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# (54) ELECTROWETTING-BASED CONCENTRATION HOMOGENIZATION MICROFLUIDIC CHIP AND CONCENTRATION HOMOGENIZATION METHOD

(57) An electrowetting-based concentration homogenization microfluidic chip and a concentration homogenization method, the chip comprising a microfluidic chip body provided with a microchannel; the microchannel is used to transport, by using electrowetting pipetting technology, liquid to move along the microchannel; the microfluidic chip body is provided with a sample region, a reagent storage region, a dilution region, a concentration quantification region, an accurate sampling region and a homogenization region; and the sample region, the reagent storage region, the dilution region, the concentration quantification region, the accurate sampling region and the homogenization region communicate with one another by means of the microchannel. The method comprises: 1. adding a plurality of original samples to respective sample regions; 2. adding a reagent to a reagent storage region; 3. using electrowetting pipetting technology to move the reagent to a dilution region, and moving the original samples to the dilution region to complete set gradient dilution; 4. manipulating the diluted sample to move to a concentration quantification region for measuring; 5. selecting, according to homogenization, an original sample as a mother liquor, and 6. separating a required volume by means of an accurate sampling region and moving same to a homogenization region to complete concentration homogenization.

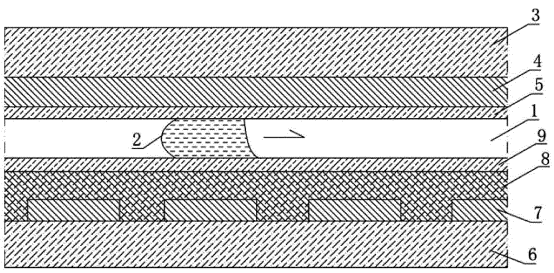


Fig. 1

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## Description

### CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the priority to Chinese Patent Application No. 202111217576.X, titled "ELECTROWETTING-BASED CONCENTRATION HOMOGENIZATION MICROFLUIDIC CHIP AND CONCENTRATION HOMOGENIZATION METHOD", filed on October 19, 2021 with the China National Intellectual Property Administration, which is incorporated herein by reference in its entirety.

### FIELD

**[0002]** The present application relates to an electrowetting-based microfluidic chip, and in particular relates to a microfluidic chip for electrowetting-based homogenization of concentration and a concentration homogenization method.

### BACKGROUND

**[0003]** Detection for multiple mixed samples (hereinafter referred to as mixed-sample detection), having the advantages of time saving, cost reduction and efficiency improving, is a detection means commonly used in the fields such as gene sequencing, pharmaceutical synthesis, biochemical analysis and diagnosis. Since the initial concentrations of the samples are different before the mixed-sample detection, after mixing, the concentrations of some samples will be too high and the concentrations of some other samples will be too low in the same mixed system. The signal of the low-concentration samples is apt to be covered and lost, and the signal of the high-concentration samples may easily exceed the range of detection, resulting in inaccuracy of the overall detection. Concentration homogenization is a method for mixing samples, where samples with different concentrations are mixed according to a certain proportion, to make the samples in the same mixed system have the same quality or concentration. This method can effectively solve the above problems.

**[0004]** The operation process of concentration homogenization mainly includes the following three steps of quantifying concentration, calculation and comparison, and precise mixing: 1) concentration quantification: the initial concentrations of different samples are detected by using quantitative detection instrument; 2) calculation and comparison: the required volumes of the different samples are calculated according to the results of concentration quantification and the required value of the homogenized concentration; 3) precise mixing: based on the calculation results, the required volumes are accurately pipetted from the samples and then mixed.

**[0005]** In the above existing method for concentration homogenization, the concentration detection is generally achieved based on quantitative detection instruments, and the pipetting and mixing of the samples are performed manually, which has shortcomings such as a low degree of automation and complexity in operation, thereby seriously lowering the efficiency of mixed-sample detection. Besides, the manual method often results in a large deviation and low accuracy in pipetting volumes due to the inherent deviation of pipetting tools and insufficient proficiency of operators. For example, hanging liquid or reagent residues often occurs during pipetting using conventional pipettes.

**[0006]** Due to advantages of low sample consumption, fast reaction, high detection efficiency, effective heat transfer and mass transfer, no cross-contamination, and easy integration with other technological equipment and the like, microfluidic systems are widely used in fields such as chemical analysis, biomedicine, food hygiene and environmental monitoring. In microfluidic systems, electrowetting (EW) pipetting technology is a new type of method for droplets manipulation to control the surface tension of droplets using electricity. That is, by changing the voltage between the droplet and the insulating substrate, the wettability of the droplet on the substrate is changed, that is, the contact angle is changed, leading to the droplet deformation and displacement. The electrowetting pipetting technology breaks the dependence of pipetting system on traditional mechanical arms, pipelines with pumps and valves, and complex flow channels, and can realize highly flexible and accurate manipulation of reagents through digital programming. However, there are no relevant reports to date on methods for using microfluidic chips to manipulate samples in a microchannel of microfluidic chip by electrowetting pipetting for concentration homogenization.

### SUMMARY

**[0007]** An object of the present disclosure is to provide a microfluidic chip for electrowetting-based concentration homogenization, and another object of the present disclosure is to provide a method for concentration homogenization using the microfluidic chip, to solve problems such as large deviation of pipetting, low accuracy and low efficiency caused by manual pipetting which is limited by the proficiency of the operators and the inherent deviation of the pipette.

**[0008]** In order to achieve the above objects, the present disclosure provides the following technical solutions.

**[0009]** A microfluidic chip for electrowetting-based concentration homogenization according to the present disclosure includes a microfluidic chip body provided with a microchannel used to make a liquid move along the microchannel by electrowetting pipetting technology, where the microfluidic chip body is further provided with sample regions, a reagent storage region, dilution regions, a concentration quantification region, an accurate sampling region and a homogenization region; and the sample regions, the reagent storage region, the dilution

regions, the concentration quantification region, the accurate sampling region and the homogenization region communicate with one another via the microchannel; and where

each of the sample regions is used for storage of an original sample;

the reagent storage region is used for storage of a reagent, where the reagent is used to dilute the original samples according to a set gradient;

each of the dilution regions is used for preparing a diluted sample by mixing the reagent and the corresponding original sample entering the dilution region via the microchannel;

the concentration quantification region is used for concentration determination of the diluted sample entering the concentration quantification region via the microchannel;

the accurate sampling region is used to obtain a set volume of each of the original samples or/and a set volume of each of the diluted samples; and

the concentration homogenization region is used for selecting the suitable original samples or the diluted samples as mother liquids and calculating required volumes of the mother liquids for concentration homogenization according to a set total volume and a set concentration required for concentration homogenization and based on concentrations of the original samples and concentrations of the diluted samples obtained by detection or calculation, and moving the required volumes of the mother liquids to the concentration homogenization region by using electrowetting pipetting technology, to complete the concentration homogenization.

**[0010]** Preferably, the microfluidic chip body includes an upper substrate and a lower substrate arranged spaced apart in an up-down direction, and the microchannel for liquid movement is formed between the upper substrate and the lower substrate. The upper substrate includes an upper insulating substrate, a common electrode and an upper hydrophobic layer arranged in the listed sequence from top to bottom. The lower substrate includes a lower insulating substrate, a drive electrode array, a dielectric layer and a lower hydrophobic layer arranged in the listed sequence from bottom to top.

**[0011]** A concentration homogenization method applicable to the microfluidic chip for electrowetting-based concentration homogenization according to the present disclosure includes the following steps:

step 1, the plurality of original samples are added to the sample regions respectively;

step 2, the reagent is added to the reagent storage region;

step 3, by using the electrowetting pipetting technology, a set volume of the reagent is moved to each of the dilution regions, and a set volume of each of the original samples is moved to the corresponding dilution region via the microchannel, and the reagent and the original sample in the corresponding dilution region are mixed, to prepare a corresponding diluted sample of the diluted samples, and completing set gradient dilution in sequence;

step 4, by using the electrowetting pipetting technology, the diluted samples in the dilution regions are manipulated respectively in sequence, to make a set volume of each of the diluted samples move to a concentration quantification region for electrochemical detection or fluorescence detection, final concentrations of the diluted samples are directly obtained or calculated based on detection results, and the concentrations of the diluted samples and the concentrations of the corresponding original samples are calculated based on dilution factors of the diluted samples in the dilution regions;

step 5, based on the set total volume and the set concentration required for concentration homogenization, the original samples or the diluted samples with appropriate concentrations are selected as the mother liquids, and the required volumes of the mother liquids are calculated;

step 6, by using the electrowetting pipetting technology, the required volumes of mother liquids are accurately separated from the mother liquids via the accurate sampling region, and are moved to the homogenization region for mixing, to complete the concentration homogenization.

**[0012]** The method according to the present disclosure is easy to operate and has a high degree of automation, which reduces errors in manual operation, and the homogenization process can be completed within 1 hour, which saves time and greatly improves the detection efficiency. The use of electrowetting pipetting technology realizes accurate pipetting with a volume deviation of less than 3%, which solves the problem of inaccurate pipetting results caused by the insufficient proficiency of the operators or the inherent deviation of the pipettes in conventional pipetting.

## BRIEF DESCRIPTION OF DRAWINGS

**[0013]**

Fig. 1 is a schematic view showing the structure of a microfluidic chip body according to the present dis-

closure; and

Fig. 2 is a schematic view showing the arrangement of sample regions, a reagent storage region, dilution regions, a concentration quantification region, an accurate sampling region, a homogenization region and a microchannel according to the present disclosure.

## DETAILED DESCRIPTION OF THE EMBODIMENTS

**[0014]** The embodiments of the present disclosure are described in detail below with reference to the attached drawings. The embodiments are implemented on the premise of the technical solutions of the present disclosure, which provide detailed implementation solutions and specific operation processes, but the scope of protection of the present disclosure is not limited to the following embodiments.

**[0015]** As shown in Fig. 1, a microfluidic chip for electrowetting-based concentration homogenization according to the present disclosure includes a microfluidic chip body provided with the a microchannel 1, and the microchannel 1 is used to make a droplet 2 move along the microchannel 1 by using electrowetting pipetting technology.

**[0016]** The microfluidic chip body includes an upper substrate and a lower substrate arranged spaced apart in an up-down direction, and the microchannel 1 for movement of the droplet 2 is formed between the upper substrate and the lower substrate. The upper substrate includes an upper insulating substrate 3, a common electrode 4 and an upper hydrophobic layer 5 arranged in the listed sequence from top to bottom. The lower substrate includes a lower insulating substrate 6, a drive electrode array 7, a dielectric layer 8 and a lower hydrophobic layer 9 arranged in the listed sequence from bottom to top.

**[0017]** The microfluidic chip body further provided with sample regions, a reagent storage region, dilution regions, a concentration quantification region, an accurate sampling region and a homogenization region, where the sample regions, the reagent storage region, the dilution regions, the concentration quantification region, the accurate sampling region and the homogenization region communicate with one another via the microchannel 1.

**[0018]** As shown in Fig. 2, each of the sample regions is used for storing an original sample. In this embodiment, four sample regions are provided, which are indicated by 10.1, 10.2, 10.3 and 10.4.

**[0019]** The reagent storage region 11 is used for storing a reagent, and the reagent is used to dilute the original samples according to a set gradient.

**[0020]** Each of the dilution regions is used to mix the reagent and the original sample entering the dilution region via the microchannel 1, to prepare a diluted sample. In this embodiment, gradient dilution with two stages is taken as an example, each sample region corresponds to two dilution regions, namely, a first dilution region 12.1 and a second dilution region 12.2. Of course, gradient dilution with three or more stages can be selected according to the needs.

**[0021]** The concentration quantification region 13 is used for concentration determination of the diluted sample entering this region via the microchannel 1 by electrochemical detection or fluorescence detection.

**[0022]** The accurate sampling region 14 is used to obtain a set volume of each of the original samples or/and a set volume of each of the diluted samples.

**[0023]** The concentration homogenization region 15 is used to select the original samples or the diluted samples as mother liquids and calculate required volumes of the mother liquids for concentration homogenization according to a set total volume and a set concentration required for concentration homogenization and based on concentrations of the original samples and concentrations of the diluted samples obtained by detection or calculation, and the required volumes of the mother liquids are moved to this region by using the electrowetting pipetting technology.

**[0024]** The concentration homogenization method applicable to the microfluidic chip for electrowetting-based concentration homogenization according to the present disclosure includes the following steps:

step 1, the four original samples are pre-added to the sample regions 10.1, 10.2, 10.3, and 10.4, respectively;

step 2, the reagent is pre-added to the reagent storage region 11;

step 3, by using the electrowetting pipetting technology, set volumes of the original samples in the sample regions 10.1, 10.2, 10.3, and 10.4 are moved to the corresponding first dilution regions 12.1 via the microchannel respectively, and set volumes of the reagent are moved to the first dilution regions 12.1 respectively, and the above set volumes of the reagent and the above set volumes of original samples are correspondingly mixed to prepare first diluted samples, and then by using the electrowetting pipetting technology, set volumes of the first diluted samples are moved to the second dilution regions 12.2 via the microchannel 1, and set volumes of the reagent are moved to the second dilution regions 12.2, the above set volumes of first diluted samples and the above set volumes of reagent are correspondingly mixed, to prepare second diluted samples, and the gradient dilution with two stages of each of the original samples is completed;

step 4, by using the electrowetting pipetting technology, the second diluted samples in the respective second dilution regions are manipulated in sequence to make set volumes of the second diluted samples

move to the concentration quantification region 13 for electrochemical detection or fluorescence detection, the concentrations of the second diluted samples are directly obtained or calculated based on the detection results, and the concentrations of the corresponding first diluted samples and the concentrations of the original samples are calculated based on the dilution factors of the second diluted samples in the second dilution regions;

step 5, based on the set total volume and the set concentration required for concentration homogenization, the original samples or the diluted samples with appropriate concentrations are selected as mother liquids, and the required volumes of the mother liquids are calculated; and

step 6, by using the electrowetting pipetting technology, the required volumes of the mother liquids are accurately separated from the mother liquids via the accurate sampling region 14 and moved to the homogenization region 15 for mixing, to complete the concentration homogenization.

## Claims

1. A microfluidic chip for electrowetting-based concentration homogenization, comprising a microfluidic chip body provided with a microchannel configured to make a liquid move along the microchannel by electrowetting pipetting technology, wherein the microfluidic chip body is further provided with sample regions, a reagent storage region, dilution regions, a concentration quantification region, an accurate sampling region and a homogenization region; and the sample regions, the reagent storage region, the dilution regions, the concentration quantification region, the accurate sampling region and the homogenization region communicate with one another via the microchannel; and wherein

   each of the sample regions is provided for storage of an original sample;
   the reagent storage region is provided for storage of a reagent, wherein the reagent is used to dilute the original samples according to a set gradient;
   each of the dilution regions is provided for preparing a diluted sample by mixing the reagent and the corresponding original sample entering the dilution region through the microchannel;
   the concentration quantification region is provided for concentration determination of the diluted sample entering the concentration quantification region via the microchannel;
   the accurate sampling region is provided for obtaining a set volume of each of the original samples or/and a set volume of each of the diluted samples; and
   the concentration homogenization region is provided for selecting the original samples or the diluted samples as mother liquids and calculating required volumes of the mother liquids for concentration homogenization according to a set total volume and a set concentration required for concentration homogenization and based on concentrations of the original samples and concentrations of the diluted samples obtained by detection or calculation, and moving the required volumes of the mother liquids to the concentration homogenization region by electrowetting pipetting technology, to complete the concentration homogenization.

2. The microfluidic chip for electrowetting-based concentration homogenization according to claim 1, wherein

   the microfluidic chip body comprises an upper substrate and a lower substrate arranged spaced apart in an up-down direction, and the microchannel for liquid movement is formed between the upper substrate and the lower substrate; and wherein
   the upper substrate comprises an upper insulating substrate, a common electrode and an upper hydrophobic layer arranged in the listed sequence from top to bottom; the lower substrate comprises a lower insulating substrate, a drive electrode array, a dielectric layer and a lower hydrophobic layer arranged in the listed sequence from bottom to top.

3. A concentration homogenization method applicable to the microfluidic chip for electrowetting-based concentration homogenization according to claim 1, comprising the following steps:

   step 1, adding the plurality of original samples to the sample regions respectively;
   step 2, adding the reagent to the reagent storage region;
   step 3, by using the electrowetting pipetting technology, moving a set volume of the reagent to each of the dilution regions, and moving a set volume of each of the original samples to the corresponding dilution region via the microchannel, and mixing the reagent and the original sample in the corresponding dilution region, to prepare a corresponding diluted sample of the diluted samples, and completing set gradient dilution in sequence;
   step 4, by using the electrowetting pipetting technology, manipulating the diluted samples in the dilution regions respectively in sequence to

make a set volume of each of the diluted samples move to the concentration quantification region for concentration detection, directly obtaining or calculating the concentrations of the diluted samples based on detection results, and calculating the concentrations of the diluted samples and the concentrations of the corresponding original samples based on dilution factors of the diluted samples in the dilution regions;

step 5, based on the set total volume and the set concentration required for concentration homogenization, selecting the original samples or the diluted samples as the mother liquids, and calculating the required volumes of the mother liquids; and

step 6, by using the electrowetting pipetting technology, accurately separating the required volumes from the mother liquids via the accurate sampling region and moving the required volumes of the mother liquids to the homogenization region for mixing, to complete the concentration homogenization.

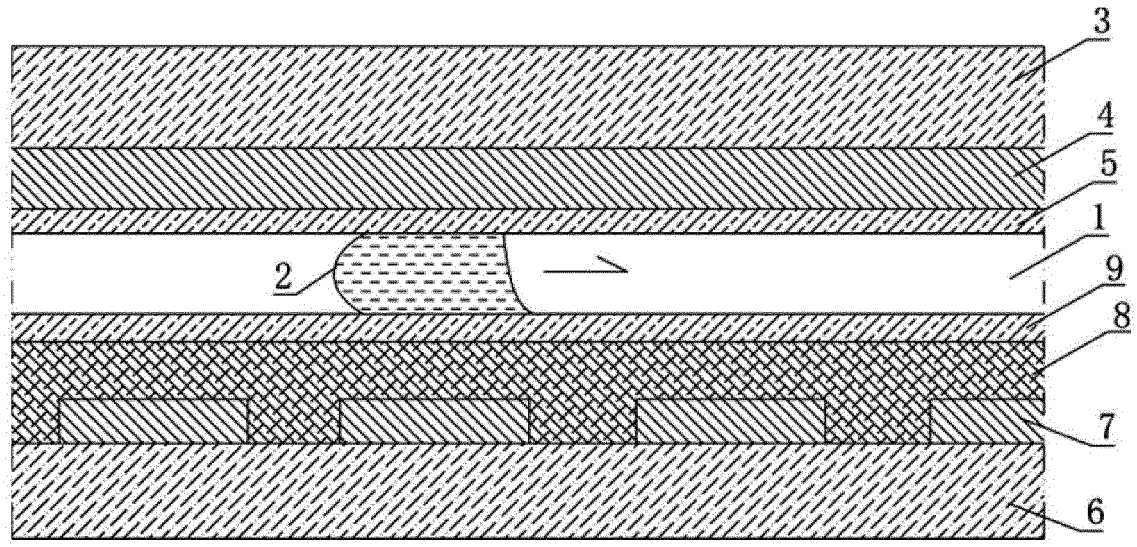


Fig. 1

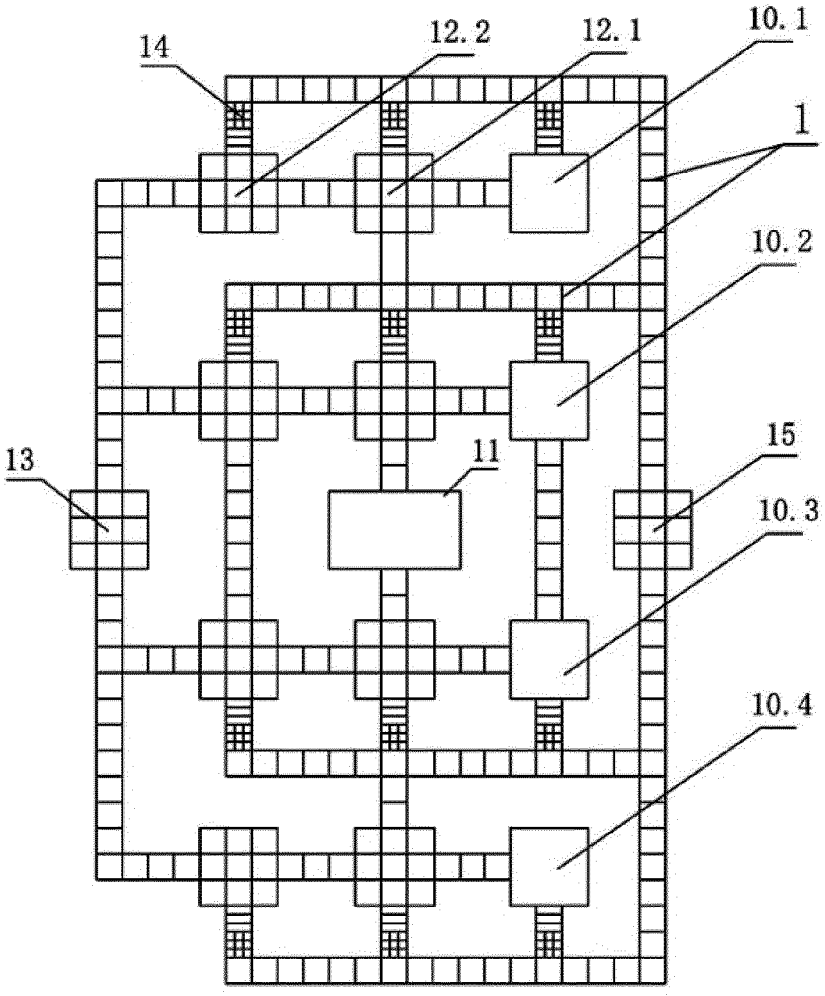


Fig. 2

## INTERNATIONAL SEARCH REPORT

International application No.
**PCT/CN2022/091636**

**A. CLASSIFICATION OF SUBJECT MATTER**

B01L 3/00(2006.01)i

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)

B01L3，G01N；CPC：B01L2400/0427

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)

CNTXT; WPABSC; WPABS; ENTXT; CJFD; DWPI; ENTXTC; VEN; VCN; CNKI: 微流, 微通道, 芯片, 电润湿, 浓度, 均一化, 均匀化, 样品, 样本, 试样, 稀释, 定量, 计算, micro+, chip+, electrowetting, concentration, homogeniz+, normaliz+, sample, dilut+, quantif+, calculat+, next-generation, sequencing, NGS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
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| Y | US 2019329258 A1 (TECAN TRADING AG) 31 October 2019 (2019-10-31)<br>description, paragraphs [0062] and [0080]-[0086], and figures 1-14 | 1-3 |
| Y | CN 113252632 A (CHENGDU HONGYI LIGHT WING BIOENGINEERING CO. LTD. et al.) 13 August 2021 (2021-08-13)<br>claims 1 and 5-7 | 1-3 |
| Y | US 2017073729 A1 (LIIUMINA INC.) 16 March 2017 (2017-03-16)<br>description, paragraphs [0023]-[0025], [0046], and [0079]-[0086], and figure 1.2 | 1-3 |
| A | WO 2006132211 A1 (HITACHI HIGH-TECHNOLOGIES CORP. et al.) 14 December 2006 (2006-12-14)<br>entire document | 1-3 |
| A | US 2021054443 A1 (NANJINGJINSIRUI SCIENCE & TECH BIOLOGY CORP.) 25 February 2021 (2021-02-25)<br>entire document | 1-3 |

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| **01 August 2022** | **08 August 2022** |
| Name and mailing address of the ISA/CN<br>**China National Intellectual Property Administration (ISA/CN)**<br>**No. 6, Xitucheng Road, Jimenqiao, Haidian District, Beijing 100088, China**<br>Facsimile No. **(86-10)62019451** | Authorized officer<br><br>Telephone No. |

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/091636

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 2013105319 A1 (BHATTACHARYA BHARGAB B et al.) 02 May 2013 (2013-05-02) entire document | 1-3 |

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**INTERNATIONAL SEARCH REPORT**
**Information on patent family members**

International application No.
**PCT/CN2022/091636**

| Patent document cited in search report | Publication date (day/month/year) | Patent family member(s) | Publication date (day/month/year) |
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