



(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 153(4) EPC

(43) Date of publication:
05.01.2022 Bulletin 2022/01

(51) Int Cl.:
C12M 1/34 ^(2006.01) **C12M 1/00** ^(2006.01)
C12Q 1/6851 ^(2018.01)

(21) Application number: **20779964.4**

(86) International application number:
PCT/CN2020/081497

(22) Date of filing: **26.03.2020**

(87) International publication number:
WO 2020/192742 (01.10.2020 Gazette 2020/40)

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME
Designated Validation States:
KH MA MD TN

(72) Inventors:
• **GAO, Yibo**
Shenzhen, Guangdong 518000 (CN)
• **SONG, Qi**
Shenzhen, Guangdong 518000 (CN)
• **WEN, Weijia**
Shenzhen, Guangdong 518000 (CN)

(30) Priority: **27.03.2019 CN 201910235034**

(74) Representative: **Grünecker Patent- und Rechtsanwälte**
PartG mbB
Leopoldstraße 4
80802 München (DE)

(71) Applicant: **Shenzhen Shineway Technology Corporation**
Shenzhen, Guangdong Province 518000 (CN)

(54) **SELF-DRIVEN MICROFLUIDIC CHIP AND METHOD FOR USING SAME**

(57) The present invention provides a self-driven microfluidic chip and a use method thereof. The self-driven microfluidic chip of the present invention comprises a base and a cover, and a sample inlet, a sample outlet, a plurality of reaction chambers and one or a plurality of primary channels are formed between the base and the cover; the sample inlet and the sample outlet can be communicated with the outside, the primary channels are communicated with the sample inlet and the sample outlet, and a liquid inlet channel and a liquid outlet channel are arranged between each reaction chamber and one primary channel; the internal surfaces of the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels, which are located on the base and/or the cover, are hydrophilic surfaces, the cross sectional area of each liquid inlet channel is less than that of each liquid outlet channel, and in the liquid flow direction in the primary channels, each liquid inlet channel is located in front of each liquid outlet channel; and after the solution of the sample to be tested enters the reaction chambers, the primary channels can be filled with an oil phase reagent to close the liquid inlet channels and the liquid outlet channels. The self-driven microfluidic chip of the present invention realizes the self-driven flow sampling of the solution of the sample to be tested without external drive equipment.

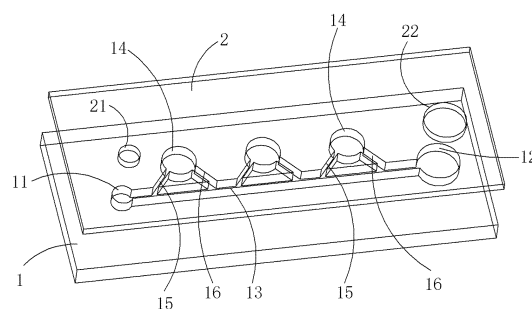


FIG. 1

Description

[0001] The present application is proposed based on the Chinese patent application with the application number of 201910235034.1 and the application date of March 27th, 2019 and claims the priority of the Chinese patent application, the disclosures of which are hereby incorporated by reference.

Technical Field

[0002] The present invention relates to the field of microfluidic chips, and particularly relates to a self-driven microfluidic chip and a method for using the same.

Background

[0003] In the past 20 years, the number of new cancer cases in China has exceeded 2.2 million each year. At the same time, the number of new cancer cases is still increasing at a rate of 3%-5% each year. Cancer has become the first cause of death affecting lives and health of the Chinese people. The development of cancer requires several steps of gene mutation, generation of cancer cells, formation of tumors and metastatic spread. The current cancer diagnosis methods mainly include pathological examination, imaging examination and so on, and can obtain more accurate testing results only after the tumor grows to a certain size and the cancer develops to the middle and late stages, and the best treatment time for patients is often missed. If mutated genes can be detected in the proliferation process of cancer cells to bring forward the diagnosis time, the survival rates of patients can be increased. Therefore, a nucleic acid testing method with high sensitivity and high accuracy is urgently needed.

[0004] At present, conventional nucleic acid testing methods are to amplify target nucleic acid molecules to achieve signal amplification, mainly including polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), and the most widely used technology is real-time fluorescent quantitative PCR technology. In the PCR methods, the third-generation digital PCR method has higher testing sensitivity. In the method, DNA molecules to be tested are dispersed in thousands of reaction units in advance, and the DNA concentration of an initial sample can be obtained by counting the fluorescence intensity changes of each reaction unit before and after PCR amplification, which can realize the quantitative testing of a nucleic acid sample with an ultra-low concentration. Compared with traditional nucleic acid testing technologies, the digital PCR has higher testing sensitivity and wider testable dynamic range and can obtain more accurate testing results in various fields such as analysis of cancer markers, non-invasive prenatal testing, environmental monitoring and food safety monitoring.

[0005] The microfluidic chip technology is to integrate traditional biochemical analysis on a few square centimeters even smaller chip and complete testing analysis in the micro-nano scale channel and micro-cavity in the chip, and is more suitable for use in combination with single nucleic acid molecule testing method to realize high-sensitivity and low-dosage testing. In the related control of a microfluidic chip, a device such as syringe or air pump or electric pump is usually used to drive a reagent to flow in the chip, which makes chip operation complicated and requires corresponding instruments to realize functions.

Summary

[0006] In view of this, the present invention aims to propose a self-driven microfluidic chip capable of realizing self-driven sample loading without external drive equipment.

[0007] To achieve the above purpose, the technical solution of the present invention is realized as follows.

[0008] A self-driven microfluidic chip, used for accommodating solution of a sample to be tested, comprises a base and a cover, at least one of which is made of colorless transparent material; the cover is fitted and fixedly connected to one surface of the base, and a sample inlet, a sample outlet, a plurality of reaction chambers and one or a plurality of primary channels are formed between the base and the cover; and the sample inlet and the sample outlet can be communicated with the outside, the primary channels are communicated with the sample inlet and the sample outlet, and a liquid inlet channel and a liquid outlet channel are arranged between each reaction chamber and one primary channel.

[0009] The internal surfaces of the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels, which are located on the base and/or the cover, are hydrophilic surfaces, the cross sectional area of each liquid inlet channel is less than that of each liquid outlet channel, and in the liquid flow direction in the primary channels, each liquid inlet channel is located in front of each liquid outlet channel so that the solution of the sample to be tested that enters through the sample inlet can be driven by capillary force to enter the reaction chambers.

[0010] Moreover, after the solution of the sample to be tested enters the reaction chambers, the primary channels can be filled with an oil phase reagent to close the liquid inlet channels and the liquid outlet channels.

[0011] Further, the base is composed of a substrate, or the base is composed of a substrate and a reaction layer fitted on the substrate.

[0012] Further, the substrate and the reaction layer are silicon chips, and the cover is a glass sheet.

[0013] Further, the sample inlet, the sample outlet, the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels are formed on the base by means of etching.

[0014] Further, each primary channel has the width of 5 μm to 5 mm and the height of 5 μm to 3 mm.

[0015] Further, each liquid inlet channel has the width of 5 μm to 3 mm and the height of 5 μm to 3 mm; and each liquid outlet channel has the width of 5 μm to 3 mm and the height of 5 μm to 3 mm.

[0016] Further, the cross sectional areas of the liquid inlet channels, the liquid outlet channels and the primary channels increase in sequence.

[0017] Further, the oil phase reagent is one or a mixture of mineral oil, silicone oil, fluorocarbon oil and paraffin oil.

[0018] Further, the plurality of primary channels are arranged, and a waste liquid pool is arranged between the plurality of primary channels and the sample outlet.

[0019] A self-driven microfluidic chip comprises a base, a cover, a sample inlet, a sample outlet, a plurality of reaction chambers, at least one primary channel, a plurality of liquid inlet channels, a plurality of liquid outlet channels, a sample inlet through hole and a sample outlet through hole;

[0020] The cover is fitted and fixedly connected to the surface of the base; the sample inlet, the sample outlet, the plurality of reaction chambers and the at least one primary channel are arranged between the base and the cover, and the at least one primary channel is communicated with the sample inlet and the sample outlet; a liquid inlet channel and a liquid outlet channel are arranged between each reaction chamber and the corresponding primary channel; and the sample inlet through hole and the sample outlet through hole are arranged on the cover so that the sample inlet and the sample outlet can be communicated with the outside.

[0021] Further, the self-driven microfluidic chip is used for accommodating solution of the sample to be tested, and the internal surfaces of the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels, which are located on the base and/or the cover, are hydrophilic surfaces; the cross sectional area of each liquid inlet channel is less than that of each liquid outlet channel, and the cross sectional area of each liquid outlet channel is less than that of each primary channel; and in the liquid flow direction in the primary channels, each liquid inlet channel is located in front of each liquid outlet channel so that the solution of the sample to be tested that enters through the sample inlet can be driven by capillary force to enter the reaction chambers.

[0022] Further, after the solution of the sample to be tested enters the reaction chambers, the primary channels can be filled with an oil phase reagent to close the reaction chambers.

[0023] Further, the oil phase reagent is one or more of mineral oil, silicone oil, fluorocarbon oil and paraffin oil.

[0024] Further, along the liquid flow direction in the primary channels, the included angle between each liquid inlet channel and each primary channel is less than 90°, and the included angle between each liquid outlet channel and each primary channel is greater than or equal to

90°.

[0025] Further, each primary channel has the width of 5 μm to 5 mm and the height of 5 μm to 3 mm; each liquid inlet channel has the width of 5 μm to 3 mm and the height of 5 μm to 3 mm; and each liquid outlet channel has the width of 5 μm to 3 mm and the height of 5 μm to 3 mm.

[0026] Further, each primary channel has the width of 10 μm to 500 μm and the height of 10 μm to 500 μm ; each liquid inlet channel has the width of 5 μm to 500 μm and the height of 5 μm to 500 μm ; and each liquid outlet channel has the width of 5 μm to 500 μm and the height of 5 μm to 500 μm .

[0027] Further, the base is composed of a substrate, or the base is composed of a substrate and a reaction layer fitted on the substrate.

[0028] Further, the substrate and the reaction layer are silicon chips, and the cover is a glass sheet.

[0029] Further, hollow channels and reaction chambers are etched on the reaction layer.

[0030] Further, the sample inlet, the sample outlet, the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels are formed on the base by means of etching.

[0031] Further, the plurality of primary channels are arranged, and a waste liquid pool is arranged between the plurality of primary channels and the sample outlet.

[0032] A PCR testing method, using the self-driven microfluidic chip, is characterized by comprising the following steps.

a. Mixing DNA or RNA of a sample to be tested with a nucleic acid amplification reaction reagent containing fluorescent probe or fluorescent dye to obtain a solution of the sample to be tested.

b. Adding the solution of the sample to be tested at the sample inlet of the self-driven microfluidic chip so that the solution of the sample to be tested can be driven by capillary force to flow into the primary channels first and then flow into the liquid inlet channels, the reaction chambers and the liquid outlet channels in sequence until all the channels and the reaction chambers are filled.

c. Adding an oil phase reagent incompatible with water at the sample inlet so that the oil phase reagent can be driven by pressure to flow to the sample outlet along the primary channels and the solution of the sample to be tested in the primary channels is driven by the oil phase reagent to flow to the sample outlet until the primary channels are filled with the oil phase reagent, wherein the reaction chambers are separately isolated.

d. Closing the sample inlet and the sample outlet.

e. Placing the self-driven microfluidic chip in a real-time fluorescent quantitative PCR system for real-time fluorescent acquisition and testing to obtain a fluorescence signal curve; or, placing the self-driven microfluidic chip in an in situ PCR system for digital

PCR amplification reaction, after reaction, using a fluorescent acquisition system to conduct fluorescent imaging on the area of the reaction chambers, acquiring the fluorescence intensity signal of each reaction chamber, setting a fluorescence intensity threshold, if the threshold is exceeded, the reaction chamber is positive, counting the number of positive reaction chambers, and using Poisson distribution for derivation and calculation to obtain the original copy number of the tested sample, thereby realizing absolute quantitative testing.

[0033] Compared with the prior art, the present invention has the following advantages.

[0034] For the self-driven microfluidic chip of the present invention, the solution of the sample to be tested that enters through the sample inlet flows forwards from the sample inlet under the action of capillary force of the channels, and enters the reaction chambers under the action of capillary force of the liquid inlet channels when reaching the liquid inlet channels, and due to the front and back position relation and different cross sectional areas of the liquid inlet channels and the liquid outlet channels, the solution of the sample to be tested is subjected to a greater capillary force in the liquid inlet channels so as to be capable of entering the corresponding reaction chambers from the liquid inlet channels and pushing the air in the reaction chambers to flow to the primary channels from the liquid outlet channels. After the solution of the sample to be tested is added continuously, all the reaction chambers can be filled. In this way, the self-driven flow sampling of the solution of the sample to be tested can be realized without external drive equipment, and the present invention also can further prevent the tested sample from being polluted by impurities in the environment so as to avoid environmental pollution from the amplified sample.

[0035] The present invention also provides a method for using the self-driven microfluidic chip, comprising the following steps.

- a. Mixing DNA or RNA of a sample to be tested with a nucleic acid amplification reaction reagent containing fluorescent probe or fluorescent dye to obtain solution of the sample to be tested.
- b. Adding the solution of the sample to be tested at the sample inlet of the self-driven microfluidic chip so that the solution can be driven by capillary force to flow into the primary channels first and then flow into the liquid inlet channels, the reaction chambers and the liquid outlet channels in sequence until all the channels and the reaction chambers are filled.
- c. Adding an oil phase reagent incompatible with water at the sample inlet so that the oil phase reagent can be driven by pressure to flow to the waste liquid pool along the primary channels and the solution of the sample to be tested in the primary channels is driven by the oil phase reagent to flow to the waste

liquid pool until the primary channels are filled with the oil phase reagent, wherein the reaction chambers are separately isolated.

d. Closing the sample inlet and the sample outlet.

e. Placing the chip in a real-time fluorescent quantitative PCR system for real-time fluorescent acquisition and testing to obtain a fluorescence signal curve; or, placing the chip in an in situ PCR system for digital PCR amplification reaction, after reaction, using a fluorescent acquisition system to conduct fluorescent imaging on the area of the reaction chambers, acquiring the fluorescence intensity signal of each reaction chamber, setting a fluorescence intensity threshold, if the threshold is exceeded, the reaction chamber is positive, counting the number of positive reaction chambers, and using Poisson distribution for derivation and calculation to obtain the original copy number of the tested sample, thereby realizing absolute quantification.

[0036] The method for using the self-driven microfluidic chip of the present invention can realize real-time fluorescent quantitative PCR testing or digital PCR testing without a microvalve or a micropump or other structures, the experiment cost is low, the periphery of each reaction chamber is sealed, and the use method also has the advantage of good evaporation and fusion prevention effects.

Description of Drawings

[0037] Drawings forming a part of the present invention are used for providing further understanding of the present invention. Exemplary embodiments of the present invention and the description are used for explaining the present invention, but do not constitute a limitation to the present invention. In the drawings:

Fig. 1 is a schematic diagram of a self-driven microfluidic chip of embodiment 1 of the present invention; Fig. 2 is a schematic diagram of a self-driven microfluidic chip of embodiment 2 of the present invention; Fig. 3 is a local enlarged view of Position I in Fig. 2.

Reference Signs:

[0038] 1 - base, 11 - sample inlet, 12 - sample outlet, 13 - primary channel, 14 - reaction chamber, 15 - liquid inlet channel, 16 - liquid outlet channel, 17 - waste liquid pool, 2 - cover, 21 - sample inlet through hole, and 22 - sample outlet through hole.

Detailed Description

[0039] It should be explained that if there is no conflict, the embodiments in the present invention and the features in the embodiments can be mutually combined.

[0040] The present invention will be described in detail

below by reference to the drawings and in conjunction with the embodiments.

Embodiment 1

[0041] The embodiment relates to a self-driven microfluidic chip which is used for accommodating the solution of the sample to be tested so as to carry out various testing operations such as real-time fluorescent quantitative PCR testing or digital PCR testing. As shown in Fig. 1, the self-driven microfluidic chip comprises a base 1 and a cover 2, at least one of which is made of colorless transparent material, the colorless transparent material shall have low fluorescence, and the characteristic of low fluorescence of the embodiment is generally that the excited fluorescence intensity of the material is less than that of fluorescein with a concentration of $10\text{e-}8$ mol/L under the irradiation of excitation wavelength of the fluorescence probe such as FAM, HEX, VIC, CY3, TAMRA, ROX and CY5 used in the PCR reagent. Due to the characteristic of low fluorescence, the solution of the sample to be tested can be received by a testing instrument.

[0042] The base 1 is preferably made of material which has good biocompatibility and is not easy to adsorb such substances as nucleic acid, and also preferably made of material with good heat conduction effect. Specifically, in the embodiment, the cover 2 is a transparent glass sheet, and the base 1 is a silicon chip. Of course, in other embodiments of the present invention, the base 1 and the cover 2 can be both made of transparent material such as glass sheet, or the base 1 and the cover 2 also can be made of other materials meeting the arrangement requirements of the microfluidic chip of the embodiment.

[0043] In Fig. 1, to clearly show the structure of the cover 2 and the base 1, the cover 2 and the base 1 are separated by a certain distance. In practical application, the cover 2 is fitted and fixedly connected to the surface of the base 1, and the cover 2 and the base 1 can be connected by means of hot pressing, laser welding, ultrasonic welding, low temperature bonding, electrostatic bonding and adhesive bonding according to different materials.

[0044] Also as shown in Fig. 1, a sample inlet 11, a sample outlet 12, a plurality of reaction chambers 14 and one primary channel 13 are formed between the base 1 and the cover 2. Specifically, the sample inlet 11, the sample outlet 12, the plurality of reaction chambers 14 and one primary channel 13 are formed on the surface of the base 1 by means of etching. A sample inlet through hole 21 and a sample outlet through hole 22 respectively aligned up and down with the sample inlet 11 and the sample outlet 12 are arranged on the cover 2 so that the sample inlet 11 and the sample outlet 12 can be communicated with the outside. Both ends of each primary channel 13 are respectively communicated with the sample inlet 11 and the sample outlet 12, and the solution of the sample to be tested can flow to the sample outlet 12 through the primary channels 13 and flow out through

the sample outlet 12 after being dropped in the sample inlet 11 through the sample inlet through hole 21. A liquid inlet channel 15 and a liquid outlet channel 16 are arranged between each reaction chamber 14 and the primary channel 13.

[0045] The sample inlet 11 of the embodiment is preferably circular, the diameter thereof is preferably 0.5 mm, 1 mm, 1.2 mm, 1.5 mm or 2 mm, and the height can be 0.1 mm, 0.2 mm or 0.3 mm. Similarly, the sample outlet 12 is also preferably circular, the diameter is preferably 0.5 mm, 1 mm, 2 mm, 3 mm, 4 mm or 5 mm, and the height can be 0.1 mm, 0.2 mm or 0.3 mm.

[0046] It should be noted that the sample inlet 11, the sample outlet 12, the plurality of reaction chambers 14, the primary channels 13, the liquid inlet channels 15 and the liquid outlet channels 16 can be arranged on the base 1 or the cover 2, or simultaneously processed on the base 1 and the cover 2. After the cover 2 is fitted and fixed on the surface of the base 1, the sample inlet 11, the sample outlet 12, the plurality of reaction chambers 14, the primary channels 13, the liquid inlet channels 15 and the liquid outlet channels 16 are located between the base 1 and the cover 2, and at this time, the reaction chambers 14, the primary channels 13, the liquid inlet channels 15 and the liquid outlet channels 16 are closed between the base 1 and the cover 2 and only can be communicated with the outside atmosphere through the sample inlet 11 and the sample outlet 12.

[0047] In addition, it should be also explained in the embodiment that the base 1 can be composed of a substrate, and the structure of the microfluidic chip composed of the substrate and the cover 2 is shown as above. Alternatively, the base of the embodiment also can be composed of a substrate and a reaction layer fitted on the substrate, hollow channels and reaction chambers can be etched on the reaction layer to be used as a bottom layer corresponding to the substrate of the reaction layer, and the cover is used as an upper layer so that the microfluidic chip has a three-layer structure, which is different from the two-layer structure of the base 1 only composed of a substrate. The substrate and the reaction layer are preferably silicon chips.

[0048] In the embodiment, the internal surfaces of the primary channels 13, the reaction chambers 14, the liquid inlet channels 15 and the liquid outlet channels 16, which are located on the base 1 and/or the cover 2, are hydrophilic surfaces, the cross sectional area of each liquid inlet channel 15 is less than that of each liquid outlet channel 16, and in the liquid flow direction in the primary channels 13, each liquid inlet channel 15 is located in front of each liquid outlet channel 16, this is to say, the liquid in the primary channels 13 will touch the liquid inlet channels 15 first and then the liquid outlet channels 16 when flowing. The hydrophilic surfaces can be composed of silicon dioxide layers.

[0049] With the above arrangement, the solution of the sample to be tested that enters through the sample inlet 11 flows forwards from the sample inlet 11 under the

action of capillary force of the primary channels 13, and enters the reaction chambers 14 under the action of capillary force of the liquid inlet channels 15 when reaching the liquid inlet channels 15, and due to the front and back position relation and different cross sectional areas of the liquid inlet channels 15 and the liquid outlet channels 16, the solution of the sample to be tested is subjected to a greater capillary force in the liquid inlet channels 15 so as to be capable of entering the corresponding reaction chambers 14 from the liquid inlet channels 15 and pushing the air in the reaction chambers 14 to flow to the primary channels 13 from the liquid outlet channels 16. After the solution of the sample to be tested is added continuously, all the reaction chambers 14 can be filled. In this way, the self-driven microfluidic chip of the embodiment realizes the self-driven flow sampling of the solution of the sample to be tested without external drive equipment.

[0050] In the embodiment, along the liquid flow direction in the primary channels 13, the included angle between each liquid inlet channel 15 and each primary channel 13 is less than 90° , and the included angle between each liquid outlet channel 16 and each primary channel 13 is greater than or equal to 90° . In this way, the solution of the sample to be tested can enter the liquid inlet channels 15 more smoothly. Moreover, in the embodiment, the cross sectional areas of the liquid inlet channels 15, the liquid outlet channels 16 and the primary channels 13 also increase in sequence. Under equal conditions, the smaller the cross sectional area of a channel is, the greater the internal capillary force is, so the cross sectional area of each liquid inlet channel 15 is less than that of each liquid outlet channel 16, which can ensure that the capillary force in the liquid inlet channels 15 is higher than that in the liquid outlet channels 16. The solution of the sample to be tested can enter the reaction chambers 14 continuously through the liquid inlet channels 15 and discharge air in the reaction chambers 14 through the liquid outlet channels 16, and the liquid flow in the liquid inlet channels 15 per unit time is not lower than that in the liquid outlet channels 16.

[0051] In the embodiment, the intersections of the liquid inlet channels 15 and the liquid outlet channels 16 with the primary channels 13 are arranged at intervals so that when the solution of the sample to be tested flows along the primary channels 13, a sufficient amount of solution can first enter the liquid inlet channels 15 and press the air in the reaction chambers 14 into the liquid outlet channels 16. The air can prevent the solution of the sample to be tested from entering the liquid outlet channels 16, so the reaction chambers 14 are easier to be filled.

[0052] In the embodiment, each primary channel 13 has the width of $5\ \mu\text{m}$ to $5\ \text{mm}$ and the height of $5\ \mu\text{m}$ to $3\ \text{mm}$, and preferably, each primary channel 13 can have the width of $10\ \mu\text{m}$ to $500\ \mu\text{m}$, for example, $20\ \mu\text{m}$, $50\ \mu\text{m}$, $100\ \mu\text{m}$, $200\ \mu\text{m}$, $250\ \mu\text{m}$, $300\ \mu\text{m}$ and $400\ \mu\text{m}$, and the height of $10\ \mu\text{m}$ to $500\ \mu\text{m}$, for example, $20\ \mu\text{m}$, $50\ \mu\text{m}$, $100\ \mu\text{m}$, $200\ \mu\text{m}$, $250\ \mu\text{m}$, $300\ \mu\text{m}$ and $400\ \mu\text{m}$.

$100\ \mu\text{m}$, $200\ \mu\text{m}$, $250\ \mu\text{m}$, $300\ \mu\text{m}$ and $400\ \mu\text{m}$.

[0053] Each liquid inlet channel 15 has the width of $5\ \mu\text{m}$ to $3\ \text{mm}$ and the height of $5\ \mu\text{m}$ to $3\ \text{mm}$, and preferably, each liquid inlet channel 15 can have the width of $5\ \mu\text{m}$ to $500\ \mu\text{m}$, for example, $10\ \mu\text{m}$, $20\ \mu\text{m}$, $50\ \mu\text{m}$, $100\ \mu\text{m}$, $200\ \mu\text{m}$, $250\ \mu\text{m}$, $300\ \mu\text{m}$ and $400\ \mu\text{m}$, and the height of $5\ \mu\text{m}$ to $500\ \mu\text{m}$, for example, $10\ \mu\text{m}$, $20\ \mu\text{m}$, $50\ \mu\text{m}$, $100\ \mu\text{m}$, $200\ \mu\text{m}$, $250\ \mu\text{m}$, $300\ \mu\text{m}$ and $400\ \mu\text{m}$.

[0054] Each liquid outlet channel 16 has the width of $5\ \mu\text{m}$ to $3\ \text{mm}$ and the height of $5\ \mu\text{m}$ to $3\ \text{mm}$, and preferably, each liquid outlet channel 16 can have the width of $5\ \mu\text{m}$ to $500\ \mu\text{m}$, for example, $10\ \mu\text{m}$, $20\ \mu\text{m}$, $50\ \mu\text{m}$, $100\ \mu\text{m}$, $200\ \mu\text{m}$, $250\ \mu\text{m}$, $300\ \mu\text{m}$ and $400\ \mu\text{m}$, and the height of $5\ \mu\text{m}$ to $500\ \mu\text{m}$, for example, $10\ \mu\text{m}$, $20\ \mu\text{m}$, $50\ \mu\text{m}$, $100\ \mu\text{m}$, $200\ \mu\text{m}$, $250\ \mu\text{m}$, $300\ \mu\text{m}$ and $400\ \mu\text{m}$.

[0055] In the embodiment, after the solution of the sample to be tested enters the reaction chambers 14, an oil phase reagent can be filled in the primary channels 13. The type of the oil phase reagent is not limited, and can be one or a mixture of mineral oil, silicone oil, fluorocarbon oil and paraffin oil. Since the reaction chambers 14, the liquid inlet channels 15 and the liquid outlet channels 16 are filled with the solution of the sample to be tested, which is aqueous and incompatible with oil, the oil phase reagent will not enter the liquid inlet channels 15 and the liquid outlet channels 16 so that the solution of the sample to be tested can be kept dispersed and blocked in the reaction chambers by the oil phase reagent. After sample loading, an adhesive tape with high transparency and high temperature resistance is stuck on the surface of the chip to close the sample inlet 11 and the sample outlet 12.

[0056] The self-driven microfluidic chip of the embodiment can realize non-driven sample injection by means of the design of the structure and the surface tension of the material without an external drive pump and other drive equipment, has simple and convenient sample loading process, provides more convenient operating modes for nucleic acid testing in conventional experiment environments and various special complex experiment environments, and also greatly reduces the testing cost. Meanwhile, the chip made by the method has high sample injection speed and can increase the testing speed and shorten the waiting time.

[0057] In addition, the sample loading amount of the solution of the sample to be tested required by the self-driven microfluidic chip of the embodiment is in μL , and the demand for the sample and the reagent is low, which can reduce the degree of difficulty in acquisition of the sample to be tested and reduce the usage amount of the reagent and the cost. The solution of the sample to be tested is injected and dispersed in the closed chip, then amplification and result testing can be carried out without taking out the sample, and the whole process is performed without exposure to the external environment, which can effectively prevent the tested sample from being polluted by impurities in the environment and also

avoid environmental pollution from the amplified sample.

[0058] Compared with traditional nucleic acid testing chips for real-time fluorescent quantitative PCR, the self-driven microfluidic chip of the embodiment has the function of reagent dispersion and can realize traditional nucleic acid testing and digital nucleic acid testing. The digital nucleic acid testing method can realize absolute quantitative nucleic acid testing with higher sensitivity and higher accuracy, and theoretically can realize single molecule nucleic acid testing. The self-driven microfluidic chip can be widely applied in the fields of early cancer screening, cancer companion diagnostic, non-invasive prenatal screening, and organ transplant and matching, which require high testing sensitivity and high accuracy.

[0059] In addition, compared with the existing digital PCR platforms, the self-driven microfluidic chip of the embodiment does not need a sample inlet instrument, or a microvalve or a micropump or other structures made on the chip, which can greatly reduce the production cost of the chip. Meanwhile, the periphery of each reaction chamber is sealed, and each reaction chamber is only connected with the primary channel, so the self-driven microfluidic chip has better evaporation and fusion prevention effects than droplet and microslot digital PCR chips. At the same time, each reaction chamber has fixed volume, higher uniformity, and better testing sensitivity and accuracy.

Embodiment 2

[0060] The embodiment relates to a self-driven microfluidic chip, which has roughly the same structure as the self-driven microfluidic chip in embodiment 1, and the difference is that as shown in Fig. 2 and Fig. 3, in the embodiment, a plurality of primary channels 13 are arranged, a waste liquid pool 17 is arranged between the plurality of primary channels 13 and the sample outlet 12, and the structure of each reaction chamber 14 is square. The solution of the sample to be tested or the oil phase reagent in each primary channel 13 can enter the waste liquid pool 17 and then flow to the sample outlet 12.

[0061] In addition, when the self-driven microfluidic chip of the embodiment is used for real-time fluorescent quantitative PCR testing or digital PCR nucleic acid testing, the specific use method thereof comprises the following steps, and the solution of the sample to be tested below can be commercial HBV DNA fragments. The self-driven microfluidic chip of embodiment 1 can also be used with reference to the steps described below.

[0062] Specifically, the use method of the self-driven microfluidic chip of the embodiment comprises:

Step a: mixing DNA or RNA of a sample to be tested with a nucleic acid amplification reaction reagent containing fluorescent probe or fluorescent dye to obtain solution of the sample to be tested;

Step b: adding the solution of the sample to be tested at the sample inlet of the self-driven microfluidic chip

so that the solution can be driven by capillary force to flow into the primary channels first and then flow into the liquid inlet channels, the reaction chambers and the liquid outlet channels in sequence until all the channels and the reaction chambers are filled; Step c: adding an oil phase reagent incompatible with water at the sample inlet so that the oil phase reagent can be driven by pressure to flow to the waste liquid pool along the primary channels and the solution of the sample to be tested in the primary channels is driven by the oil phase reagent to flow to the waste liquid pool until the primary channels are filled with the oil phase reagent, wherein the reaction chambers are separately isolated;

Step d: closing the sample inlet and the sample outlet;

Step e: placing the chip in a real-time fluorescent quantitative PCR system for real-time fluorescent acquisition and testing to obtain a fluorescence signal curve; or, placing the chip in an in situ PCR system for digital PCR amplification reaction, after reaction, using a fluorescent acquisition system to conduct fluorescent imaging on the area of the reaction chambers, acquiring the fluorescence intensity signal of each reaction chamber, setting a fluorescence intensity threshold, if the threshold is exceeded, the reaction chamber is positive, counting the number of positive reaction chambers, and using Poisson distribution for derivation and calculation to obtain the original copy number of the tested sample, thereby realizing absolute quantification.

[0063] The self-driven microfluidic chip of the embodiment can realize real-time fluorescent quantitative PCR testing or digital PCR testing without a microvalve or a micropump or other structures, the experiment cost is low, the periphery of each reaction chamber is sealed, and the self-driven microfluidic chip has good evaporation and fusion prevention effects.

[0064] Apparently, the above embodiments are only examples made for clear description, and do not define the embodiments. For those ordinary skilled in the art, other variations or changes in other forms can also be made based on the above description. Not all of the embodiments are enumerated herein. Apparent variations or changes derived therefrom are still within the protection scope of the present invention.

Claims

1. A self-driven microfluidic chip, used for accommodating solution of a sample to be tested, comprising a base and a cover, at least one of which is made of colorless transparent material; the cover is fitted and fixedly connected to one surface of the base, and a sample inlet, a sample outlet, a plurality of reaction chambers and one or a plurality of primary channels

are formed between the base and the cover; and the sample inlet and the sample outlet can be communicated with the outside, the primary channels are communicated with the sample inlet and the sample outlet, and a liquid inlet channel and a liquid outlet channel are arranged between each reaction chamber and one primary channel;

the internal surfaces of the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels, which are located on the base and/or the cover, are hydrophilic surfaces, the cross sectional area of each liquid inlet channel is less than that of each liquid outlet channel, and in the liquid flow direction in the primary channels, each liquid inlet channel is located in front of each liquid outlet channel so that the solution of the sample to be tested that enters through the sample inlet can be driven by capillary force to enter the reaction chambers; moreover, after the solution of the sample to be tested enters the reaction chambers, the primary channels can be filled with an oil phase reagent to close the liquid inlet channels and the liquid outlet channels.

2. The self-driven microfluidic chip according to claim 1, wherein the base is composed of a substrate, or the base is composed of a substrate and a reaction layer fitted on the substrate.
3. The self-driven microfluidic chip according to claim 2, wherein the substrate and the reaction layer are silicon chips, and the cover is a glass sheet.
4. The self-driven microfluidic chip according to claim 1, wherein the sample inlet, the sample outlet, the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels are formed on the base by means of etching.
5. The self-driven microfluidic chip according to claim 1, wherein each primary channel has the width of 5 μm to 5 mm and the height of 5 μm to 3 mm.
6. The self-driven microfluidic chip according to claim 1, wherein each liquid inlet channel has the width of 5 μm to 3 mm and the height of 5 μm to 3 mm; and each liquid outlet channel has the width of 5 μm to 3 mm and the height of 5 μm to 3 mm.
7. The self-driven microfluidic chip according to claim 1, wherein the cross sectional areas of the liquid inlet channels, the liquid outlet channels and the primary channels increase in sequence.
8. The self-driven microfluidic chip according to claim 1, wherein the oil phase reagent is one or a mixture

of mineral oil, silicone oil, fluorocarbon oil and paraffin oil.

9. The self-driven microfluidic chip according to claim 1, wherein the plurality of primary channels are arranged, and a waste liquid pool is arranged between the plurality of primary channels and the sample outlet.
10. A method for using the self-driven microfluidic chip of claim 1, wherein the method comprises the following steps:
 - a. mixing DNA or RNA of a sample to be tested with a nucleic acid amplification reaction reagent containing fluorescent probe or fluorescent dye to obtain a solution of the sample to be tested;
 - b. adding the solution of the sample to be tested at the sample inlet of the self-driven microfluidic chip so that the solution can be driven by capillary force to flow into the primary channels first and then flow into the liquid inlet channels, the reaction chambers and the liquid outlet channels in sequence until all the channels and the reaction chambers are filled;
 - c. adding an oil phase reagent incompatible with water at the sample inlet so that the oil phase reagent can be driven by pressure to flow to the waste liquid pool along the primary channels and the solution of the sample to be tested in the primary channels is driven by the oil phase reagent to flow to the waste liquid pool until the primary channels are filled with the oil phase reagent, wherein the reaction chambers are separately isolated;
 - d. closing the sample inlet and the sample outlet;
 - e. placing the self-driven microfluidic chip in a real-time fluorescent quantitative PCR system for real-time fluorescent acquisition and testing to obtain a fluorescence signal curve; or, placing the self-driven microfluidic chip in an in situ PCR system for digital PCR amplification reaction, after reaction, using a fluorescent acquisition system to conduct fluorescent imaging on the area of the reaction chambers, acquiring the fluorescence intensity signal of each reaction chamber, setting a fluorescence intensity threshold, if the threshold is exceeded, the reaction chamber is positive, counting the number of positive reaction chambers, and using Poisson distribution for derivation and calculation to obtain the original copy number of the tested sample, thereby realizing absolute quantification.
11. A self-driven microfluidic chip, comprising a base, a cover, a sample inlet, a sample outlet, a plurality of reaction chambers, at least one primary channel, a plurality of liquid inlet channels, a plurality of liquid

outlet channels, a sample inlet through hole and a sample outlet through hole;

the cover is fitted and fixedly connected to the surface of the base; the sample inlet, the sample outlet, the plurality of reaction chambers and the at least one primary channel are arranged between the base and the cover, and the at least one primary channel is communicated with the sample inlet and the sample outlet; a liquid inlet channel and a liquid outlet channel are arranged between each reaction chamber and the corresponding primary channel; and the sample inlet through hole and the sample outlet through hole are arranged on the cover so that the sample inlet and the sample outlet can be communicated with the outside.

12. The self-driven microfluidic chip according to claim 11, wherein the self-driven microfluidic chip is used for accommodating a solution of the sample to be tested, and the internal surfaces of the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels, which are located on the base and/or the cover, are hydrophilic surfaces;

the cross sectional area of each liquid inlet channel is less than that of each liquid outlet channel, and the cross sectional area of each liquid outlet channel is less than that of each primary channel;

in the liquid flow direction in the primary channels, each liquid inlet channel is located in front of each liquid outlet channel so that the solution of the sample to be tested that enters through the sample inlet can be driven by capillary force to enter the reaction chambers.

13. The self-driven microfluidic chip according to claim 12, wherein after the solution of the sample to be tested enters the reaction chambers, the primary channels can be filled with an oil phase reagent to close the reaction chambers.
14. The self-driven microfluidic chip according to claim 13, wherein the oil phase reagent is one or a mixture of mineral oil, silicone oil, fluorocarbon oil and paraffin oil.
15. The self-driven microfluidic chip according to claim 13, wherein along the liquid flow direction in the primary channels, an included angle between each liquid inlet channel and each primary channel is less than 90° , and an included angle between each liquid outlet channel and each primary channel is greater than or equal to 90° .
16. The self-driven microfluidic chip according to claim 13, wherein each primary channel has the width of $5\text{ }\mu\text{m}$ to 5 mm and the height of $5\text{ }\mu\text{m}$ to 3 mm ; each

liquid inlet channel has the width of $5\text{ }\mu\text{m}$ to 3 mm and the height of $5\text{ }\mu\text{m}$ to 3 mm ; and each liquid outlet channel has the width of $5\text{ }\mu\text{m}$ to 3 mm and the height of $5\text{ }\mu\text{m}$ to 3 mm .

17. The self-driven microfluidic chip according to claim 13, wherein each primary channel has the width of $10\text{ }\mu\text{m}$ to $500\text{ }\mu\text{m}$ and the height of $10\text{ }\mu\text{m}$ to $500\text{ }\mu\text{m}$; each liquid inlet channel has the width of $5\text{ }\mu\text{m}$ to $500\text{ }\mu\text{m}$ and the height of $5\text{ }\mu\text{m}$ to $500\text{ }\mu\text{m}$; and each liquid outlet channel has the width of $5\text{ }\mu\text{m}$ to $500\text{ }\mu\text{m}$ and the height of $5\text{ }\mu\text{m}$ to $500\text{ }\mu\text{m}$.
18. The self-driven microfluidic chip according to claim 13, wherein the base is composed of a substrate, or the base is composed of a substrate and a reaction layer fitted on the substrate.
19. The self-driven microfluidic chip according to claim 18, wherein the substrate and the reaction layer are silicon chips, and the cover is a glass sheet.
20. The self-driven microfluidic chip according to claim 18, wherein hollow channels and reaction chambers are etched on the reaction layer.
21. The self-driven microfluidic chip according to claim 13, wherein the sample inlet, the sample outlet, the primary channels, the reaction chambers, the liquid inlet channels and the liquid outlet channels are formed on the base by means of etching.
22. The self-driven microfluidic chip according to claim 11, wherein a plurality of primary channels are arranged, and a waste liquid pool is arranged between the plurality of primary channels and the sample outlet.
23. A PCR testing method, using the self-driven microfluidic chip of any of claims 11-22, wherein the method comprises the following steps:
- mixing DNA or RNA of a sample to be tested with a nucleic acid amplification reaction reagent containing fluorescent probe or fluorescent dye to obtain a solution of the sample to be tested;
 - adding the solution of the sample to be tested at the sample inlet of the self-driven microfluidic chip so that the solution of the sample to be tested can be driven by capillary force to flow into the primary channels first and then flow into the liquid inlet channels, the reaction chambers and the liquid outlet channels in sequence until all the channels and the reaction chambers are filled;
 - adding an oil phase reagent incompatible with water at the sample inlet so that the oil phase reagent can be driven by pressure to flow to the

sample outlet along the primary channels and the solution of the sample to be tested in the primary channels is driven by the oil phase reagent to flow to the sample outlet until the primary channels are filled with the oil phase reagent, wherein the reaction chambers are separately isolated; 5

d. closing the sample inlet and the sample outlet;

e. placing the self-driven microfluidic chip in a real-time fluorescent quantitative PCR system for real-time fluorescent acquisition and testing to obtain a fluorescence signal curve; or, placing the self-driven microfluidic chip in an in situ PCR system for digital PCR amplification reaction, after reaction, using a fluorescent acquisition system to conduct fluorescent imaging on the area of the reaction chambers, acquiring the fluorescence intensity signal of each reaction chamber, setting a fluorescence intensity threshold, if the threshold is exceeded, the reaction chamber is positive, counting the number of positive reaction chambers, and using Poisson distribution for derivation and calculation to obtain the original copy number of the tested sample, thereby realizing absolute quantitative testing. 25

30

35

40

45

50

55

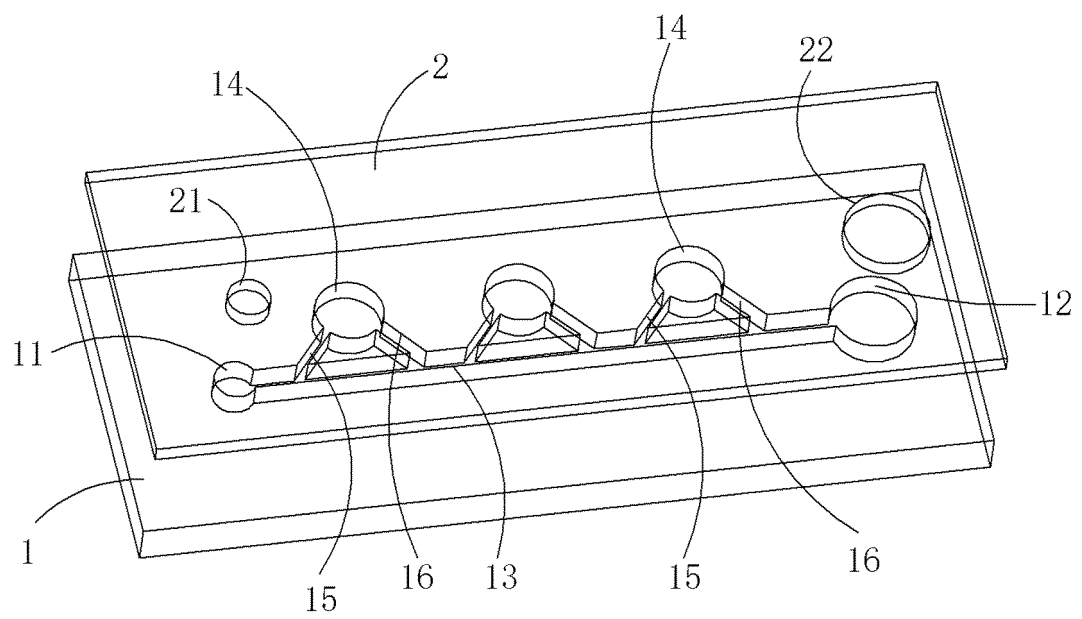


FIG. 1

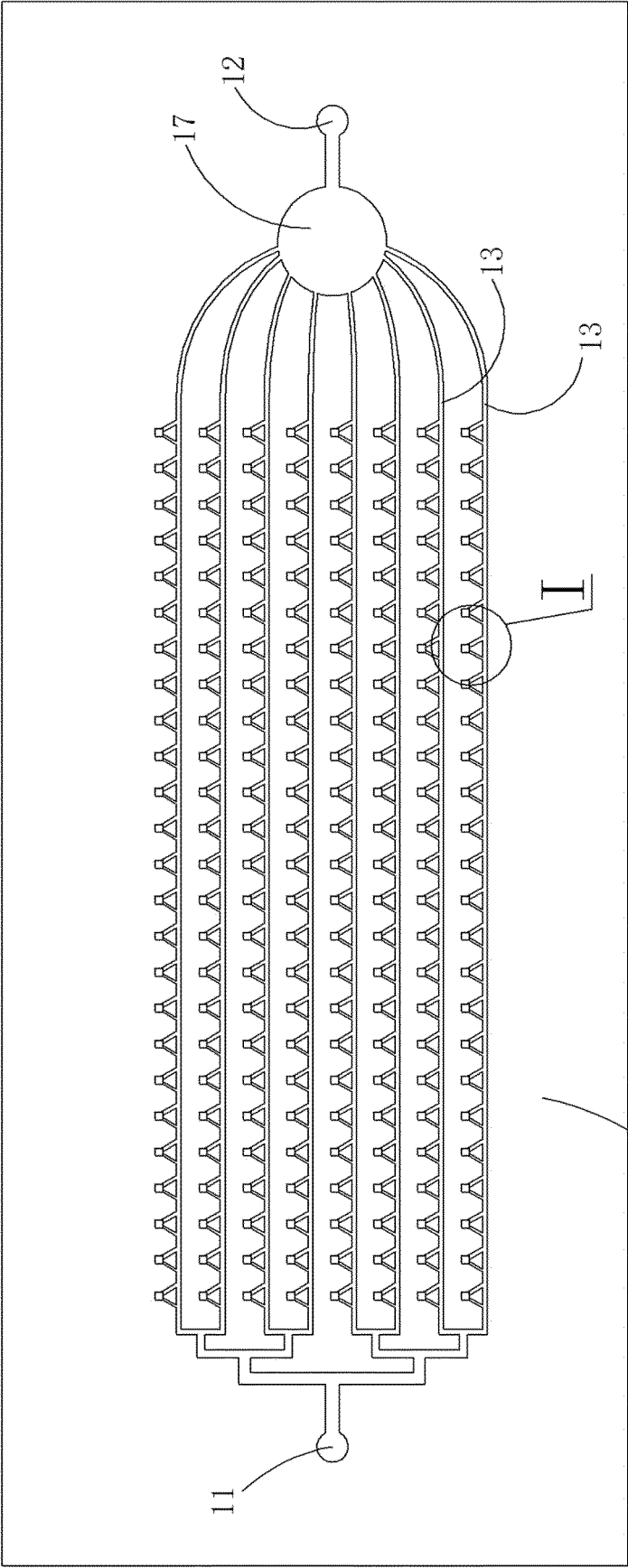


FIG. 2

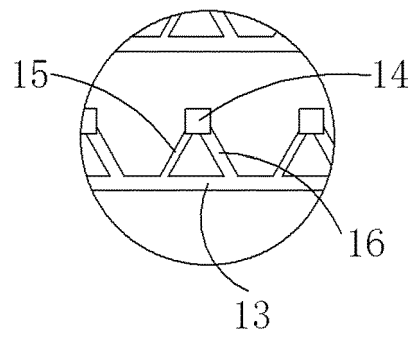


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/081497

5	A. CLASSIFICATION OF SUBJECT MATTER	
	C12M 1/34(2006.01)i; C12M 1/00(2006.01)i; C12Q 1/6851(2018.01)i	
	According to International Patent Classification (IPC) or to both national classification and IPC	
	B. FIELDS SEARCHED	
10	Minimum documentation searched (classification system followed by classification symbols)	
	C12M C12Q	
	Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched	
15	Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)	
	CNABS; CNMED; CNKI; CNTXT; 百度学术和检索词, Baidu Xueshu and Search Terms: 毛细力, 毛细管力, 芯片, 聚合酶链式反应, 通道, 横截面积等; DWPI; SIPOABS; EPTXT; USTXT; WOTXT; JPTXT; PUBMED; ISI WEB OF SCIENCE和检索词, ISI WEB OF SCIENCE and Search Terms: capillary force?, chip?, channel?, PCR, polymerase chain reaction, cross sectional area etc.	
20	C. DOCUMENTS CONSIDERED TO BE RELEVANT	
	Category*	Citation of document, with indication, where appropriate, of the relevant passages
		Relevant to claim No.
	X	CN 105026932 A (SIEMENS HEALTHCARE DIAGNOSTICS INC.) 04 November 2015 (2015-11-04) see description, paragraphs [0022]-[0081], and figures 1-5
25	PX	CN 109929749 A (SHENZHEN SHINEWAY HI-TECH CO., LTD.) 25 June 2019 (2019-06-25) see claims 1-10, and description, paragraphs [0005]-[0063]
	A	US 2009197274 A1 (SEIKO EPSON CORPORATION) 06 August 2009 (2009-08-06) see description, paragraphs [0024]-[0054]
30	A	CN 102277294 A (ZHEJIANG UNIVERSITY) 14 December 2011 (2011-12-14) see description, specific embodiment
	A	CN 104263631 A (AKONNI BIOSYSTEMS, INC.) 07 January 2015 (2015-01-07) see description, specific embodiment
35	A	CN 107475074 A (SHENZHEN SHINEWAY HI-TECH CO., LTD.) 15 December 2017 (2017-12-15) see description, specific embodiment
	☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.	
40	* 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
	11 June 2020	30 June 2020
50	Name and mailing address of the ISA/CN	Authorized officer
	China National Intellectual Property Administration (ISA/CN) No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088 China	
55	Facsimile No. (86-10)62019451	Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2020/081497

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 105026932 A	04 November 2015	US 2016318019 A1	03 November 2016
		KR 20150130523 A	23 November 2015
		KR 101653701 B1	02 September 2016
		EP 2972331 A1	20 January 2016
		WO 2014151450 A1	25 September 2014
		CN 105026932 B	13 June 2017
		EP 2972331 A4	04 January 2017
		US 10105701 B2	23 October 2018
		EP 2972331 B1	17 October 2018
CN 109929749 A	25 June 2019	None	
US 2009197274 A1	06 August 2009	JP 2009178146 A	13 August 2009
		US 8092999 B2	10 January 2012
		JP 4556194 B2	06 October 2010
CN 102277294 A	14 December 2011	CN 102277294 B	17 April 2013
CN 104263631 A	07 January 2015	None	
CN 107475074 A	15 December 2017	None	

Form PCT/ISA/210 (patent family annex) (January 2015)

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- CN 201910235034 [0001]