007) (88) Date of publication of the international search report:
US 60/920,337 (CIP) 21 February 2008
Filed on 26 March 2007 (26.03.2007) US 60/808,614 (CIP)
Filed on 25 May 2006 (25.05.2006)
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

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<th>B01F13/00</th>
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<th>B01L3/00</th>
<th>G01N15/14</th>
<th>G01N33/50</th>
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According to International Patent Classification (IPC) or to both national classification and IPC.

**B. MINIMUM DOCUMENTATION SEARCHED**

<table>
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<td>BOIF BOIJ BOIL GOIN</td>
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<th>Category*</th>
<th>Citation of document, with Indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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*1 Further documents are listed in the continuation of Box C

**K** 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 document but published on or after the priority date
  *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  *O* document referring to an oral disclosure, use, exhibition or other means
  *P* document published prior to the international filing date but later than the priority date claimed

**Date of the actual completion of the international search**

5 October 2007

**Date of mailing of the International search report**

18/12/2007

**Name and mailing address of the ISA/AEPI**

European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Tx 31 651 epo nl, Fax (+31-70) 340-3016

**Authorized officer**

Real Cabrera, Rafael
**INTERNATIONAL SEARCH REPORT**

**DOCUMENTS CONSIDERED TO BE RELEVANT**

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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>A</td>
<td>figures 7A,12H,13C</td>
<td>3,4,15, 19,20</td>
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<td>A</td>
<td>SONG ET AL: &quot;A Microrfluidic System for Controlling Reaction Networks in Time&quot;</td>
<td>1-28</td>
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<td>ANGEWANDTE CHEMIE, WILEY-VCH, WEINHEIM, DE,</td>
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<td>vol. 42, no. 7, 17 February 2003 (2003-02-17), pages 768-772, XP002289726</td>
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<td></td>
<td>ISSN: 1433-7851</td>
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<td>figure 5a</td>
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INTERNATIONAL SEARCH REPORT

INTERNATIONAL application No
PCT/US2007/011462

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1 □ Claims Nos., because they relate to subject matter not required to be searched by this Authority, namely

2 □ Claims Nos., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3 □ Claims Nos., because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows

see additional sheet

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. □ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ Required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos. 1 - 28

Remark on Protest □ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee

□ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

□ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-28

   Method of coalescing droplets of fluids using an electric field using a main channel having an expanded portion.

2. claims: 29-50

   Method of forming a droplet emulsion library of a sample fluid.

3. claims: 51-52

   Method of forming a uniformed sized droplet emulsion library using a periodic array of geometric parameters defining an obstacle matrix.

4. claims: 53-83

   Method for solidifying a droplet or nanoreactor using a solidifying agent.

5. claims: 84-102,130-133

   Method for introducing sample fluid to a microfluidic substrate after combining sample fluid and at least one immiscible phase fluid within a storage means.

6. claims: 103-110

   Method of extracting biological or chemical material from within a droplet or nanoreactor by separating layers of components and using a destabilizing surfactant.


   Method of amplifying DNA flowing all components together through an inlet channel into a main channel having a heated junction.


   Method of amplifying DNA flowing a first and a second sample fluids through separate inlet channels into a main channel having a heated junction and coalescing the droplets.

Method of amplifying DNA in a microfluidic substrate having a main channel comprising a serpentine line with heating and cooling regions.

10. claims: 114,117-127,129-134,138

Method of amplifying DNA flowing a first and a second sample fluids through separate inlet channels into a main channel and coalescing the droplets.

11. claims: 115,117-138

Method of sequencing DNA in which the components added are effective to permit PCR amplification.

12. claims: 116-138

Method of sequencing DNA in which the components added are effective to permit isothermal amplification.

13. claims: 139-156

Method of detecting a single nucleotide polymorphism (SNP).

14. claim: 157

Method of forming enzyme emulsions.

15. claim: 158

Method of detecting enzyme activity using a microfluidic substrate comprising at least one inlet channel.

16. claims: 159-185

Method of detecting enzyme activity using a microfluidic substrate comprising at least two inlet channels and coalescing the droplets.

17. claims: 186,188-199

Method of detecting an aqueous solution.
18. claims: 187-199

Method of tracking an aqueous solution.
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<th>Patent document cited in search report</th>
<th>Publication date</th>
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<tr>
<td>WO 2005021151 A</td>
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<td>JP 2007503984 T</td>
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