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[Continued on next page]

(54) Title: SYSTEMS, APPARATUS AND METHODS FOR BIOCHEMICAL ANALYSIS

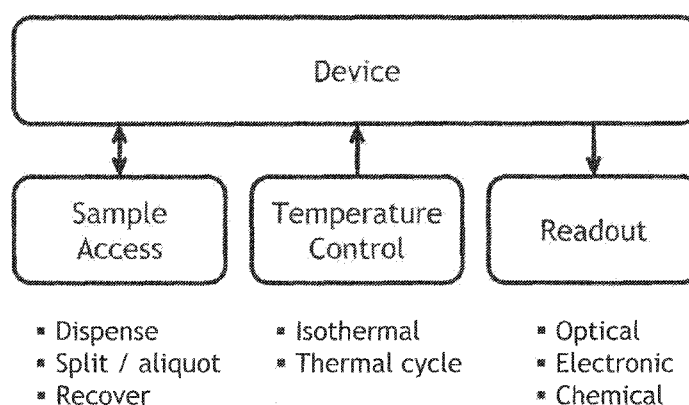


FIG. 1

(57) **Abstract:** Systems, apparatus and methods are provided for biochemical analysis of a sample (e.g., a cell or nucleic acids). Samples are analyzed for molecular information and remain accessible for subsequent analysis or testing. The systems, apparatus and methods and systems provided are useful for performing quantitative and highly parallel biochemical reactions on biological samples in a high-throughput manner.



(88) Date of publication of the international search report:
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INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68; C12M 1/34 (2012.01)

USPC - 435/6.1, 287.1

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)

IPC(8) - C12Q 1/68; C12M 1/34 (2012.01)

USPC - 435/6.1, 435/287.1

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

USPC - 435/6.11, 435/91.2, 435/287.2, 435/287.3, 435/288.3, 435/288.4

(Text Search)

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

PatBase, PubWEST (PGPB, USPT, USOC, EPAB, JPAB), US PatFT, US AppFT, PubMed and Google Patents.

Search Terms: integrated system, biochemical reaction, sample, reaction module, dispensing module, thermal module, thermal control element, detection module, recovery module, droplet, drop, PCR, reagent, reaction volume, sequencer, amplif\$, drop, heatin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0042737 A1 (KATZ et al.) 12 February 2009 (12.02.2009) Abstract, para [0009]-[0010], [0012], [0041], [0042], [0045], [0046], [0056], [0061], [0076], [0082], [0086], Fig 13A	1-8 and 10-11 ----- 9
Y	US 2004/0209288 A1 (MEHRABIAN et al.) 21 October 2004 (21.10.2004) para [0034]-[0036]	9
A	US 7,745,221 B2 (BUTLER et al.) 29 June 2010 (29.06.2010) entire document.	1-11

☐ Further documents are listed in the continuation of Box C.

* 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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46776

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 examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-11, drawn to an integrated system for performing a biochemical reaction on a sample comprising: a. a reaction module comprising: a device comprising a plurality of discrete reaction volumes, said reaction volumes comprising a reagent and a sample, wherein said reaction volume is accessible to perform more than one biochemical reaction on said sample; b. a dispensing module for dispensing said sample in the device, wherein said dispensing module can be operably coupled with said device; c. a thermal module comprising a heating element and a thermal control element, wherein said thermal module can be operably coupled to said device; d. a detection module to detect a signal from the discrete reaction volumes; and e. a recovery module operable to individually recover at least a portion or substantially all reacted material from each of said plurality of discrete reaction volume, wherein said recovery module can be operably coupled with the device.

*****Continued in 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.:
1-11

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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Continuation of Box No. III - Observations where unity of invention is lacking:

Group II: Claims 12-17, drawn to a method for analyzing a sample, the method comprising: a. dispensing said sample and a reagent into a plurality of discrete reaction volumes; b) performing a biochemical reaction on said discrete reaction volume; c. detecting a signal from said discrete reaction volumes; and d. recovering at least a portion of or substantially all reacted material from said discrete reaction volumes.

Group III: Claims 18-22, drawn to an apparatus for receiving a device, the apparatus comprising: a base surface and a top surface and sidewalls including at least one opening for receiving said device; a pair of spaced guide rails for contacting said device to said base and preventing contact between said device and said top surface, said device configured to receive a fluid.

The inventions listed as Groups I-III 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 I-II do not include the inventive concept of an apparatus for receiving a device, the apparatus comprising: a base surface and a top surface and sidewalls including at least one opening for receiving said device; a pair of spaced guide rails for contacting said device to said base and preventing contact between said device and said top surface, said device configured to receive a fluid, as required by Group III.

Groups I and III do not include the inventive concept of a method for analyzing a sample, as required by Group II.

Groups II-III do not include the inventive concept of an integrated system for performing a biochemical reaction on a sample, as required by Group I.

Groups I-II share the technical feature of performing a biochemical reaction on a sample, wherein said sample and a reagent is dispensed into a plurality of discrete reaction volumes; a biochemical reaction being performed on said discrete reaction volumes, a signal being detected from said discrete volumes and at least a portion of or substantially all reacted material from said discrete reaction volumes are recovered.

Groups I and III share the technical feature of a device.

However, these shared technical features do not represent a contribution over the prior art as being anticipated by US 2009/0042737 A1 to Katz et al. (hereinafter 'Katz') that teaches a method for analyzing a sample (abstract; para [0009]-[0010]), the method comprising:

--a) dispensing said sample and a reagent into a plurality of discrete reaction volumes (fig 8, part 408; para [0042]-'the cells are individually encapsulated in nanoliter microvessel or droplets with a pre-determined mix of reagents suitable for amplifying a target DNA sequence in the cell. For example, the cells and PCR reagent mix can be introduced into a flow channel in a microfluidic network designed to isolate and encapsulated individual cells in a stream of water in oil drop as further discussed below', [0065]-[0066]-'In some embodiments, the flow conditions of the PCR mix are controlled such that introduction of the PCR mix also organizes the sample flow into a single file stream of cells. Alternatively, the diameter of the sample flow cell 409 can be configured to organize the sample into a single file stream of cells when the sample is introduced at the sample cell input 413. Encapsulation media inlet 412 is fluidically connected to encapsulation flow channel 407 which intersects sample flow channel 409 at an L-junction downstream of the PCR mix intersection. The encapsulation media, such as oil, flows into the stream of cells and PCR mix at the "L" junction between the sample flow channel 409 and the encapsulation flow channel 407 under flow conditions suitable to pinch off droplets of cells and PCR mix into a stream of nanoliter microvessels preferably containing a single cell surrounded by the oil encapsulation media. The flow conditions in the encapsulation flow channel 407 are controlled to ensure that zero or one cell per microvessel are preferably encapsulated', 'The stream of microvessels 408 is then loaded into a microfluidic capillary tube 420 for amplification of genetic material in the microvessels and measurement of the gene expression');

--b) performing a biochemical reaction on said discrete reaction volume (para [0066]-[0067]-'The stream of microvessels 408 is then loaded into a microfluidic capillary tube 420 for amplification of genetic material in the microvessels and measurement of the gene expression', 'Once the aluminum block 422 has been loaded with the plurality of capillary tube segments 421, the temperature of the aluminum block can be ramped from 60.degree. C. to 95.degree. C. at 1.degree. C./S to cycle the capillary tube segments 421 through the temperature profile for PCR amplification. Any suitable heating element discussed above can be used to cycle the temperature through the desired number of PCR cycles');

--c) detecting a signal from said discrete reaction volumes (para [0056]-'The microvessels 208 then flow through a serpentine microfluidic channel 210 that passes the microvessels 208 through one or more thermal zones necessary for amplification and real-time detection of amplified gene products... The microvessels 208 are passed through the temperature zones 211, 212, 213 multiple times and measured after each passage to determine the quantity of amplified product. As discussed above, the PCR mix includes probes that fluoresce at a level proportional to the quantity of amplified product. The fluorophores may be excited by a light source 214, such as fixed and scanning lasers, UV lamps, light emitting diodes, at a specific time or temperature during each PCR cycle and detected by a detector 215 such as photomultiplier tube, CCD camera, photodiodes or photodiode arrays, or other optical detector. The detector 215 measures the intensity of the fluorescence to determine the quantity of amplified product in the microreactors 208'); and

--d) recovering at least a portion of or substantially all reacted material from said discrete reaction volumes (para [0086]-'it may be desired to recover genetic material from a selected portion of the cells amplified and measured in the nanowell array, based on the phenotype and genotype measurement of the cells', 'The amplified genetic material is measured with optical detector 815 and correlated to the phenotype information for each nanowell location. The genetic material may then be harvested from the nanowells of interest based on the correlated phenotype-genotype information by using a robotic micro-pipet 820 to extract material from the individually addressable wells of interest').

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Katz further teaches a device for performing said method above (para [0009]-[0011], [0042], [0056], [0065]-[0067], [0086]).

As said shared technical features were known at the time of the invention, these cannot be considered special technical features that could otherwise unify the Groups.

Groups I-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.