PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 13174-716.600	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 18/49415	International filing date *(day/month/year)* 04 September 2018 (04.09.2018)	(Earliest) Priority Date *(day/month/year)* 01 September 2017 (01.09.2017)
Applicant MIROCULUS INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

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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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-40 and 98-115, drawn to cartridges for a digital microfluidics (DMF) apparatus.
Group II: Claims 41-59 and 82-89, drawn to digital microfluidics (DMF) reader devices.
Group III: Claims 60-66, drawn to methods of preventing droplet evaporation within an air-matrix digital microfluidic (DMF) apparatus.
Group IV: Claims 67-75 drawn to methods of dispensing a predetermined volume of fluid into an air gap of an air-matrix digital microfluidics (DMF) apparatus.
Group V: Claims 76-81 drawn to a method for controlling a digital microfluidics (DMF) apparatus.
Group VI: Claims 90-97 drawn to a method of detecting the location and identity of a material in an air gap of a digital microfluidics (DMF) cartridge.

-- Please See Supplemental Box --

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 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. ☒ No 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.:

1-40 and 98-115

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.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - B01L 3/00, B81B 1/00, C12M 1/00, C12M 1/38, G01N 27/403, H01L 21/768 (2018.01)
CPC - B01L 3/5027, B01L 9/527, B01L 2200/06, B01L 2200/10, B01L 2300/0645, B01L 2300/0819, B01L 2300/161, B01L 2300/165, B81B 1/00, B81B 2201/05, C12M 23/16, B01L 2200/027, B01L 2300/048, B01L 2300/0848, B01L 2300/0874, B01L 2300/0877, B01L 2300/18, B01L 2300/1822, B01L 2400/0415, B01L 2400/0427, G01N 27/403, G01N 27/4143, G01N 2223/34, H01L 21/7682, H01L 29/0649, H01L 51/44, H01L 2221/10

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)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/197013 A1 (MIROCULUS INC.) 08 December 2016 (08.12.2016), para [00011],[00017], [00021], [00022], [00068], [00078]	1-40, 98-115
A	US 2015/0144489 A1 (TECAN TRADINGA AG) 28 May 2015 (28.05.2015), Fig. 2; Table 1; para [0018], [0081], [0089], [0093], [0094]	1-40, 98-115
A	WO 2011/062557 A1 (GONG) 26 May 2011 (26.05.2011), pg 8, para 3 to pg 11, para 2	1-40, 98-115
A	US 2009/0203063 A1 (WHEELER et al.) 13 August 2009 (13.08.2009), para [0008], [0055], [0064], [0079], [0087]	1-40, 98-115
Y, P	WO 2018/126082 A1 (MIROCULIS INC.) 05 July 2018 (05.07.2018), para [0006]-[0099]	1-40, 98-115

☐ Further documents are listed in the continuation of Box C. ☐ 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
"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
"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
04 December 2018	31 DEC 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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Special Technical Features

The inventions listed as Groups I, II, III, IV, V, and VI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups II, III, IV, V, and VI do not require a cartridge having a bottom and a top, the cartridge comprising: a sheet of dielectric material having a first side and a second side, the first side forming an exposed bottom surface on the bottom of the cartridge, wherein at least the second side of the sheet of dielectric material comprises a first hydrophobic surface; a top plate having a first side and a second side and a thickness therebetween; a ground electrode on the first side of the top plate; a second hydrophobic surface on the first side of the top plate covering the ground electrode; and an air gap separating the first hydrophobic layer and the second hydrophobic layer, wherein the air gap comprises a separation of greater than 280 micrometers (as in Claim 1);
a cartridge for a digital microfluidics (DMF) apparatus, the cartridge having a bottom and a top, the cartridge comprising: a flexible sheet of dielectric material having a first side and a second side, the first side forming an exposed bottom surface on the bottom of the cartridge; a first hydrophobic layer on the second side of the sheet of dielectric material; a top plate having a first side and a second side and a thickness therebetween; a ground electrode on the first side of the top plate, wherein the ground electrode comprises a grid pattern formed of a non-transparent material forming a plurality of open cells along the first side of the top plate; a second hydrophobic layer on the first side of the top plate covering the ground electrode; and an air gap separating the first hydrophobic layer and the second hydrophobic layer, wherein the air gap comprises a separation of greater than 400 micrometers (as in Claim 17);
a cartridge for a digital microfluidics (DMF) apparatus, the cartridge having a bottom and a top, the cartridge comprising: a sheet of dielectric material having a first side and a second side, the first side forming an exposed bottom surface on the bottom of the cartridge; a first hydrophobic layer on the second side of the sheet of dielectric material; a top plate having a first side and a second side and a thickness therebetween; a ground electrode on the first side of the top plate; a second hydrophobic layer on the first side of the top plate covering the ground electrode; an air gap separating the first hydrophobic layer and the second hydrophobic layer; a microfluidics channel formed in or on the second side of the top plate, wherein the microfluidics channel extends along the second side of the top plate; an opening between the microfluidics channel and the air gap; and a cover covering the microfluidics channel, wherein the cover includes one or more access ports for accessing the microfluidics channel (as in Claim 26); and
a cartridge for a digital microfluidics (DMF) apparatus, the cartridge having a bottom and a top, the cartridge comprising: a sheet of dielectric material having a first side and a second side, the first side forming an exposed bottom surface on the bottom of the cartridge; a first hydrophobic layer on the second side of the sheet of dielectric material; a top plate having first side and a second side and a thickness therebetween; a ground electrode on first side of the top plate; a second hydrophobic layer on the first side of the top plate covering the ground electrode; an air gap separating the first hydrophobic layer and the second hydrophobic layer, wherein the air gap comprises a separation of greater than 500 micrometers; a first microfluidics channel and a second microfluidics channel, wherein the first and second microfluidics channels are formed in the second side of the top plate, wherein the first and second microfluidics channels extend along the second side of the top plate; a first opening between the first microfluidics channel and the air gap and a second opening between the second microfluidics channel and the air gap, wherein the first and second openings are adjacent to each other within about 2 cm; and a cover covering the microfluidics channel, wherein the cover includes one or more access ports for accessing the microfluidics channel (as in Claim 40); and
a cartridge for a digital microfluidics (DMF) apparatus, the cartridge having a bottom and a top, the cartridge comprising: a sheet of dielectric material having a first side and a second side, the first side forming an exposed bottom surface on the bottom of the cartridge, wherein at least the second side of the sheet of dielectric material comprises a first hydrophobic surface; a tensioning frame holding the sheet of dielectric material in tension so that it is substantially flat; a top plate having a first side and a second side and a thickness therebetween; a ground electrode on the first side of the top plate; a second hydrophobic surface on the first side of the top plate covering the ground electrode; and an air gap separating the first hydrophobic layer and the second hydrophobic layer, wherein the air gap comprises a separation of greater than 280 micrometers (as in Claim 98), as required by Group I.

Groups I, III, IV, V, and VI do not require a digital microfluidics (DMF) reader device configured to operate with a disposable cartridge having a bottom dielectric surface, a top plate with a ground electrode, and an air gap between the bottom dielectric and the top plate, the device comprising: a seating surface for seating the disposable cartridge; a first plurality of drive electrodes on the seating surface, wherein all or some of the drive electrodes comprises an opening therethrough; a plurality of vacuum ports, wherein each vacuum port is coupled to one or more of the openings through the drive electrodes; a vacuum pump for applying a vacuum to the vacuum ports; and a control for applying energy to sequentially activate and de-activate one or more selected drive electrodes to move a droplet within the air gap of the cartridge along a desired path within the air gap, wherein the DMF reader is configured to apply the vacuum to the vacuum ports to secure each drive electrode to the bottom dielectric of the disposable cartridge when the disposable cartridge is placed on the seating surface (as in Claim 41); and
a digital microfluidics (DMF) reader device, the device comprising: a seating surface for seating the disposable cartridge; a plurality of drive electrodes on the seating surface, wherein at least some of the drive electrode comprises an opening therethrough; a plurality of vacuum ports, wherein each vacuum port is coupled to one or more of the openings through the drive electrodes; a vacuum pump for applying a vacuum to the vacuum ports; and a control for applying energy to sequentially activate and de-activate one or more selected drive electrodes to move a droplet within the air gap of the cartridge along a desired path within the air gap, wherein the DMF reader is configured to apply the vacuum to the vacuum ports to secure each drive electrode to the bottom dielectric of the disposable cartridge to retain the disposable cartridge on the seating surface (as in Claim 59); and
a digital microfluidics (DMF) reader device configured to operate with a disposable cartridge having a bottom dielectric surface, a top plate with a ground electrode, and an air gap between the bottom dielectric and the top plate, the device comprising: a seating surface for seating the disposable cartridge on an upper surface; a first plurality of drive electrodes on the seating surface, wherein all or some of the drive electrodes comprises an opening therethrough; a thermal control for applying thermal energy to a first region of the seating surface; a plurality of thermal vias, wherein the thermal vias comprise a thermally conductive material and are in thermal communication with the first region of the seating surface but are electrically isolated from the subset of electrodes and further wherein the thermal vias are in thermal communication with the thermal control; a plurality of vacuum ports, wherein each vacuum port is coupled to one or more of the openings through the drive electrodes; a vacuum pump for applying a vacuum to the vacuum ports; and a control for applying energy to sequentially activate and de-activate one or more selected drive electrodes to move a droplet within the air gap of the cartridge along a desired path within the air gap (as in Claim 82), as required by Group II.

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Groups I, II, IV, V, and VI do not require a method of preventing droplet evaporation within an air-matrix digital microfluidic (DMF) apparatus, the method comprising: introducing an aqueous reaction droplet into an air gap of the air-matrix DMF apparatus which is formed between a first plate and a second plate of the airmartix DMF apparatus; sequentially energizing driving electrodes on or in the first plate to move the aqueous reaction droplet within the air gap of the air-matrix DMF apparatus so that it combines with a droplet of nonpolar fluid within the air gap of the airmatrix DMF apparatus, forming a coated reaction droplet in which that the nonpolar fluid coats the aqueous reaction droplet and protects the reaction droplet from evaporation; and sequentially energizing the driving electrodes to move the coated reaction droplet within the air gap of the air-matrix DMF apparatus (as in Claim 60); and
a method of preventing droplet evaporation within an air-matrix digital microfluidic (DMF) apparatus, the method comprising: introducing an aqueous reaction droplet into an air gap of the air-matrix DMF apparatus which is formed between a first plate and a second plate of the airmatrix DMF apparatus; sequentially energizing driving electrodes on or in the first plate to move the aqueous reaction droplet within the air gap of the air-matrix DMF apparatus so that it combines with a droplet of nonpolar fluid within the air gap of the airmatrix DMF apparatus, forming a coated reaction droplet in which that the nonpolar fluid coats the aqueous reaction droplet and protects the reaction droplet from evaporation, wherein the nonpolar fluid is liquid at between 10 degrees C and 100 degrees C, further wherein the volume of the nonpolar fluid is less than the volume of the aqueous reaction droplet; and sequentially energizing the driving electrodes to move the coated reaction droplet within the air gap of the air-matrix DMF apparatus (as in Claim 66), as required by Group III.

Groups I, II, III, V, and VI do not require a method of dispensing a predetermined volume of fluid into an air gap of an air-matrix digital microfluidics (DMF) apparatus, wherein the air gap is greater than 400 micrometers wide, further wherein the DMF apparatus comprises a plurality of driving electrodes adjacent to the air gap, the method comprising: flooding a portion of the air gap with the fluid from a port in communication with the air gap; applying energy to activate a first driving electrode adjacent to the portion of the air gap that is flooded; and applying suction to withdraw the fluid back into the port while the first electrode is activated, leaving a droplet having a predetermined volume of the fluid in the air gap adjacent to the activated first electrode (as in Claim 67);
a method of dispensing a predetermined volume of fluid into an air gap of an air-matrix digital microfluidics (DMF) apparatus, wherein the air gap is greater than 400 micrometers wide, further wherein the DMF apparatus comprises a plurality of driving electrodes adjacent to the air gap, the method comprising: flooding a portion of the air gap with the fluid from a port in communication with the air gap; applying energy to activate a first driving electrode or a first group of contiguous driving electrodes adjacent to the portion of the air gap that is flooded, wherein the first driving electrode or the first group of contiguous driving electrodes are spaced apart from the port by one or more driving electrodes that are not activated; and applying suction to withdraw the fluid back into the port while the first electrode or first group of contiguous electrodes are activated, leaving a droplet of the fluid in the air gap adjacent to the first electrode or first group of contiguous electrodes (as in Claim 75), as required by Group IV.

Groups I, II, III, IV, and VI do not require a method for controlling a digital microfluidics (DMF) apparatus, the method comprising: providing a graphical user interface comprising a menu of fluid handling control commands, including one or more of: move, heat, remove, cycle, wait, breakoff, mix and dispense; receiving a fluid handling protocol comprising user-selected fluid handling control commands; calculating a path for moving fluid within an air gap of the DMF apparatus based on the fluid handling protocol, wherein the path minimizes the amount of overlap in the path to avoid contamination; and executing the fluid handing protocol using the DMF apparatus based on the calculated path (as in Claim 76), as required by Group V.

Groups I, II, III, IV, and V do not require a method of detecting the location and identity of a material in an air gap of a digital microfluidics (DMF) cartridge, the method comprising: disconnecting a reference electrode on a first side of the air gap of the DMF cartridge from a driving circuit; setting the voltage of one or more drive electrodes of an array of drive electrodes on a second side of the air gap to a high voltage while setting all other drive electrode of the array of drive electrodes to ground; sensing the voltage at the reference electrode; determining a capacitance between the first side of the air gap and the second side of the air gap based on the voltage sensed at the reference electrode; and Identifying the material in the air gap adjacent to the one or more drive electrodes based on the determined capacitance (as in Claim 90), as required by Group VI.

Shared Common Features

The only feature shared by Groups I, II, III, IV, V, and VI that would otherwise unify the groups is a digital microfluidics (DMF) apparatus, an air gap, and electrodes. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is obvious over WO 2016/197103 A1 (Miroculus Inc.). Miroculus Inc. discloses a digital microfluidics (DMF) apparatus (para [00017]), an air gap (para [00017]), and electrodes (para [00017]).

The only feature shared by Groups I, II, IV, and VI that would otherwise unify the groups is a port in communication with an air gap. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by Miroculus Inc. Miroculus Inc. discloses a port in communication with an air gap (para [00068]).

The only feature shared by Groups I and II that would otherwise unify the groups is a disposable cartridge having a bottom dielectric surface, a top plate with a ground electrode, and an air gap between the bottom dielectric and the top plate. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 2015/0144489 A1 (Tecan Trading AG). Tecan Trading AG discloses a disposable cartridge (para [0018]) having a bottom dielectric surface (Fig. 2; para [0094], dielectric layer, 24.), a top plate with a ground electrode (Fig. 2; para [0089], cover plate, 12, of electrode array, 9, connected to a ground potential.), and an air gap between the bottom dielectric and the top plate (Fig. 2; para [0081], [0089], [0093], [0094], gap, 6... as shown without liquid droplets, 23, located between said dielectric layer, 24, and cover plate, 12.).

The only feature shared by Groups I and IV that would otherwise unify the groups is an air gap is greater than 400 micrometers wide. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by Tecan Trading AG. Tecan Trading AG disclose an air gap is greater than 400 micrometers wide (Table 1; gap, 6, with height of 0.2-2.0 mm or 200 micrometers to 2,000 micrometers.).

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The only feature shared by Groups III and IV that would otherwise unify the groups is an air matrix. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by Miroculus Inc. discloses an air matrix (para [00015]).

As the technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I, II, III, IV, V, and VI therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.