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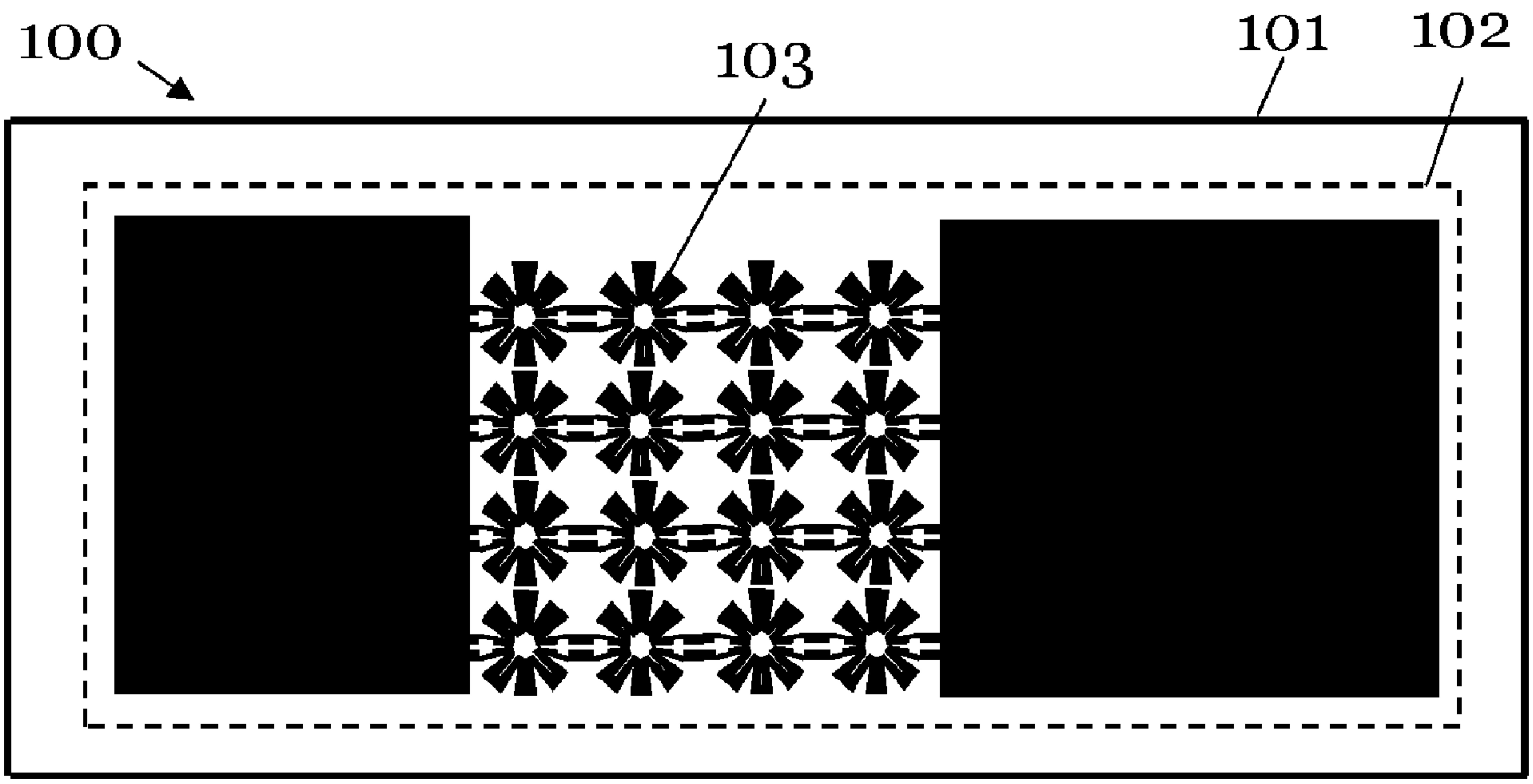

FIG 1

(57) Abrégé/Abstract:
A micro-fluidic device (100) for deflecting objects in a liquid, the device comprising: a substrate (101) for providing an object-containing liquid thereon; and a microbubble generator (e.g. a heating element) (102), having at least one microbubble generating

(57) Abrégé(suite)/Abstract(continued):
element (e.g. at least one micro-heater) (103), located on a surface of the substrate and in direct contact with the liquid when provided on the substrate (101); characterized in that: the at least microbubble generating element (103) is adapted to deflect a single object in the liquid through generation of a plurality of microbubblesby each of them. Further, systems (200) for sorting objects using the micro-fluidic device (100) and methods for fabricating a micro-fluidic device (100) are presented.

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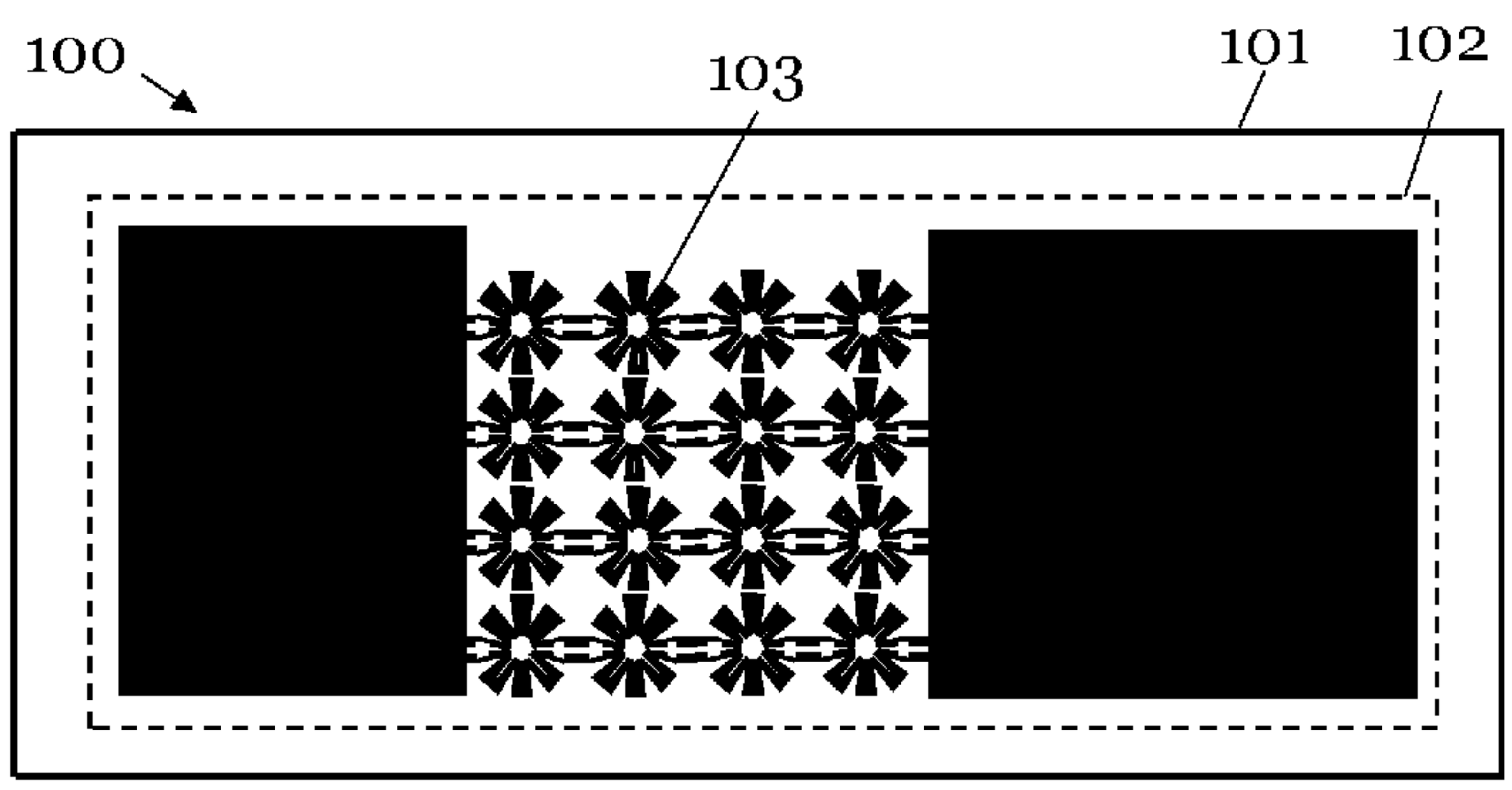

FIG 1

(57) **Abstract**: A micro-fluidic device (100) for deflecting objects in a liquid, the device comprising: a substrate (101) for providing an object-containing liquid thereon; and a microbubble generator (e.g. a heating element) (102), having at least one microbubble generating element (e.g. at least one micro-heater) (103), located on a surface of the substrate and in direct contact with the liquid when provided on the substrate (101); characterized in that: the at least microbubble generating element (103) is adapted to deflect a single object in the liquid through generation of a plurality of microbubblesby each of them. Further, systems (200) for sorting objects using the micro-fluidic device (100) and methods for fabricating a micro-fluidic device (100) are presented.

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Microbubble generator device, systems and method to fabricate

Field of the Invention

The field of the invention is micro-fluidic devices. In particular, the invention is related to jet flow generators for micro-fluidic systems. More in particular, the invention is related to flow cytometry devices.

Background to the Invention

Cell sorting is a technique whereby cells are separated based on their properties. Typically, in a first stage, cells propagate through a fluidic channel and are characterized, for example based on size. In a second stage, based on the characterization of each cell, each cell is sorted by deflecting that cell towards an outlet of the fluidic channel.

In prior art devices the deflection of cells is performed by generating a microbubble which creates a jet flow in the fluidic channel. The magnitude of the force of the jet flow on a cell determines to which outlet of the fluidic channel the cell is deflected.

A first problem in these prior art devices is the time required to generate a microbubble. Because cell sorting applications require high throughput, it is essential that microbubbles can be generated in a short span of time to keep up with the velocity of cells propagating through the fluidic channel.

A second problem relates to the temperature required to create microbubbles. To bring a liquid to its boiling point on a micro scale, a higher temperature is required than the boiling temperature of bulk liquid. This phenomenon is known as superheating. The requirement of higher temperatures leads to higher energy consumption and, related to the first problem, to a longer duration of the heating to create microbubbles.

A third problem relates to the accuracy of the sorting. The accuracy is related to the controllability of the generated microbubbles. In order to increase the accuracy it is required that there is total control over the force created by the jet flow. In current prior art devices, this is lacking.

There is a need for a micro-fluidic device which solves at least some of the problems stated above.

Summary of the Invention

It is an object of the invention to create a microbubble generator which allows accurate generation of a jet flow in a liquid.

Particular and preferred aspects of the invention are set out in the accompanying independent and dependent claims. Features from the dependent claims may be combined with features of the independent claims and with features of other dependent claims as appropriate and not merely as explicitly set out in the claims. Embodiments of the micro-bubble generator are discussed in claims 1 to 13. In claims 14 to 16 embodiments of systems for sorting objects such as cells, using the micro-bubble generator, are discussed. In claims 17 to 22, embodiments of methods for fabricating a microbubble generator are discussed.

It is an advantage of embodiments of the invention that a microbubble generator is provided which generates microbubbles in a very short span of time thereby making it useful for sorting objects such as cells at high speed, e.g. at least 10.000 cells/s. Hence, it is an advantage of embodiments of the invention that a cell sorting system is provided enabling such high throughput. It is an advantage of embodiments of the invention that a microbubble generator is provided which is inexpensive, easy and simple to fabricate. It is an advantage of embodiments of the invention that a microbubble generator is provided which may be fabricated using semiconductor technology, e.g. CMOS compatible processing steps.

In a first aspect, the present invention provides a micro-fluidic device for deflecting objects in a liquid, comprising:

- a substrate for providing thereon an object-containing liquid; and
- a microbubble generator, having at least one microbubble generating element located on a surface of the substrate and in direct contact with the liquid when provided on the substrate;

characterized in that:
the at least one microbubble generating element is adapted to deflect a single object in the liquid through generation of a plurality of microbubbles by each of them.

According to an embodiment of the invention, the at least one microbubble generating element comprises a first series of connected microstructures; each microstructure adapted for generating a microbubble in the liquid when the microbubble generator is activated.

According to embodiments of the present invention, the microbubble generator may be a heating element and the at least one microbubble generating element may be at least one micro-heater located on a surface of the substrate and in direct contact with the liquid when provided on the substrate, characterized in that the at least one micro-heater is adapted to deflect a single object in the liquid through the generation of a plurality of microbubbles by each of the micro-heaters.

Other embodiments of the present invention may provide microbubbles by other means, like cavitation. For example, embodiments of the present invention may comprise an electrolytic unit as microbubble generator, comprising at least one set of electrodes with the function of a microbubble generating element. In this case, activating a microbubble generator may comprise inducing a voltage in the electrolytic unit, for example. The electrodes may be shaped such that at least two microstructures generate microbubbles simultaneously when a voltage is applied to the electrodes.

According to an embodiment of the invention, the at least one microbubble generating element is adapted so at least two microstructures are activated when the microbubble generating element is activated. In embodiments of the present invention comprising a micro-heater, the micro-heater is shaped such that at least two microstructures heat up simultaneously when an electrical current flows through the at least one micro-heater.

According to an embodiment of the invention, the at least one micro-heater further comprises a second series of connected microstructures connected in parallel to the first series of connected microstructures; and the at least one micro-heater is shaped such that at least two microstructures of different series of connected microstructures heat up simultaneously when an electrical current flows through the micro-heater.

According to an embodiment of the invention, the device further comprises a non-conductive layer, located in between the liquid and the microbubble generator, e.g. the heating element, having a plurality of cavities fabricated down to the microbubble generator, e.g. the heating element, wherein each cavity is aligned with a corresponding microstructure.

According to an embodiment of the invention, one of the cavities comprises at least one sharp corner.

According to an embodiment of the invention, a cross-section, parallel to the substrate, of one of the cavities is triangular or rectangular shaped.

According to an embodiment of the invention, each microstructure comprises a portion having a cross-sectional dimension of 10 micrometers or smaller.

According to an embodiment of the invention, the microbubble generator comprises only one conductive, e.g. metal, layer. For example, the conductive layer may be comprised in a heating element.

According to an embodiment of the invention, the device further comprises a controller connected to the microbubble generator and configured to monitor a parameter or parameters related to the generation of microbubbles. For example, in embodiments comprising a heating element a microbubble generator and at least one micro-heater as microbubble generating element, the controller may be configured to monitor the temperature of the heating element or configured to monitor the resistance of the heating element. In some embodiments of the present invention, it may control both temperature and resistance, or any other parameter related to microbubble nucleation and generation. In embodiments comprising an electrolytic unit, the controller may be configured to monitor the voltage, and/or any other parameter related to microbubble generation via electrolysis, such as current or capacitance.

According to an embodiment of the invention comprising a heating element as a microbubble generator, the heating element comprises a SiC layer for preventing deformation of the heating element.

In a second aspect of the present invention, a system for sorting objects is presented, comprising a first micro-fluidic device as described in the above paragraphs and a first micro-fluidic channel; wherein the first micro-fluidic device is positioned to deflect single objects propagating in the first micro-fluidic channel by generating microbubbles.

According to an embodiment of the invention, the system further comprises a second micro-fluidic channel fluidically connected to the first micro-fluidic channel; wherein the first micro-fluidic device is positioned in the second micro-fluidic channel and configured for deflecting single objects propagating in the first micro-fluidic channel by generating microbubbles.

According to an embodiment of the invention, the system further comprises a third micro-fluidic channel fluidically connected to the first micro-fluidic channel, wherein the second and the third micro-fluidic channel are aligned and positioned at opposite sides of the first micro-fluidic channel; and further comprising a second micro-fluidic device positioned in the third micro-fluidic channel and wherein the first and the second micro-fluidic devices are configured for deflecting single objects propagating in the first micro-fluidic channel.

According to an embodiment of a third aspect of the present invention, the first and the second micro-fluidic devices are further configured to synchronously deflect single objects propagating in the first micro-fluidic channel.

A method for fabricating a micro-fluidic device is presented, the method comprising: providing a substrate; providing a conductive, e.g. metal, layer on top of the substrate; patterning a microbubble generator, e.g. a heating element, having at least one microbubble generating element, e.g. at least one micro-heater, in the conductive layer;

characterized in that:

patterning the microbubble generator comprises patterning a microbubble generating element comprising a series of microstructures, wherein each microstructure is adapted for generating a microbubble when activated.

In embodiments of the present invention, patterning of the microbubble generator having at least one microbubble generating element may comprise fabricating the at least one microbubble generating element such that its shape allows at least two microstructures of the at least one microbubble generating element to generate microbubbles simultaneously when the at least one microbubble generating element is activated.

According to an embodiment of the invention, the patterning of the at least one microbubble generating element may comprise patterning of at least one micro-heater, which comprises fabricating the at least one micro-heater such that its shape allows at least two microstructures of the at least one micro-heater to heat up simultaneously, thereby generating microbubbles, when an electrical current flows through the at least one micro-heater.

According to an embodiment of the invention, the patterning of the at least one microbubble generating element may comprise patterning of at least one set of electrodes of an electrolytic unit, which comprises fabricating the at least one set of electrodes such that its shape allows at least two microstructures of the at least one set of electrodes to induce electrolysis simultaneously when a voltage is applied between the electrodes, thereby producing microbubbles.

According to an embodiment of the invention, the method further comprises providing a micro-fluidic layer on top of the microbubble generator, e.g. heating element; creating a micro-fluidic channel in the micro-fluidic layer; and closing the micro-fluidic channel with a lid.

According to an embodiment of the invention, the micro-fluidics layer is a photo-patternable polymer for attaching the lid.

According to an embodiment of the invention, a photo-patternable polymer is present in between the lid and the micro-fluidic layer, for closing the micro-fluidic channel with a lid.

For purposes of summarizing the invention and the advantages achieved over the prior art, certain objects and advantages of the invention have been described herein above. Of course, it is to be understood that not necessarily all such objects or advantages may be achieved in accordance with any particular embodiment of the invention. Thus, for example, those skilled in the art will recognize that the invention may be embodied or carried out in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objects or advantages as may be taught or suggested herein.

The above and other aspects of the invention will be apparent from and elucidated with reference to the embodiment(s) described hereinafter.

Brief Description of the Drawings

The invention will now be described further, by way of example, with reference to the accompanying drawings, in which:

FIG 1 illustrates a micro-fluidic device according to an embodiment of the invention

FIG 2 illustrates an embodiment of a single micro-heater

FIG 3 illustrates an embodiment of a single micro-heater

FIG 4 illustrates an embodiment of an electrode couple.

FIG 5 illustrates an embodiment of a matrix of microbubble generating elements.

FIG 6 illustrates an embodiment of two micro-heaters

FIG 7 illustrates an embodiment of a matrix of micro-heaters

FIG 8 illustrates an embodiment of a single micro-heater

FIG 9 illustrates an embodiment of two micro-heaters

FIG 10 illustrates an embodiment of a matrix of micro-heaters

FIG 11 illustrates an embodiment of a matrix of electrodes of an electrolytic unit.

FIG 12 are SEM images of a micro-heater and a micro-structure

FIG 13 illustrates an embodiment of a micro-heater and cross-sectional views of a micro-heater

FIG 14 illustrates an embodiment of a single micro-heater covered by a layer having nucleation cavities

FIG 15 illustrates an embodiment of a system for sorting cells

FIG 16 illustrates an embodiment of a system for sorting cells

FIG 17 illustrates an embodiment of the invention with two micro-fluidic devices implementing a jet flow regime

FIG 18 illustrates an alternative embodiment of the invention with two micro-fluidic devices implementing a jet flow regime

FIG 19 illustrates a jet flow regime of a single microfluidic device and of two micro-fluidic devices.

FIG 20 illustrates a jet flow regime of two micro-fluidic devices

FIG 21 illustrates a process flow for creating a micro-fluidic device according to an embodiment of the invention

FIG 22 illustrates a process flow for creating a micro-fluidic device according to an embodiment of the invention

The drawings are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes. The dimensions and the relative dimensions do not necessarily correspond to actual reductions to practice of the invention.

In the different drawings, the same reference signs refer to the same or analogous elements. Any reference signs in the claims shall not be construed as limiting the scope.

Description of the Invention

The device as presented in this disclosure provides solutions to the problems of prior art devices as described above.

Throughout the description reference is made to “a micro-fluidic device” and “a micro-fluidic device for deflecting objects in a liquid”. This may refer to “a micro-bubble generator device” or “a jet flow generator device”.

Throughout the description reference is made to “a micro-fluidic channel”. This may refer to channels suitable for transporting particles of microscopic size such as cells. The dimensions of these channels, e.g. the width and depth may vary from 10 nm to 100 micrometers.

Firstly, the problem related to the duration of time required to generate a jet flow is solved by generating a plurality of microbubbles generated by microstructures, which conjointly create the jet flow. By generating multiple microbubbles instead of generating a single microbubble, the size of the microbubbles required to generate that jet flow can be reduced. The reduced size of the microbubbles requires less power to generate these microbubbles. The smaller the microbubble, the lower the energy, for instance the temperature, required to create that microbubble. Thus, as an advantage, a heating element configured to generate a plurality of microbubbles can generate microbubbles faster because it takes less time to bring the heating element to the required temperature. Analogously, an electrolytic unit configured to generate a plurality of microbubbles may generate also microbubbles faster by applying a small voltage. As an additional advantage, such device provides a faster response time when it is triggered, for example by supplying a current or voltage pulse to the microbubble generator, compared to systems using a large microbubble to create the jet flow.

This is particularly advantageous when the device is used in an in-flow cell sorting device. The faster the response time, the higher the velocity of objects in the cell sorting device can be, resulting in a higher throughput. As an additional advantage, power consumption can be reduced because of the lower required energy to generate microbubbles. For example, lower temperature may be required in a heating element, or a lower voltage may be required in an electrolytic unit. This is useful for on-chip cell sorting devices because of the highly parallelized architecture which comprises a plurality of devices for sorting cells, all of which need to be connected to a power supply.

Secondly, the problem related to the energy required to create microbubbles is solved by using a layer comprising multiple structures, also known as artefacts, which are aligned with microstructures adapted to generate the plurality of microbubbles. For example, it solves the problem of superheating in case of heating elements, and advantageously reduces the amount of voltage needed to nucleate a stable microbubble (e.g. a bubble that does not detach) in case of electrolytic units for microbubble generation. When a surface is smooth, in case of heating elements, superheat is considerably high, demanding approximately 350 °C for water vapour microbubble nucleation. The superheat, however, can be drastically reduced by surface artefacts (defects) or artificial micro structures serving as microbubble nucleation centres. The artefacts are irregularities, for example cavities, in the layer which contribute to the reduction of the superheat in case of heating elements. Whereas a relative high temperature is needed to create microbubbles in a liquid, this temperature is reduced by providing this layer on top of these microstructures. The reduction of the superheat allows microbubbles to be generated at lower temperatures. Again, a lower temperature means that the duration of time needed to bring the heating element to the required temperature is reduced. Thus, a device having such a layer features a faster response time. Likewise, an electrolytic unit needs a determined voltage necessary to generate microbubbles, but these tend to detach at small sizes. Additional artefacts on the surfaces help in microbubble nucleation, obtaining a minimum amount of microbubbles on the surface for a lower voltage, hence reducing the risk of microbubble detachment.

Thirdly, the problem related to the accuracy of the sorting is solved by simultaneously creating a plurality of microbubbles instead of creating a single microbubble to create the jet flow. Also, large microbubbles require a much longer time to collapse compared to small microbubbles because of the smaller surface/volume ratio, thus resulting in long microbubble life time and low sorting throughput. The process of generating small microbubbles is more controllable than the creation of a single larger microbubble. Therefore, a jet flow created by a plurality of microbubble can be accurately controlled. In cell sorting micro-fluidic devices this is an important advantage as cells propagating through micro-fluidic channels are deflected by such a jet flow. An increased control over the force created by the jet flow translates into an increased accuracy of the sorting. Also, the increased control allows the use of smaller micro-fluidic channels. Hence, more micro-fluidic channels may be fabricated in certain area.

In a **first** aspect of the invention, a micro-fluidic device for generating microbubbles in a liquid is presented. The micro-fluidic device is adapted to deflect objects in a fluid. These objects may be particles, for example particles of a biological nature such as cells or smaller sized particles.

Embodiments of the present invention are firstly described hereinafter in more detail with reference to the microbubble generator being a heating element, and the at least one microbubble generating element being at least one micro-heater. This, however, is not intended to be limiting to the present invention, as also other types of microbubble generators may be used, as described in more detail with respect to a second embodiments of the present invention.

FIRST EMBODIMENT

FIG 1 shows an example of micro-fluidic device **100** comprising a heating element **102** as a microbubble generator, comprising at least one micro-heater **103** as microbubble generating element, the at least one micro-heater **103** comprising a plurality of microstructures **104** as microbubble nucleation or generation sites. The micro-fluidic device **100** comprises a substrate **101** for providing a liquid on. A heating element **102** comprising at least one micro-heater **103** is located on a surface of the substrate **101**. The at least one micro-heater **103** is in direct contact with the liquid when it is provided on the substrate **101**. The at least one micro-heater **103** is designed such that it can generate a force in the liquid by displacing a part of the liquid. The displacement of a part of the liquid is performed by generating a plurality of micro-bubbles. Hence, a flow is created. The force of the flow in the liquid, caused by the ensemble of generated microbubbles, is suitable to deflect objects present in the liquid. Thus, the at least one micro-heater **103** is adapted to deflect a single object in the liquid by creating a force in the liquid through generation of a plurality of microbubbles.

An embodiment of the first aspect of the invention is illustrated in **FIG 1**. The micro-fluidic device **100** comprises a substrate **101.** A heating element **102** positioned on a surface of the substrate **101**. The heating element **102** is located on the surface of the substrate **101** on which a liquid may be provided. When a liquid is provided, the heating element is in direct contact with the liquid. The substrate **101** may be a semiconductor substrate, e.g. a silicon substrate. The substrate **101** may also be a glass substrate. The substrate **101** may be an inner wall of a micro-fluidic component, for example an inner wall of a micro-fluidic channel. The heating element **102** may be located on any inner wall of a micro-fluidic component, as long as the heating element **102** is in contact with a liquid when present in the micro-fluidic component. The heating element **102** is fabricated from a conducting material such as a metal, e.g. aluminium, copper, tungsten or polysilicon. The heating element **102** may advantageously be fabricated from a single metal layer. As an advantage, the thickness of the heating element **102** can be minimized and fabrication of the device can be simplified thereby reducing cost to manufacture as no additional metal layers are required. The present invention is not limited to metal layers, and any other conductive materials (such as heavily doped semiconductors) can be used.

The heating element **102** comprises at least one micro-heater **103** adapted for generating a plurality of microbubbles in a liquid. An embodiment of a micro-heater is illustrated in **FIG 2**. The at least one micro-heater **103** is a structure which is fabricated in a conductive, e.g. a metal, layer comprising the heating element **102**. For the purpose of generating a plurality of microbubbles per micro-heater **103**, each micro-heater **103** comprises a plurality microstructures **104**. Each microstructure **104** is a feature of the micro-heater **103** and is a part of the heating element **102**. Each microstructure **104** is adapted to generate a microbubble in a liquid when the micro-heater **103** heats up. This adaptation comprises that dimensions, e.g. the width or height, of each microstructure **104** are smaller than the dimensions, e.g. the width or height, of the rest of the micro-heater **103**. The selected dimensions ensure that microbubbles are generated at dedicated spots of each micro-heater **103**, each spot being the location of a microstructure **104**. The microstructures are thermally favoured for microbubble creation because of the locally higher current density hence causing joule heating. As an advantage, this assurance contributes to controllability of generated microbubbles by the microfluidic device

100. It can be accurately determined at which location a microbubble will be generated. According to an embodiment of the invention, a microstructure **104** may have a cross-sectional dimension, e.g. a width, of 10 micrometers or smaller. This means that dimensions of other parts of the micro-heater **103** are larger than 10 micrometers to ensure that microbubbles are generated at the location of the microstructures **104**.

The plurality of microstructures **104** of a single micro-heater **103** may be a first series of electrically connected microstructures. Thus, the plurality of microstructures **104** may be an array of microstructures **104** which are connected in series. The first series of electrically connected microstructures may be a meander shaped structure, e.g. a meander shaped wire, wherein specific portions of the structure have smaller dimensions than the rest of the structure, each portion being defined as a microstructure. The parts may be turns of the meander shaped structure, for example alternate turns. The meander shaped structure may be shaped such that a maximum number of turns can be achieved in a predefined area. By using such a structure, a maximum number of microbubbles can be generated on a given area. Hence, compactness of the device is increased allowing it to be used inside micro-fluidic channels. In the single micro-heater **103**, a second series of electrically connected microstructures may be electrically connected in parallel to the first series of electrically connected microstructures. A single meander shaped micro-heater **103** is illustrated in **FIG 2**.

According to an embodiment of the invention, each micro-heater **103** is a circular meander shaped structure having a plurality of microstructures **104**. Each microstructure **104** may be located at an even distance from a central point of the micro-heater **103**.

According to an embodiment of the invention, each micro-heater **103** comprises at least two microstructures **104** which are electrically connected in parallel. According to an embodiment of the invention, the micro-fluidic device **100** may comprise a series of micro-heaters, electrically connected in series. Further, the micro-fluidic device **100** may comprise a plurality of series of micro-heaters, electrically connected in parallel. The plurality of series of micro-heaters may be positioned staggered for achieving homogeneous distribution of the heat and increasing stability of the microbubble generation and control over the force generated by the microbubbles.

According to an embodiment of the invention, each micro-heater **103** is lip-shaped. Such a shape is illustrated in **FIG 3**. The two outer sharp corners of the lip-shaped structure are two microstructures **104**. In such an embodiment, the micro-heater **103** has a first and a second curved portion. Both portions are curved away from each other thereby forming a cavity in the middle (both portions are unconnected at the cavity). One end of the first curved portion is only connected to one end of a second curved portion thereby forming a sharp tip. The other end of the first curved portion is only connected to the other end of the second curved portion thereby forming a sharp tip. By connecting both portions, a lip-shaped micro-heater **103** is formed having two sharp tips (which are the microstructures **104**). Because the microstructures **104** are located at both ends of the lip-shaped micro-heater **103**, as an advantage, heat generated in one microstructure **104** does not influence the other. This allows for a homogeneous distribution of the heat and therefore an increased stability of the microbubble generation and control over the force generated by the microbubbles.

When the heating element **102** comprises a plurality of lip-shaped micro-heaters **103**, the lip-shaped micro-heaters **103** can be positioned staggered. This is illustrated in **FIG 5**. As an advantage, more-micro-heaters **103** can be positioned on a given area. In semiconductor devices this allows for a reduction of silicon material which decreases the cost of the device. Also, when more micro-heaters **103** can be positioned in the same area, a jet flow can be generated with smaller microbubbles. Hence, the jet flow can be created faster and with better control which contributes to a higher cell sorting throughput.

A plurality of micro-heaters **103**, **103a** may be electrically connected to each other in series. Such an embodiment is illustrated in **FIG 6**. When a voltage pulse is supplied to the heating element, an electrical current flows through each micro-heater **103**, **103a** causing each micro-heater to heat up. When a liquid is present on the heating element, a plurality of microbubbles are generated in the liquid when the micro-heaters **103**, **103a** reach an appropriate temperature, e.g. 350 degrees Celsius.

In addition, a plurality of micro-heaters **103**, **103a**, **103b**, **103c** may be electrically connected, in parallel. The plurality of micro-heaters may be a matrix of micro-heaters **103**, **103a**, **103b**, **103c**. Such an embodiment is illustrated in **FIG 7**. As an advantage, micro-heaters may generate microbubbles simultaneously thereby contributing to increased controllability of microbubbles. Also, a plurality of microbubbles can be generated in a small area. This is especially advantageous in dense micro-fluidic systems having a plurality of micro-fluidic channels, each comprising a micro-fluidic device as presented in the first aspect of the invention.

According to an embodiment of the invention, the micro-fluidic device comprises at least one row of interconnected microbubble generating elements, e.g. micro-heaters. The micro-fluidic device may also comprise adjacent rows of interconnected micro-heaters. Hence, the micro-fluidic device comprises a matrix of micro-heaters. As an advantage, multiple micro-heaters may simultaneously create microbubbles thereby contributing to increased controllability of a jet force created by the micro-fluidic device.

According to an embodiment of the invention, each micro-heater is shaped such that at least two microstructures **104** of a single micro-heater **103** heat up simultaneously when an electrical current flows through the micro-heater. This is achieved by for example positioning the microstructures **104** symmetrically, e.g. circular, around a central point of the micro-heater **103**, for example by positioning the microstructures at even distances from that central point. In such an embodiment, each-micro-heater **103** may be star-shaped. When an electrical current flows through the micro-heater **103**, the current flows simultaneously through different parts of the micro-heater **103** thereby causing simultaneous heat up of different microstructures **104**.

A single micro-heater **103** may also comprise different series of electrically connected microstructures **104** which are unconnected at the level of the micro-heater **103** but connected at the level of the heating element **102**. Such an embodiment is illustrated in **FIG 8**. In **FIG 8**, a single micro-heater **103** is illustrated having two series of structures **103'**, **103''**. The two series **103'**, **103''** are unconnected at the level of the micro-heater. Different micro-heaters may be connected in series to each other thereby forming a chain of micro-heaters. Such an embodiment is illustrated in **FIG 9**. Two micro-heaters **103, 103a** are connected to each other. A first series of microstructures **104** of a first micro-heater **103** is connected to a first series of microstructures **104** of a second micro-heater **103a**. A second series of microstructures **104** of the first micro-heater **103** are connected to a second series of a second micro-heater **103a**. In such an embodiment, a micro-heater may be shaped such that at least two microstructures **104** of different series of connected microstructures **104** heat up simultaneously when an electrical current flows through the micro-heater **103**. As described above, in another embodiment, different micro-heaters of the heating element **102** may be connected to each other in parallel. Such an embodiment is illustrated in **FIG 10**.

A shielding layer on top of the microbubble generator may be provided for shielding at least partially the microbubble generation sites from the liquid.

In an embodiment of the invention, the heating element **102** may be covered with a shielding layer for shielding the micro-heaters **103** of the heating element **102** from the liquid. The layer may be fabricated from electrically insulating material such as SiO_2 or SiN, having a sufficient thickness such as between 20 nm and 2μm, e.g. 350 nm. Thus, there is no direct contact between the heating element and a liquid. The thickness of the shielding layer is adapted thereby allowing heating up of a liquid to generate microbubbles in that liquid.

In some embodiments of the present invention comprising heating element **102** fabricated from a single metal layer, it was surprisingly observed by the inventors that after several voltage or current pulses were supplied to the micro-heaters **103** to induce thermal vapour microbubbles, the microstructures **104** underwent a structural change. This is illustrated in the four SEM images **(a)**, **(b)**, **(c)** and **(d)** of **FIG 12**.

The upper-left image **(a)** is a SEM image of a micro-heater before supplying voltage or current pulses. The lower-left image **(b)** is a SEM image of a micro-heater after supplying voltage or current pulses. In the lower-left image **(b)** it can be noticed that an artefact **120** is formed at each microstructure **104**. The upper-right image **(c)** is a SEM image of an individual microstructure **104**. The artefact **120** being formed at a sharp corner of the microstructure **104** after voltage or current pulses are supplied to the micro-heater is illustrated in lower-right image **(d)**.

The deformation of the microstructures is advantageous because it creates artefacts which contribute to a reduction of the superheat. Each artefact can be considered as a self-induced nucleation centre contributing to the reduction of superheat. These artefacts are advantageous because the amount of power required for microbubble generation at the microstructures is reduced (approximately 10-15% from experimental findings) and the device's life time is thereby extended. The deformation may be caused by the metal of the microstructures being thermally deformed or by electromigration. This cyclic deformation takes place at every heating pulse, and finally produces irreversible structural deformation. The deformation also propagates to the adjacent shielding layer on top of the metal, thus leading to the artefact visible in the SEM picture. On the other hand, the deformation may also be take place in the shielding layer during the supply of voltage or current pulses, or it may take place by other means such as deposition, etching, mechanical action, or any other suitable means. The device as illustrated in **FIG 12** uses aluminium as metal for the heating element and SiO2 as a shielding layer.

Devices where deformation takes place are interesting to use in low-power devices because of the reduction of the superheat. Such devices may be used in e.g. disposable devices because of their short-term usage and low cost as less materials are needed and it is easier to manufacture. The present invention is not limited to deformations in the metal, and they may be present in general in the conductive layer, or not be present at all.

When long term usage is envisaged, the device may further comprise a robust capping layer. According to an embodiment of the invention, the capping layer is positioned on top of the heating element **102**. The capping layer may comprise silicon carbide, e.g. may be a SiC layer. The SiC prevents deformation of the heating element. The use of SiC is advantageous as it is thermally and chemically stable, thermally more conductive than SiO2 or SiN and it may be deposited as a smoother surface with fewer uncontrolled artefacts.

In spite of the aforementioned thermal & mechanical properties, SiC is not a good material for electrical insulation. The resistivity is around 1e3 ohm cm, and decreases at higher temperatures. Thus, a sole SiC capping layer cannot be placed directly above the conductive heating elements. To electrically insulate the conductive layer of the heating element from the SiC layer, the aforementioned shielding layer is present in between. As discussed earlier, the shielding layer may be an electrically insulating layer such as e.g. a SiO_2 and/or a SiN layer. The shielding layer also contributes to thermal deformation tolerance and control. A suitable material for the shielding layer is SiN, due to its electrical resistivity of approximately 1e15 ohm cm, which is much better than SiC. The SiN layer can be regarded as electrically non-conductive. The SiN layer may have a thickness between 20 nm and 2 micrometers, e.g. 300 nm. Alternatively the buffer layer may be a SiO_2 layer having a thickness between 20 nm and 2 micrometers. The thickness of the SiO_2 layer may be e.g. 350 nm.

FIG 13 shows a micro-heater **1300** and two possible cross-sectional views **1301, 1302** of different embodiments of the micro-heater, the position of the cross-section being indicated in **1300** by a dashed line, and the view being indicated by two arrows. In the cross-sectional view **1301**, the micro-heater comprises: a conductive, e.g. metal, layer **102**, a layer **121** comprising SiO_2 or a SiN or a mixture thereof, and a capping layer **122**. In the cross-sectional view **1302**, the micro-heater comprises: a conductive, e.g. metal, layer **102**, a SiO_2 layer **114,** a SiN layer **121**, and a capping layer **122**.

According to an embodiment of the invention, the micro-fluidic device further comprises a non-conductive layer **105** which is located, e.g. deposited, on top of the microbubble generator. In the following discussion, an example will be shown of a non-conductive layer **105** on top of a heating element **102**. The non-conductive layer, may be the top layer which is in direct contact with the liquid. The non-conductive layer may be positioned on top of the capping layer. The non-conductive layer may have a thickness between 20-100 micrometer, e.g. 30 micrometer. Such an embodiment is illustrated in **FIG 14**. The non-conductive layer may be at least a dielectric or a polymeric layer. The non-conductive layer **105** features a plurality of cavities **106** which are fabricated down to the heating element **102**. The position of each cavity **106** is aligned with the position of a microstructure **104** of a micro-heater **103**. For example, when using a heating element **102** with

100 micro-heaters **103**, each micro-heater **103** having 10 microstructures **104**; the non-conductive layer features 1000 cavities wherein each cavity is located above or aligned with a microstructure **104**. The cavities **106** are through-holes or blind holes in the non-conductive layer **105**. Thus, a liquid provided on the conductive layer **105** has access to the micro-heaters of the heating element or at least access to the shielding layer covering the micro-heaters.

It is an advantage of the invention that the provision of cavities above micro-heaters contributes to the reduction of superheat. By providing cavities, the required temperature to generate microbubbles is lowered which allows faster creation of microbubbles. In applications such as flow cytometry a high throughput of cells is required. In such applications micro-fluidic devices as presented in this disclosure can be used to deflect characterized cells to different directions for sorting purposes. The faster the micro-fluidic device can generate microbubbles, the faster deflection of cells can be performed and the higher the throughput of the flow cytometry system can be.

It was observed by the inventors that not only the presence of cavities in the neighbourhood of microstructures contributes to the reduction of the superheat but also the shape of the cavities. It was observed by the inventors that if the cavities feature a sharp corner, e.g. a corner smaller or equal than 90 degrees, after the generation of a first microbubble, small pockets of air remain or are trapped in this sharp corner of the cavity. It was determined that when generating further microbubbles, these small pockets contribute to the further reduction of the superheat because microbubbles may be easily generated from those trapped pockets of air at a lower temperature. According to an embodiment of the invention, the cross-section of the cavities are preferentially triangular shaped. Such a geometrical shape is advantageous as it may feature 3 sharp corners. Alternatively, the cross section may be rectangular shaped. Any cavity with a cross-sectional shape having at least one sharp corner is suitable for reducing the superheat. This further reduction of the superheat further contributes to lower power consumption and faster generation of microbubbles. As described above, this is advantageous in micro-fluidic systems for sorting cells.

According to an embodiment of the invention, the heating element **102** may be connected to a module that is configured to monitor parameters related to microbubble generation, for example it may be configured to monitor the temperature of the heating element **102**. The module may be a sensor, e.g. an on-chip sensor, positioned and configured to sense the temperature of the heating element. Alternatively the heating element **102** may be connected to a module that is configured to monitor the resistance of the heating element **102**. The module may be an electronic circuit electrically connected to the heating element and configured to measure the resistance of the heating element. The temperature of the heating element or the thermal resistance of the heating element may be monitored, for example when a voltage or current pulse is applied to the heating element. This allows the voltage pulse, which is needed to bring the heating element to the required temperature, to be adjusted to the actual condition of the heating element. This improves the accuracy of the micro-fluidic device. Also, power consumption can be further reduced. The current and resistance of the heating element may be simultaneously monitored in some embodiments of the present invention.

A power unit adapted to the microbubble generator (e.g. a current source, either constant, variable or even alternate) may be integrated in some embodiments of the present invention, for example for in-field applications such as in-situ studies, or connection means may be provided for connecting the unit to an external source.

SECOND EMBODIMENT

Other embodiments of the present invention may be adapted to generate microbubbles by other means, such as for instance by electrolysis. The microbubble generator may comprise an electrolytic unit, comprising at least one set of electrodes as microbubble generating element. Each set of electrodes is adapted for generating a plurality of microbubbles, for example by the shape of microstructures present in the set of electrodes. A set of electrodes comprise at least two electrodes adapted to produce electrolysis when a potential difference (voltage) is applied between the at least two electrodes of the set. In such cases, the connections of the microbubble generating elements may be adapted to create a voltage in a set of electrodes, e.g. between at least two electrodes, comprising microstructures, hence creating electrolysis in the liquid in contact therewith, and producing microbubbles. For example, the microbubbles may comprise oxygen and hydrogen if the liquid comprises water.

FIG 4 shows a system comprising a set of electrodes, in this case two electrodes **401, 401'** using the same lip-shaped structures as in **FIG 3** or **FIG 13**, which may also comprise microstructures **104**. Each of the two electrodes is connected to a voltage of opposite polarity (or to a positive voltage and to ground, respectively), obtaining a potential difference between the tip of the microstructure **104** of one electrode **401** and the tip of the microstructure **104** of the other electrode **401'**. This potential difference, which can be variable, may be used to produce microbubbles by electrolysis of the fluid. This system advantageously presents low power consumption for producing microbubbles, for example gas microbubbles (e.g. hydrogen and oxygen in case of aqueous fluids).

A plurality of sets of electrodes may be positioned in rows, adapting the connections so that if one row of electrodes is connected to a first electric potential, the following (neighbouring) row is connected to the opposite electric potential, hence creating a voltage between the rows and generating microbubbles via electrolysis. The size of the microbubble may be controlled by controlling the voltage, thereby contributing to increased controllability of a jet force created by the micro-fluidic device. The electrodes may have the same shape as the micro-heaters of the first embodiment, for example lip-shaped, or a circular meander shaped structure having a plurality of microstructures as shown in the **FIG 11**.

Other features, analogous to the features of the embodiments comprising heating elements, may be applicable to embodiments comprising electrolytic units. For example, the advantages of the artefacts observed in the microstructures of the heating elements may also apply to electrolytic units or to any other way of microbubble generation, the artefacts being nucleation sites for microbubbles (formed by electrolysis).

The non-conductive layer **105** with shaped cavities **106** of **FIG 14** can also be applied to a microfluidic device comprising an electrolytic unit. A monitoring module may also be included, the module being configured to monitor the voltage of an electrolytic unit, or the capacitance, or the current, or variations thereof, hence determining the presence of microbubbles and controlling their growth. Voltage may be provided as a static or variable voltage.

In a **second** aspect of the invention, systems **200** for sorting cells are presented. The systems described in the second aspect of the invention may be used to sort any object which fits in the micro-fluidic channels of such systems. An embodiment of such a system is illustrated in **FIG 15**. The system comprises a micro-fluidic channel **107** for providing and propagating cells. The system **200** may be a flow cytometry device wherein cell identification is performed based on the fluorescence of objects or based on recorded images of cells (e.g. holograms) propagating in the micro-fluidic channel.

The system **200** comprises a micro-fluidic device **100** as described in the first aspect of the invention. The micro-fluidic device **100** may be positioned in the micro-fluidic channel **107** of the system. Such an embodiment is illustrated in **FIG 15**. The micro-fluidic device **100** may be positioned on any inner wall of the micro-fluidic channel **107**, ensuring that there is direct contact between the liquid and the micro-fluidic device **100**. For example, it may be positioned in the walls belonging to the closure lid of the micro-fluidic device. The micro-fluidic device **100** is positioned with respect to the micro-fluidic channel **107** such that deflection of single cells propagating or flowing in the micro-fluidic channel **107** can be performed by generating microbubbles inside the micro-fluidic channel **107** of the system **200**, wherein the jet flow (F) created in the micro-fluidic channel **107** by generated microbubbles deflects cells. The cells may be deflected such that they are directed towards different outlets **123** which are fluidically connected to the micro-fluidic channel **107**. It is an advantage of the invention that the compact design of the micro-fluidic device **100** allows integration into (inside) the micro-fluidic channel **107** of the system **200**. This is an important advantage for highly parallelized flow cytometry devices because the complete system can be integrated on a small area of a chip. In **FIG 15** it is also noticed that the micro-fluidic channel **107** is wider than the width of the micro-fluidic device **100**. This is required because the micro-fluidic device **100** creates a jet flow in the micro-fluidic channel **107** which deflects the cell towards another location in the micro-fluidic channel where no micro-fluidic device **100** is active. Hence, the propagation path of an object in the micro-fluidic channel **107** is changed to a different propagation path (propagation path of the object being indicated with an arrow **124**). To allow this, the wider micro-fluidic channel **107** is required. In such an embodiment, the micro-fluidic device **100** would not be activated if the propagation path of an object leads to the correct outlet. When the propagation path of an object does not lead to the correct outlet, the micro-fluidic device **100** is activated.

According to an embodiment of the invention, the substrate **101** of the micro-fluidic device **100** may be comprise at least part of an inner wall of the micro-fluidic channel **107** of the system **200** for sorting cells.

According to another embodiment of the invention, a system **200** for sorting cells may comprise a first micro-fluidic channel **107** for propagating cells which is fluidically connected to a second micro-fluidic channel **108**. Such an embodiment is illustrated in **FIG 16**. The first micro-fluidic channel **107** and the second micro-fluidic channel **108** may be fluidically crossing each other. The second micro-fluidic channel **108** may be positioned perpendicular to the first micro-fluidic channel **107**. The impact of the generated jet flow perpendicular on the propagation path of a cell in the first micro-fluidic channel is thereby maximal. As an advantage, power usage may be reduced. The micro-fluidic device **100** may be positioned in the second micro-fluidic channel **108** as illustrated in **FIG 16.** A jet flow created in the second micro-fluidic channel **108** deflects single cells which are propagating through the first micro-fluidic channel **107,** towards an outlet **123** of the first micro-fluidic channel **107**. Such a design allows using a large microfluidic device **100** in a wide fluidic channel or chamber **108,** than for example a more narrow channel **107** can accommodate. The second micro-fluidic channel may also comprise at least two micro-fluidic devices as illustrated in **FIG 17**. The advantage of such an embodiment is discussed below.

According to an embodiment of the invention, the system **200** for sorting cells may comprise a plurality of micro-fluidic devices **100** positioned in or on an inner wall of the micro-fluidic channel **107** used to propagate cells. Alternatively they are positioned in other micro-fluidic channels fluidically connected to the micro-fluidic channel **107**. Such embodiments contribute to the usability of the system.

According to another embodiment of the invention, a system **200** for sorting cells may comprise a first micro-fluidic channel **107** for propagating cells which is fluidically connected to a second **108** and a third micro-fluidic channel **108a**. Such an embodiment is illustrated in **FIG 18**. The first, second and third micro-fluidic channel **107, 108, 108a** may be fluidically crossing each other. The second and third micro-fluidic channel **108, 108a** may be positioned perpendicular to the first micro-fluidic channel **107**. The second and third micro-fluidic channel **108, 108a** may be fluidically connected to the first micro-fluidic channel **107** at opposite sides of the first micro-fluidic channel **107**. The impact of a generated jet flow perpendicular on the propagation path of a cell in the first micro-fluidic channel **107** is thereby maximal. As an advantage, power usage may be reduced. A first micro-fluidic device **100** may be positioned in the second micro-fluidic channel **108.** A jet flow created in the second micro-fluidic channel **108** deflects single cells which are propagating through the first micro-fluidic channel **107,** towards an outlet **123** of the first micro-fluidic channel **107**. A second micro-fluidic device **100** may be positioned in the third micro-fluidic channel **108a.** A jet flow created in the third micro-fluidic channel **108a** deflects single cells which are propagating through the first micro-fluidic channel **107,** towards an outlet **123** of the first micro-fluidic channel **107**. Such systems allow precise control over the magnitude of the force on cells in the first micro-fluidic channel **107**, generated by the jet flow. Hence, the accuracy of the sorting may be increased. Also, when two or more micro-fluidic devices conjointly create the jet flow, power requirements of each micro-fluidic device can be reduced because the jet flow is created by two or more micro-fluidic devices. Hence, smaller bubbles may be generated, enabling faster generation of the jet flow.

According to a specific embodiment of the invention, a system **200** for sorting cells comprising a micro-fluidic channel and at least two micro-fluidic devices **100** as described in the first aspect of the invention is presented. The at least two micro-fluidic devices may be positioned and configured to synchronously deflect single cells propagating in the micro-fluidic channel. It is an advantage of the invention that by synchronizing multiple micro-fluidic devices for simultaneously deflecting a single cell the sorting accuracy can be improved.

Every jet flow is composed of two phases: 1) a PUSH phase when microbubbles are created (for example, vapour microbubbles may be thermally created) that eject an outbound jet flow and 2) a PULL phase when microbubbles collapse and retract an inbound jet flow. **FIG 19** illustrates the diagrams **1901**, **1902** of a jet flow generation cycle for exemplary embodiments comprising one and two micro-fluidic devices, respectively. Diagram **1901** for a single micro-fluidic device shows a jet flow (**JF**) generation cycle **110** with the push phase **111** and the pull phase **112** as a function of time (**T**). Either one of the regimes can be used for sorting, dependent on the timing set by the user. A problem arises when two propagating cells in the micro-fluidic channel **107** are too close to each other. In such a situation, after pushing the first cell, the second cell may inevitably run into the PULL regime. As a result the second cell is wrongfully deflected to the wrong outlet. To overcome this, a further micro-fluidic device may be positioned and configured such that the force created by both micro-fluidic device may cancel out each other.

For example, one of the micro-fluidic devices may be configured to activate with a half cycle phase delay from the other micro-fluidic device. This allows delay of the pull mode by half a period. In this way, this cell is not wrongly sorted. Diagram **1902** of **FIG 19** illustrates jet flow generation cycles **110, 110a** of two micro-fluidic device with a push phase **111, 111a** and a pull phase **112, 112a**, with a half a cycle delay phase. The delay phase causes the pull phase **112** of one micro-fluidic device to coincide with the push phase **111a** of the other micro-fluidic device thereby cancelling any force. Therefore, the jet flow is nearly zero in this overlapping half cycle which precludes the wrong deflection of closely following cells after the cells to be sorted. The jet flow generation cycles as depicted in the diagram **1902** of **FIG 19** is suitable for micro-fluidic devices which are positioned at the same side of a micro-fluidic channel. For example, when implemented in the system of **FIG 16**, two micro-fluidic devices **100** are located in the micro-fluidic channel **108**, at the same side of the micro-fluidic channel **107**. This is illustrated in **FIG 17**.

Another embodiment is illustrated in **FIG 20**. In this embodiment the PULL phase of the first micro-fluidic device coincides with the PUSH phase of the second micro-fluidic device, and both align in the same physical direction at this moment. Consequently, the total jet flow is reinforced. This embodiment will work most effectively when the two micro-devices are placed on opposite sides of the micro-fluidic channel **107**. For example, when implemented in the system of **FIG 16**, two micro-fluidic devices are located in the micro-fluidic channel **108**, at the opposite sides of the micro-fluidic channel **107**. This is illustrated in **FIG 18**.

According to a **third** aspect of the invention, a method for fabrication of a micro-fluidic device as described in embodiments of the first aspect of the invention is presented. The micro-fluidic device may be fabricated using common micro-fluidic manufacture routes, e.g. semiconductor fabrication technology. For example, the micro-fluidic device may be fabricated using CMOS compatible processing steps. The method comprises providing a substrate and providing a conductive layer, e.g. a metal layer, on top of the substrate. The substrate may be a semiconductor substrate, e.g. a silicon substrate. The substrate may also be a glass substrate. The conductive layer may for example be an aluminium, copper or tungsten layer. The conductive layer may for example be formed by a stack of conductive layers, such as a Ti 20 / AlCu 200 / TiN 100 (nm) stack, or a Ti 15 / TiN 10 / W 100 / TiN 100 (nm) stack. Thereafter, a microbubble generator is created by patterning the conductive layer. The creation of the microbubble generator comprises the patterning of at least one-microbubble generating element in that conductive layer. For example, the patterning of the at least one micro-heater for a heating element **103** as microbubble generator comprises patterning a series of connected microstructures **104** in the conductive layer. Each microstructure **104** is fabricated such that its shape can generate a microbubble when the heating element **101**, and consequently each micro-heater, heats up. The fabrication of each microstructure is performed such that each microstructure comprises a portion with a dimension, e.g. the width or height, of 10 micrometers or smaller and that the rest of the micro-heater has dimensions, e.g. the width or height, greater than 10 micrometers to ensure that a microbubble is only generated at the location of the microstructure. Likewise, a patterning of at least one set of electrodes for an electrolytic unit as microbubble generator may comprise patterning a series of connected microstructures in the conductive (e.g. metal) layer, with such a shape that they can generate a microbubble when inducing a voltage between the electrodes. The method of fabrication of the at least one set of electrodes, and the shape and dimension thereof may be the same as for micro-heaters, and the electrical connections may be adapted for the generation of a voltage between the microstructures, rather than flowing a current through the microstructures.

According to an embodiment of the invention, the patterning of the at least one microbubble generation element, e.g. micro-heater, may further comprise patterning another series of connected microstructures. Such a device is described in the first aspect of the invention. The patterning of a single microbubble generation element, e.g. micro-heater, may comprise the patterning of at least two microstructures which are electrically connected in parallel. The patterning may further comprises the patterning of a series of single microbubble generation element, e.g. micro-heaters, which are electrically connected in series. For example, the patterning may further comprise the patterning of a plurality of different series of microbubble generation elements, e.g. micro-heaters, which are electrically connected in series and wherein the different series are connected in parallel.

According to an embodiment of the invention, the method may further comprise creating a capping layer on top of the microbubble generator, e.g. heating element or electrolytic unit,. The presence of the capping layer increases the robustness of the microbubble generator because it fixes the microbubble generator and ensures that deformation of the microbubble generator during usage is minimal or none. In an electrolytic unit as microbubble generator, the capping layer may protect the conductive structure. As an advantage, the life-time of the device is increased. As explained in the first aspect of the invention, when several voltage or current pulses are applied to the microbubble generator, more particularly to the one or more microbubble generating elements thereof, deformation of the microstructures is observed (see **FIG 12**). When the deformation is unwanted, it can be avoided by depositing a capping layer on top of the microbubble generator, e.g. heating element or electrolytic unit. Such a capping layer may comprise a SiC layer. The SiC layer may have a thickness between 20 nm and 2 micrometers, for example, 50 nm. The capping layer may further comprise a SiN layer wherein the SiN layer is in direct contact with the microbubble generator, e.g. heating element or electrolytic unit, and wherein the SiC layer is deposited on top of the SiN layer. In such an embodiment, the SiC layer is in direct contact with the liquid when provided. The SiN layer functions as electrical insulator. The capping layer may be deposited before a micro-fluidic channel is formed on top of the microbubble generator, e.g. heating element or electrolytic unit; thus, before e.g. a photo-patternable material is deposited on the microbubble generator.

According to an embodiment of the invention, the method may further comprises creating a non-conductive layer on top of the microbubble generator, e.g; heating element or electrolytic unit, and creating cavities **106** in the layer at the location of patterned microstructures **104**. The cavities **106** may be triangular shaped or rectangular shaped. Each cavity may be created such that it comprises at least one sharp corner, as described in the first aspect of the invention.

FIG 21 illustrates a specific embodiment of the third aspect of the invention. A method (comprising seven steps 2101-2107) to fabricate the micro-fluidic device **100** having a closed micro-fluidic component is illustrated. The steps are disclosed for a heating element **102** comprising micro-heaters, but it could be applied to any other microbubble generator such as an electrolytic unit comprising at least one set of electrodes. The micro-fluidic device **100** is fabricated by providing **2101** a substrate **101**, for example a semiconductor substrate such as e.g. a silicon substrate. On the substrate, a heating element **102** is created, for example by first providing **2102** a conductive layer, such as a metal layer **119** and patterning **2103** that metal layer **119**. After fabrication of the heating element **102**, a shielding layer **114** is deposited **2104** on top of the substrate **101** thereby covering the heating element **102**. The heating element **102** may be covered completely by the shielding layer **114**. The shielding layer **114** may be an insulating layer; it may comprise SiO_2, SiN or a mixture thereof. On top of the shielding layer **114**, a micro-fluidics layer **115** is provided **2105**. The micro-fluidics layer **115** may be fabricated from a patternable material suitable for micro-fluidic applications. The material may be PDMS or a photo-patternable adhesive suitable for wafer-level microfluidic structure fabrication. Advantageously, the material of the micro-fluidics layer **115** may be adapted to provide a strong bonding with the shielding layer **114**. If the shielding layer **114** comprises SiO_2 or SiN, the material is selected such that a strong bond with SiO_2 or SiN can be realized. Thereafter, the micro-fluidics layer **115** is patterned **2106** to create the micro-fluidic components **116** in the layer **115**. The micro-fluidics layer **115** is patterned **2106** such that the location of the micro-fluidics component **116** is in vicinity of the heating element **102** to allow heating of a liquid when present in the micro-fluidics component **116**. For example, the heating element **102** is located below the micro-fluidic component **116**. In other words, the heating element **102** may be aligned with the micro-fluidic component **116**. In an optional final step, the micro-fluidics component **116** may be closed **2107**

with a lid **117**, e.g. glass or polymer (such as polycarbonate, cyclic olefin polymer/copolymer, polypropylene) lid. Thus, the closing lid **117** is attached to the micro-fluidics layer **115** in any suitable way, for example by flip-chip bonding, anodic bonding, thermally or solvent-based polymer bonding, PDMS-Si/glass bonding or simply by adhesive tapes. It is an advantage of the invention that when using a photo-patternable adhesive, the material may be used to pattern the micro-fluidic component **116**. In addition to that, the photo-patternable adhesive may be used to attach the closing lid **117**. This dual functionality contributes to the simplicity of fabricating the device. Alternatively or additionally, the closing lid may comprise a micro-fluidics device **100**, which may also be obtained according to embodiments of the present method.

FIG 22 illustrates a specific embodiment of the third aspect of the invention. A method (comprising six steps **2201 - 2206**) to fabricate the micro-fluidic device **100** having a closed micro-fluidic component and a bondpad for supplying a voltage pulse to the heating element as microbubble generator is presented. The micro-fluidic device is fabricated by providing **2201** a substrate **101**, for example a semiconductor substrate such as e.g. a silicon substrate. On the substrate, the heating element **102** is created **2202**, for example by first providing a conductive, e.g. metal, layer and patterning that conductive layer. Additionally, on the substrate **101** a bondpad **113** is created. The bondpad **113** is created such that it is in electrical contact with the heating element **102**. For this purpose, the heating element **102** and the bondpad **113** may be fabricated from the same conductive layer. As an advantage, in a single patterning step, both the heating element **102** and the bondpad **113** may be created **2202**. Alternatively, if the material of the bondpad **113** is different from the material of the heating element **102**, a different patterning step(s) may be used to create the bondpad **113**. Further, it must be ensured that the bondpad **113** is in electrical contact with the heating element **102**. After fabrication of the heating element **102** and the bondpad **113**, a shielding layer **114** is deposited **2203** on top of the substrate **101** thereby covering the heating element **102**. The bondpad **113** is also covered by the shielding layer; however, its top surface **118** is not covered to allow later electrical connections to a power supply. The shielding layer **114** may comprise SiO_2, SiN... On top of the shielding layer, a micro-fluidics layer **115** is provided **2204**. The micro-fluidics layer **115** is positioned such that is does not cover the top surface **118** of the bondpad **113**. The micro-fluidics layer **115** may be fabricated from a patternable material suitable for micro-fluidic applications. The material may be PDMS, SU8 or a photo-patternable adhesive suitable for wafer-level microfluidic structure fabrication. It should be ensured that the material of the micro-fluidics layer **115** is adapted to provide a strong bond with the shielding layer **114**. If the shielding layer **114** is a SiO_2 or SiN layer, the material is selected such that a strong bond with SiO_2 or SiN can be realized. Thereafter, the micro-fluidics layer **115** is patterned **2205** to create the micro-fluidic component **116** in the layer **115**. The micro-fluidics layer **115** is patterned **2205** such that the location of the micro-fluidics component **116** is in vicinity of the heating element **102** to allow heating of a liquid when present in the micro-fluidics component **116**. For example, the heating element **102** is located below the micro-fluidic component **116**. In other words, the heating element **102** may be aligned with the micro-fluidic component **116**. In an optional final step, the micro-fluidics component **116** may be closed **2206** with or attached to a lid **117**, e.g. glass lid. The closing lid **117** may be bonded to the micro-fluidics layer **115**. The micro-fluidics component **116** is closed **2206** thereby not covering the bondpad **113** to allow direct electrical connectivity to a power supply later on. The step of closing the micro-fluidic component **116** is performed for example by flip-chip bonding, anodic bonding, thermally or solvent-based polymer bonding, PDMS-Si/glass bonding or simply by adhesive tapes. It is an advantage of the invention that when using a photo-patternable adhesive, the material may be used to pattern the micro-fluidic component **116**. In addition to that, the photo-patternable adhesive may be used to attach the closing lid **117**. This dual functionality contributes to the simplicity of fabricating the device.

CLAIMS

1. A micro-fluidic device (100) for deflecting objects in a liquid, the device comprising:
- a substrate (101) for providing an object-containing liquid thereon; and
- a microbubble generator (102), having at least one microbubble generating element (103, 401), located on a surface of the substrate and in direct contact with the liquid when provided on the substrate (101);

characterized in that:
the at least one microbubble generating element (103, 401) is adapted to deflect a single object in the liquid through generation of a plurality of microbubbles by each of them.

2. The micro-fluidic device according to claim 1, wherein the at least one microbubble generating element (103, 401) comprises a first series of connected microstructures (104); each microstructure (104) adapted for generating a microbubble in the liquid when the microbubble generator (102) is powered.

3. The micro-fluidic device (100) according to claim 2, wherein the microbubble generator (102) is a heating element, and the at least one microbubble generating element is at least one micro-heater (103), wherein the at least one micro-heater (103) is shaped such that at least two microstructures (104) heat up simultaneously when an electrical current flows through the at least one micro-heater (103).

4. The micro-fluidic device (100) according to any of claims 2 to 3, wherein the microbubble generator (102) is a heating element and the at least one microbubble generating element is at least one micro-heater (103), wherein the at least one micro-heater (103) further comprises a second series of connected microstructures connected in parallel to the first series of connected microstructures (104); and wherein the at least one micro-heater is shaped such that at least two microstructures of different series of connected microstructures heat up simultaneously when an electrical current flows through the micro-heater (103).

5. The micro-fluidic device (100) according to claim 2, wherein the microbubble generator (102) is an electrolytic unit comprising at least a set of electrodes (401, 401'), wherein the electrodes (401, 401') are shaped such that at least two microstructures (104) generate microbubbles simultaneously when a voltage is applied to the electrodes (401, 401').

6. The micro-fluidic device (100) according to any of claims 2 to 5, further comprising a non-conductive layer (105), located in between the liquid and the microbubble generator (102), having a plurality of cavities (106) fabricated down to the microbubble generator (102), wherein each cavity (106) is aligned with a corresponding microstructure (104).

7. The micro-fluidic device (100) according to claim 6, wherein one of the cavities (106) comprises at least one sharp corner.

8. The micro-fluidic device (100) according to claim 6 or 7, wherein a cross-section (101), parallel to the substrate (101), of one of the cavities (106) is triangular or rectangular shaped.

9. The micro-fluidic device (100) according to any of claims 2 to 8, wherein each microstructure (104) comprises a portion having a cross-sectional dimension of 10 micrometers or smaller.

10. The micro-fluidic device (100) according to any of the preceding claims, wherein the microbubble generator (102) is a heating element, wherein the microbubble generator (102) comprises only one metal layer.

11. The micro-fluidic device (100) according to any of the preceding claims, further comprising a controller connected to the microbubble generator (102) and configured to monitor at least one parameter of the microbubble generator (102) related to the generation of microbubbles.

12. The micro-fluidic device (100) according to claim 11, wherein the microbubble generator is a heating element (102), the at least one microbubble generating element is at least one micro-heater (103), the controller being configured to monitor the temperature of the heating element (102) or configured to monitor the resistance of the heating element (102).

13. The micro-fluidic device (100) according to any of the preceding claims, wherein the microbubble generator (102) comprises a SiC layer for preventing deformation of the microbubble generator.

14. A system (200) for sorting objects comprising a first micro-fluidic device (100) according to any of the preceding claims and a first micro-fluidic channel (107); wherein the first micro-fluidic device (100) is positioned to deflect single objects propagating in the first micro-fluidic channel (107) by generating microbubbles.

15. The system according to claim 14, further comprising a second micro-fluidic channel (108) fluidically connected to the first micro-fluidic channel (107); wherein the first micro-fluidic device (100) is positioned in the second micro-fluidic channel (108) and configured for deflecting single objects propagating in the first micro-fluidic channel (107) by generating microbubbles.

16. The system according to claim 15, further comprising a third micro-fluidic channel (108a) fluidically connected to the first micro-fluidic channel (107), wherein the second (108) and the third micro-fluidic channel (108a) are aligned and positioned at opposite sides of the first micro-fluidic channel (107); and further comprising a second micro-fluidic device (100) positioned in the third micro-fluidic channel (108a) and

wherein the first and the second micro-fluidic devices are configured for deflecting single objects propagating in the first micro-fluidic channel (107).

17. A method for fabricating a micro-fluidic device (100) according to any of the claims 1 to 13, the method comprising: providing a substrate (101); providing a conductive layer (119) on top of the substrate (101); patterning a microbubble generator (102) having at least one microbubble generating element (103) in the conductive layer (119);
characterized in that:
patterning the microbubble generator (102) comprises patterning a series of microstructures (104), wherein each microstructure (104) is adapted for generating a microbubble when activated.

18. The method according to claim 17, wherein patterning of the microbubble generator having at least one microbubble generating element(103) comprises fabricating the at least one microbubble generating element (103) such that its shape allows at least two microstructures (104) of the at least one microbubble generating element (103) to generate microbubbles simultaneously when the at least one microbubble generating element (103) is activated.

19. The method according to claim 18, wherein patterning of the microbubble providing having at least one microbubble generating element comprises patterning of at least one micro-heater (103) such that its shape allows at least two microstructures (104) of the at least one micro-heater (103) to heat up simultaneously when an electrical current flows through the at least one micro-heater (103).

20. The method according to claim 18, wherein patterning of the microbubble providing having at least one microbubble generating element comprises patterning of at least one set of electrodes (401, 401') such that its shape allows at least two microstructures (104) of the at least one set of electrodes (401, 401') to create eletrolysis simultaneously when a voltage is applied to the electrodes (401, 401').

21. The method according to any of the claims 17 to 20, further comprising providing a micro-fluidic layer (115) on top of the microbubble generator (102); creating a micro-fluidic channel (116) in the micro-fluidic layer (115); and closing the micro-fluidic channel with a lid (117).

22. The method according to claim 21, wherein the micro-fluidics layer (115) is a photo-patternable polymer for attaching the lid (117).

23. The method according to claim 21, wherein a photo-patternable polymer is present in between the lid and the micro-fluidic layer (115), for closing the micro-fluidic channel with a lid (117).

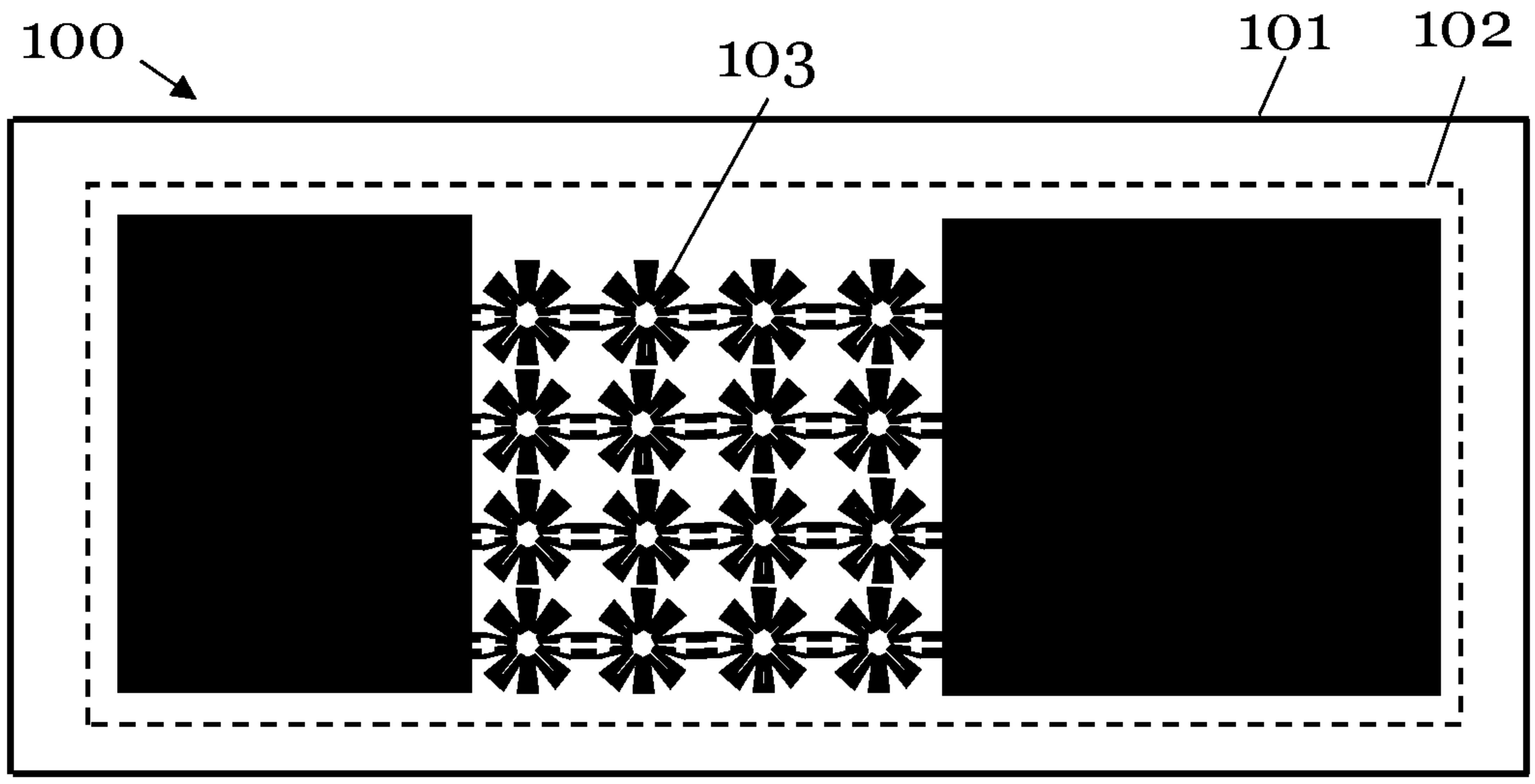


FIG 1

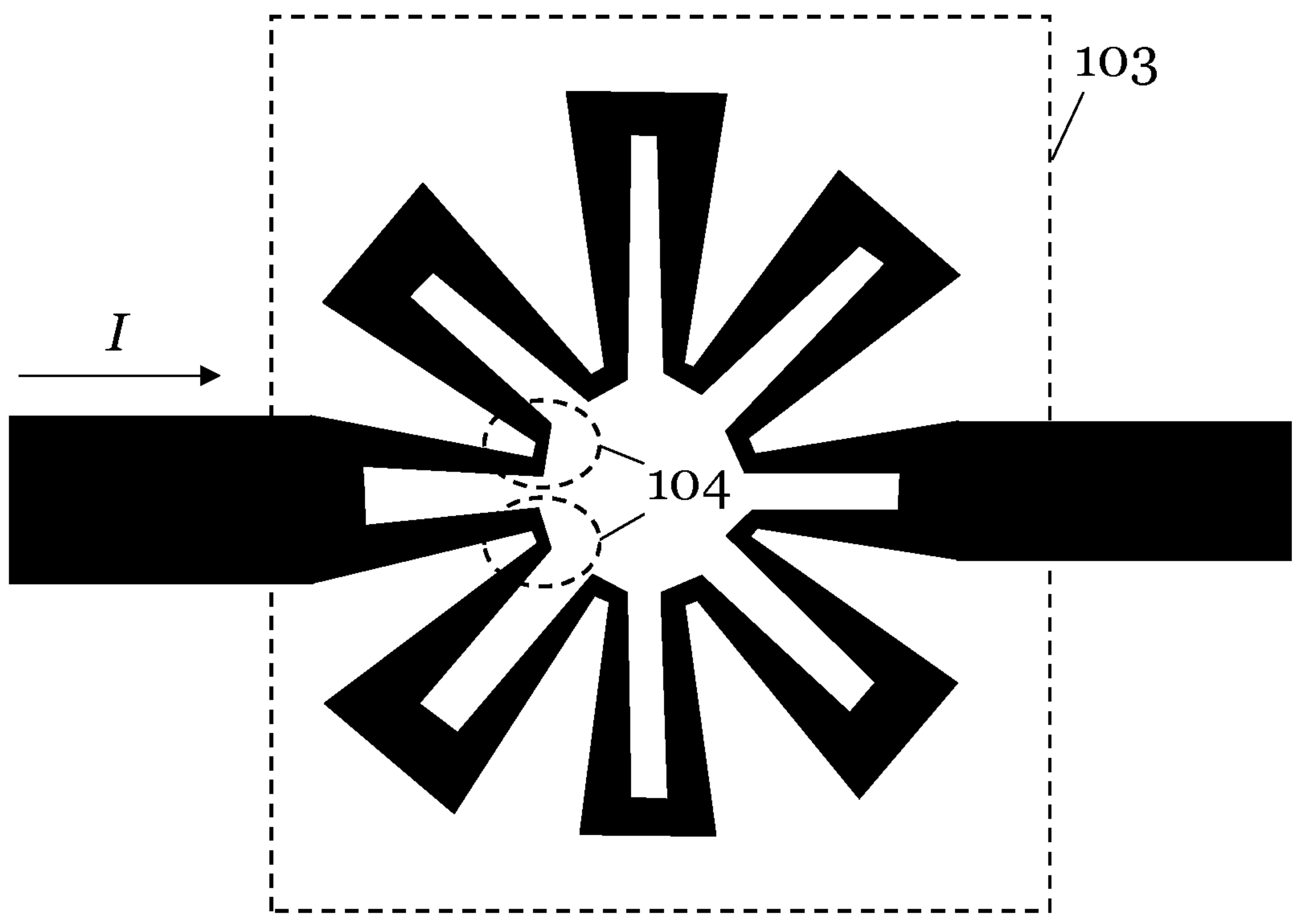


FIG 2

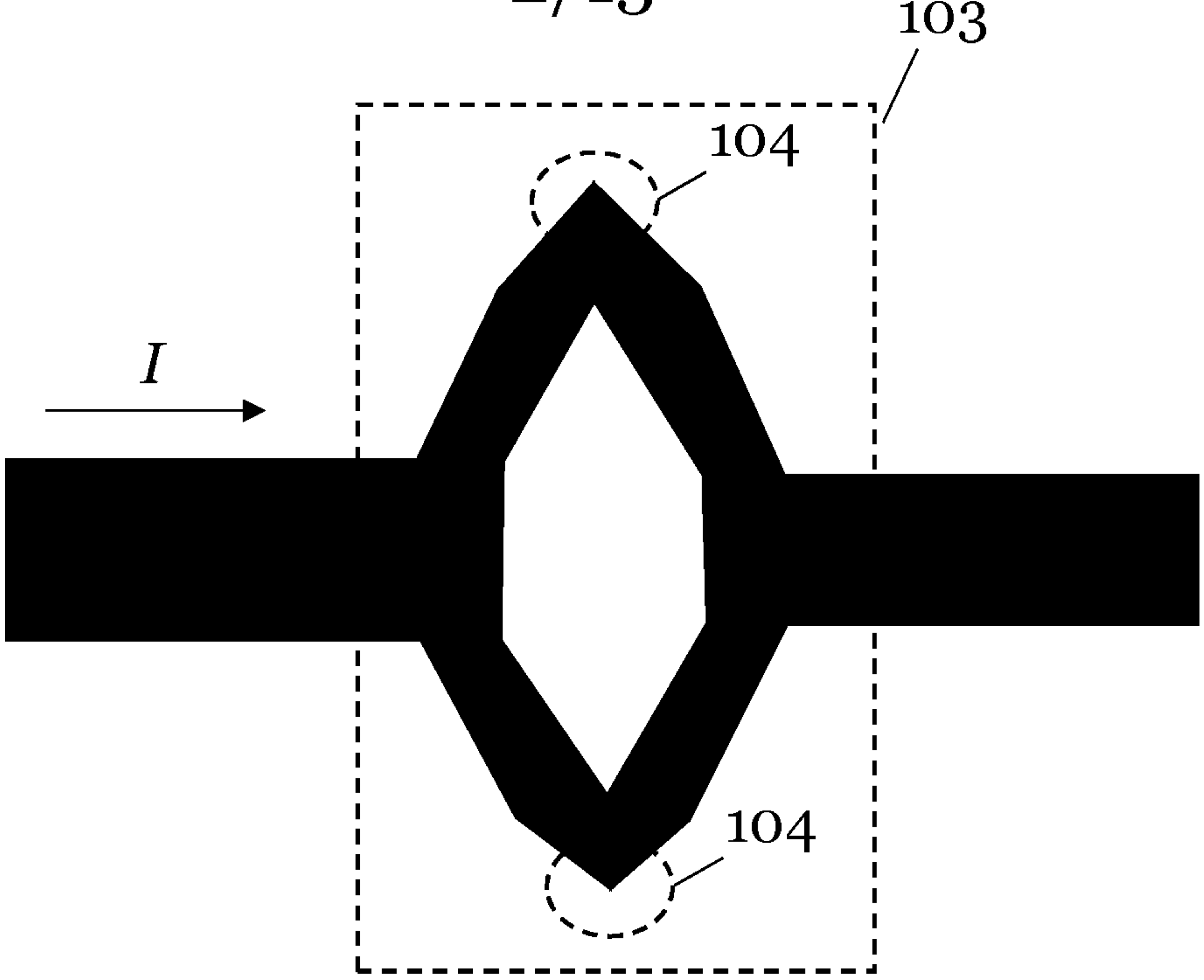


FIG 3

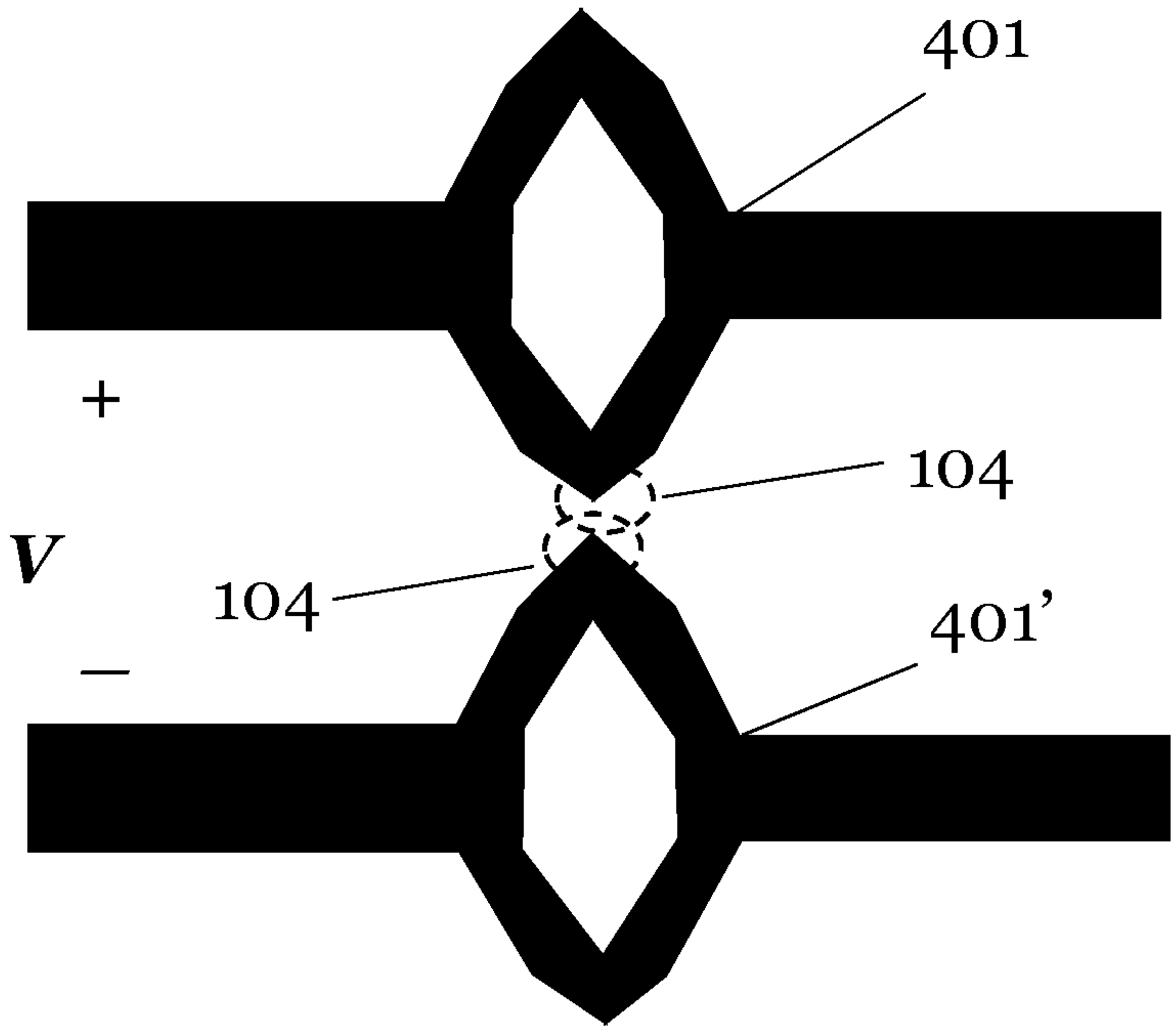


FIG 4

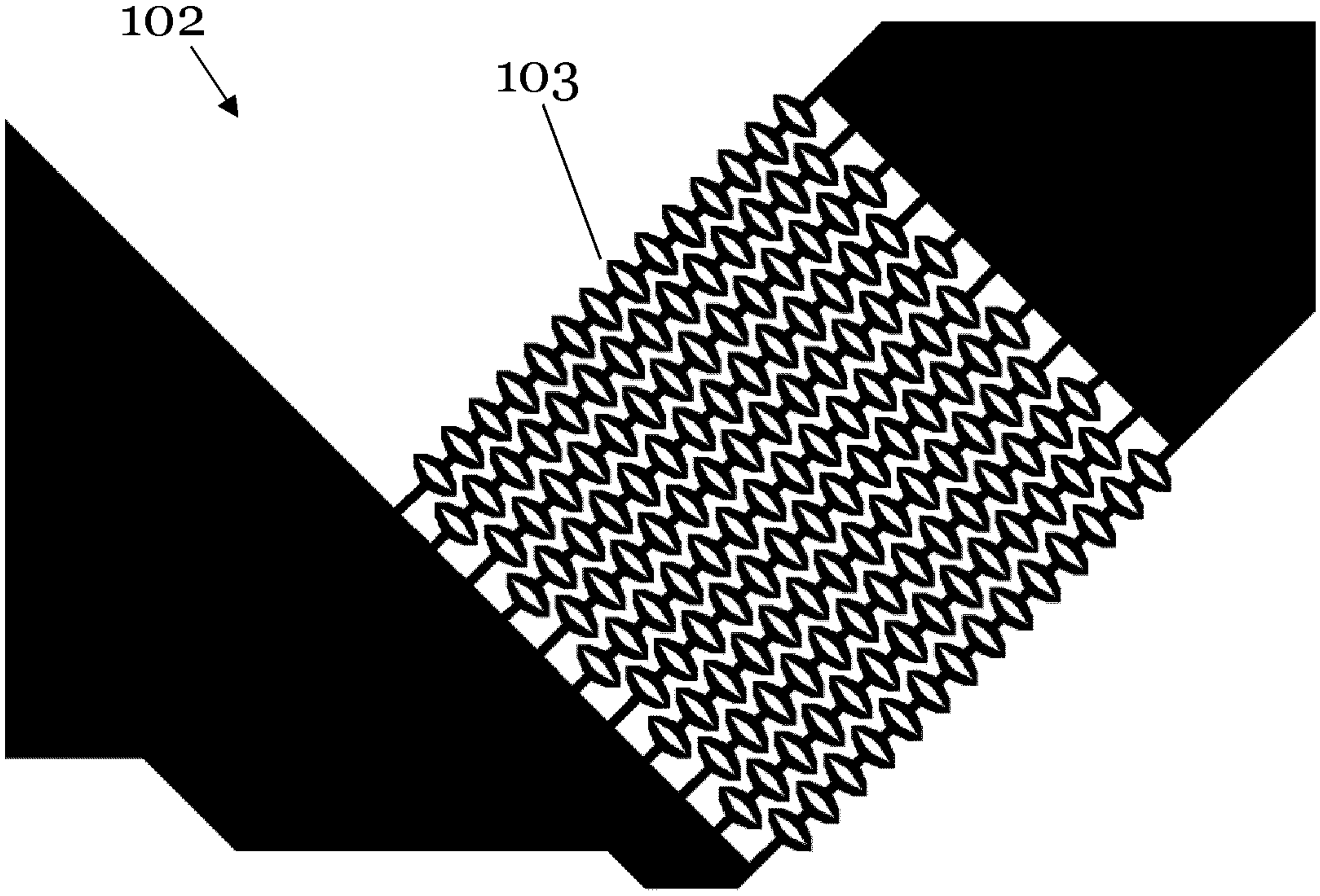


FIG 5

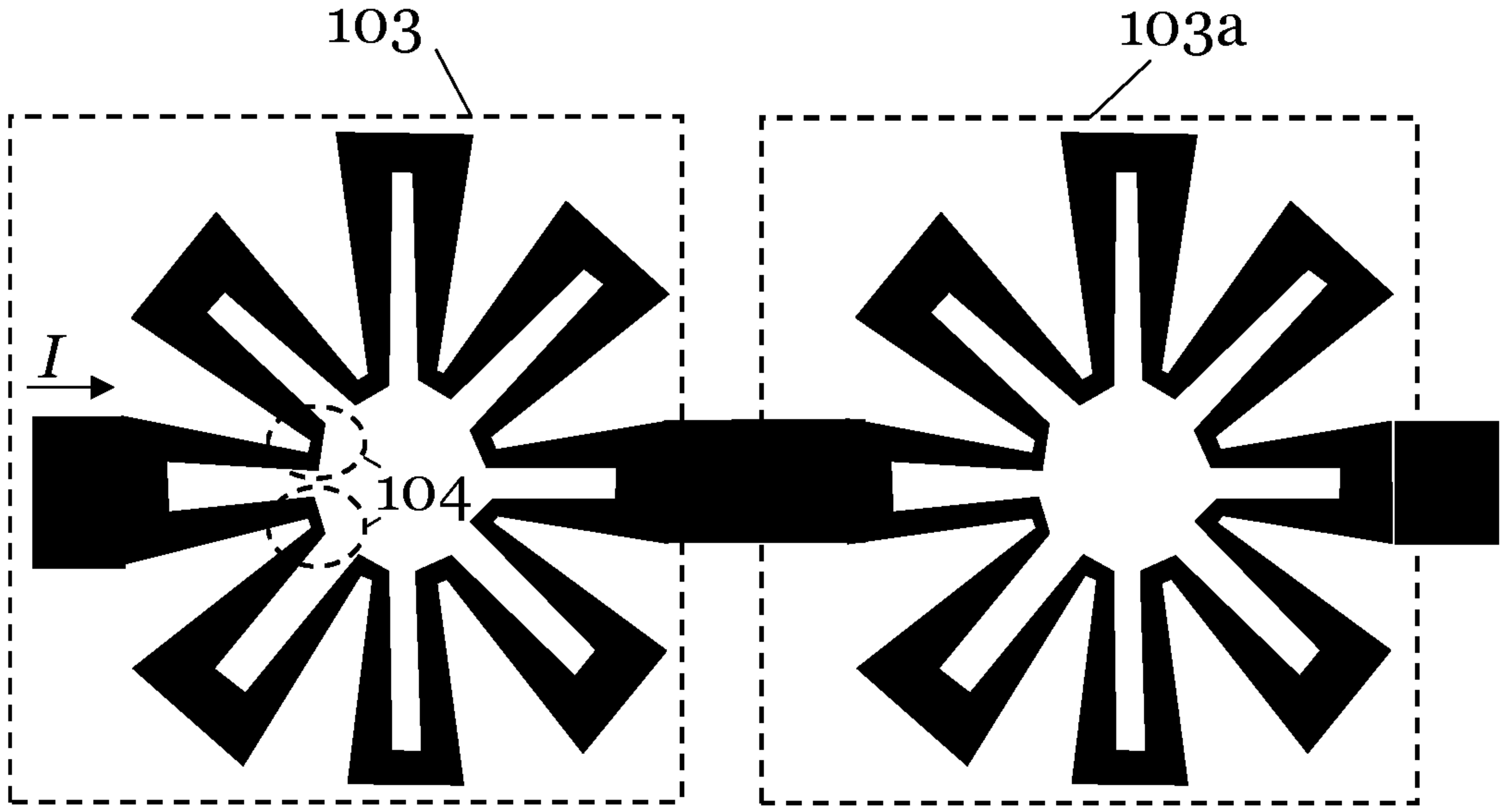


FIG 6

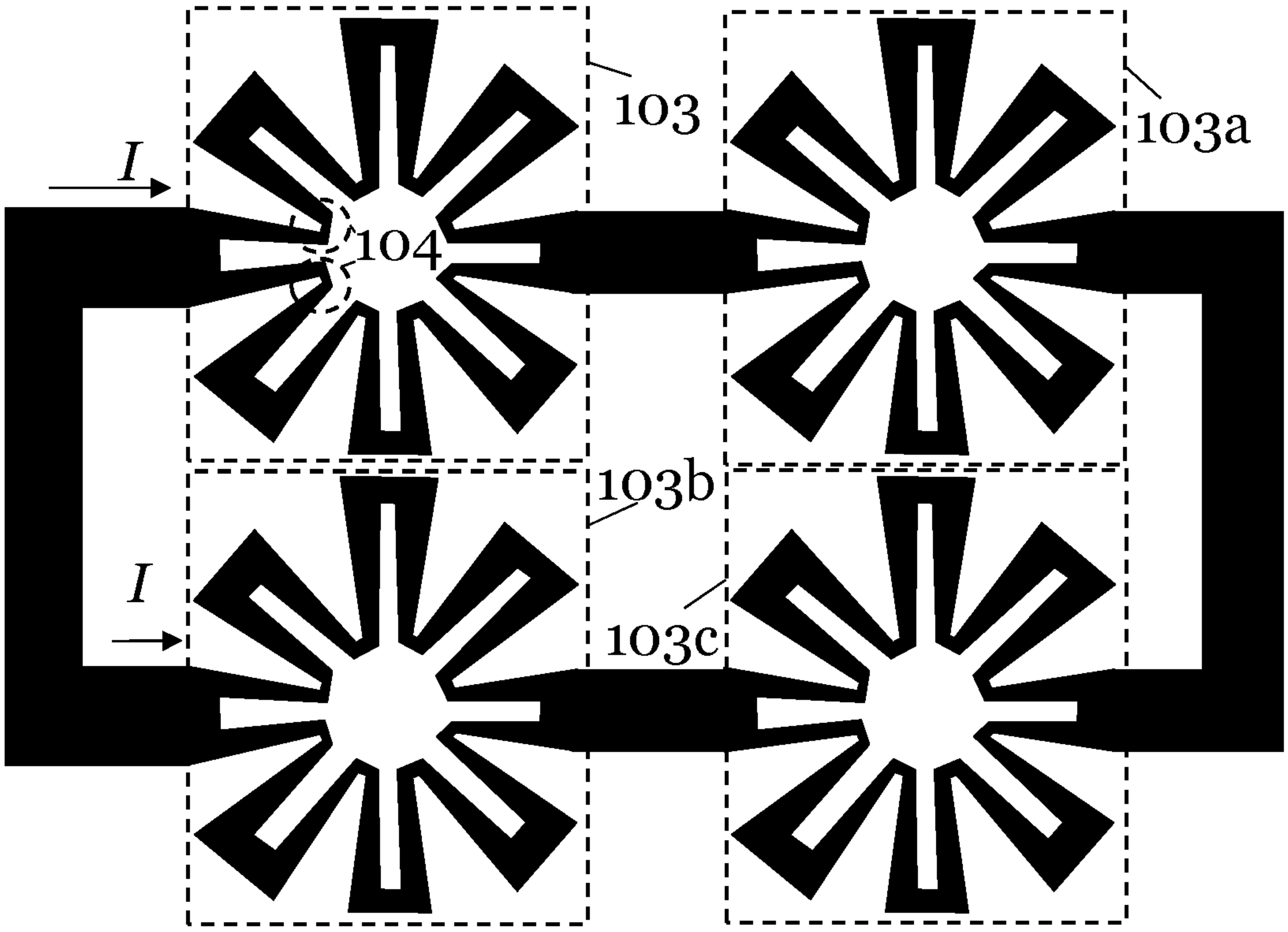


FIG 7

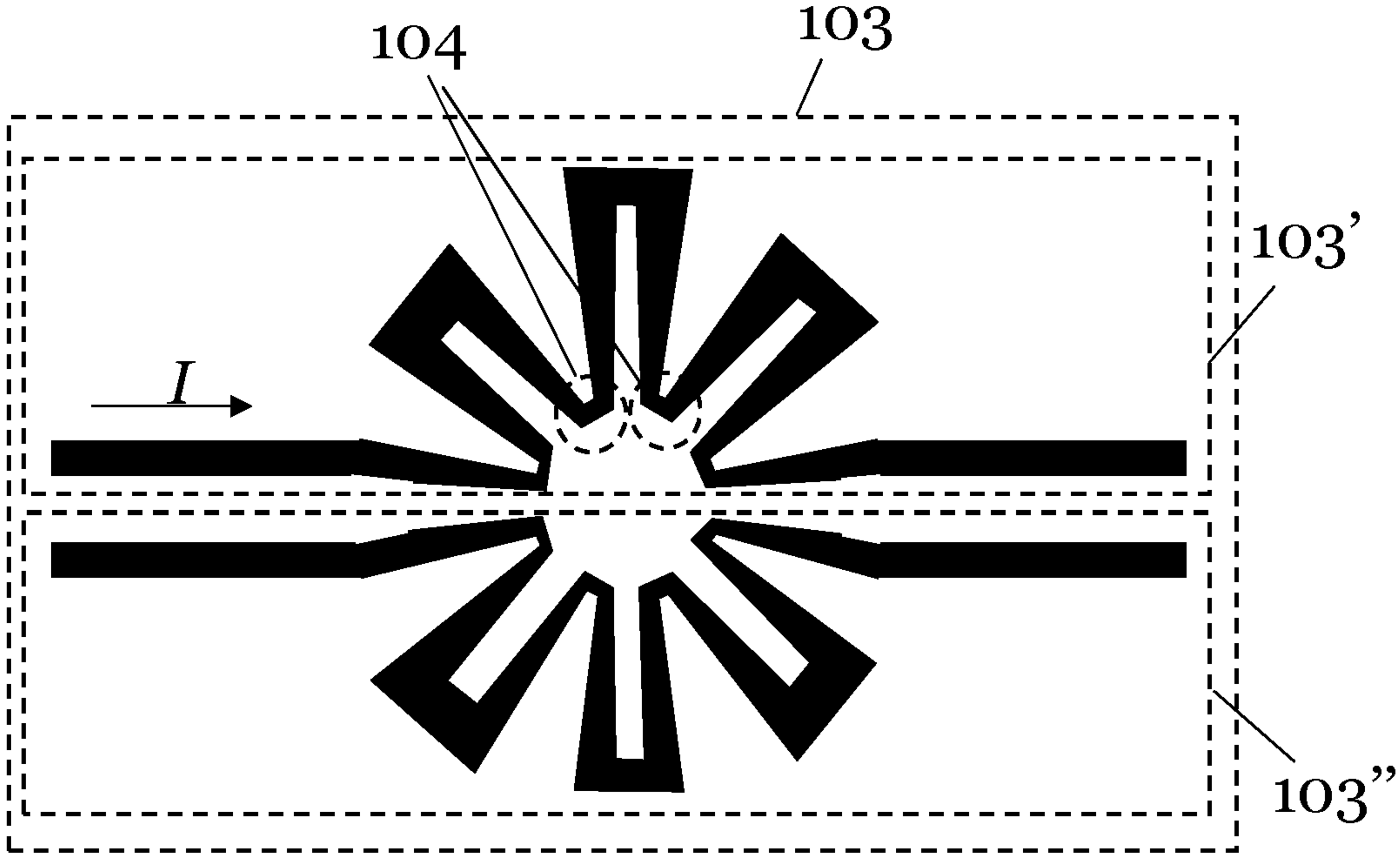


FIG 8

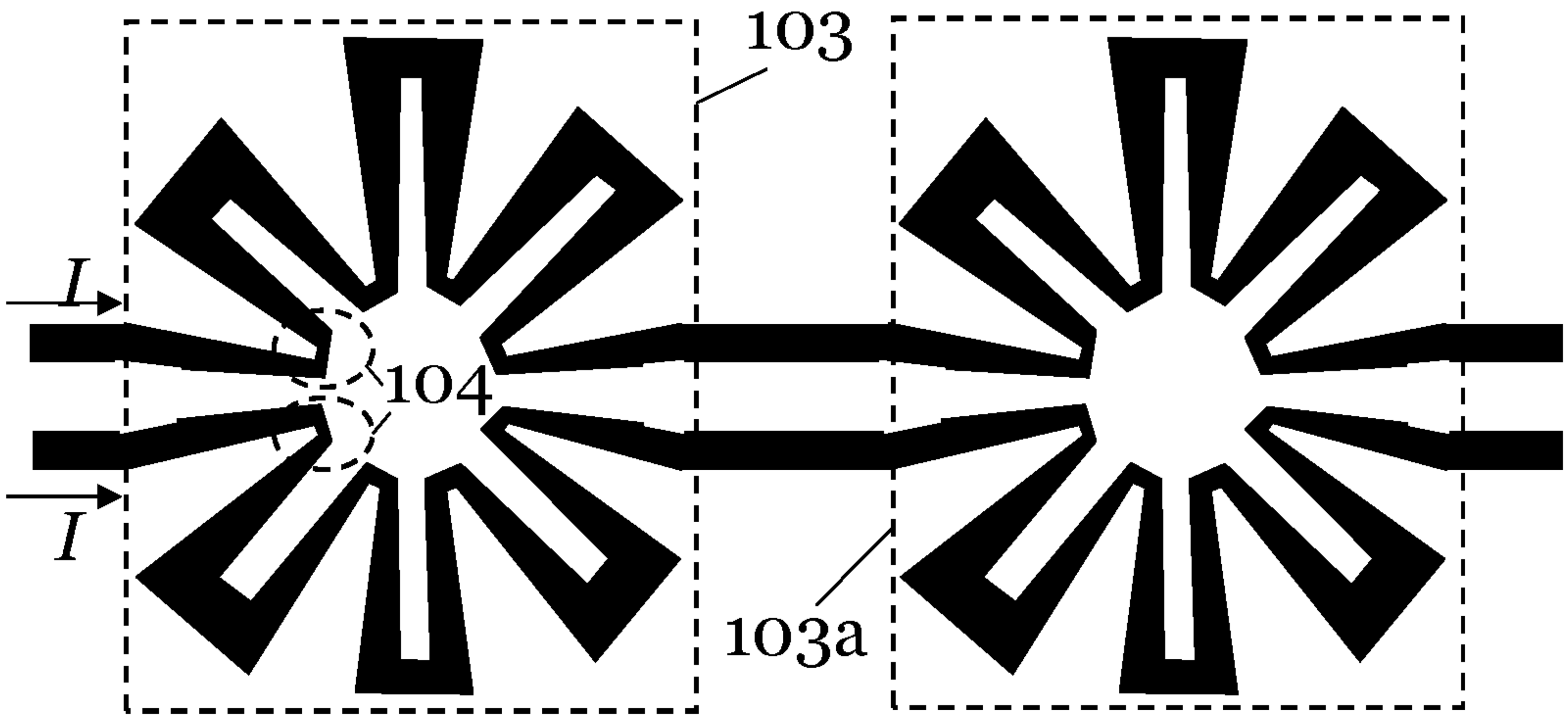


FIG 9

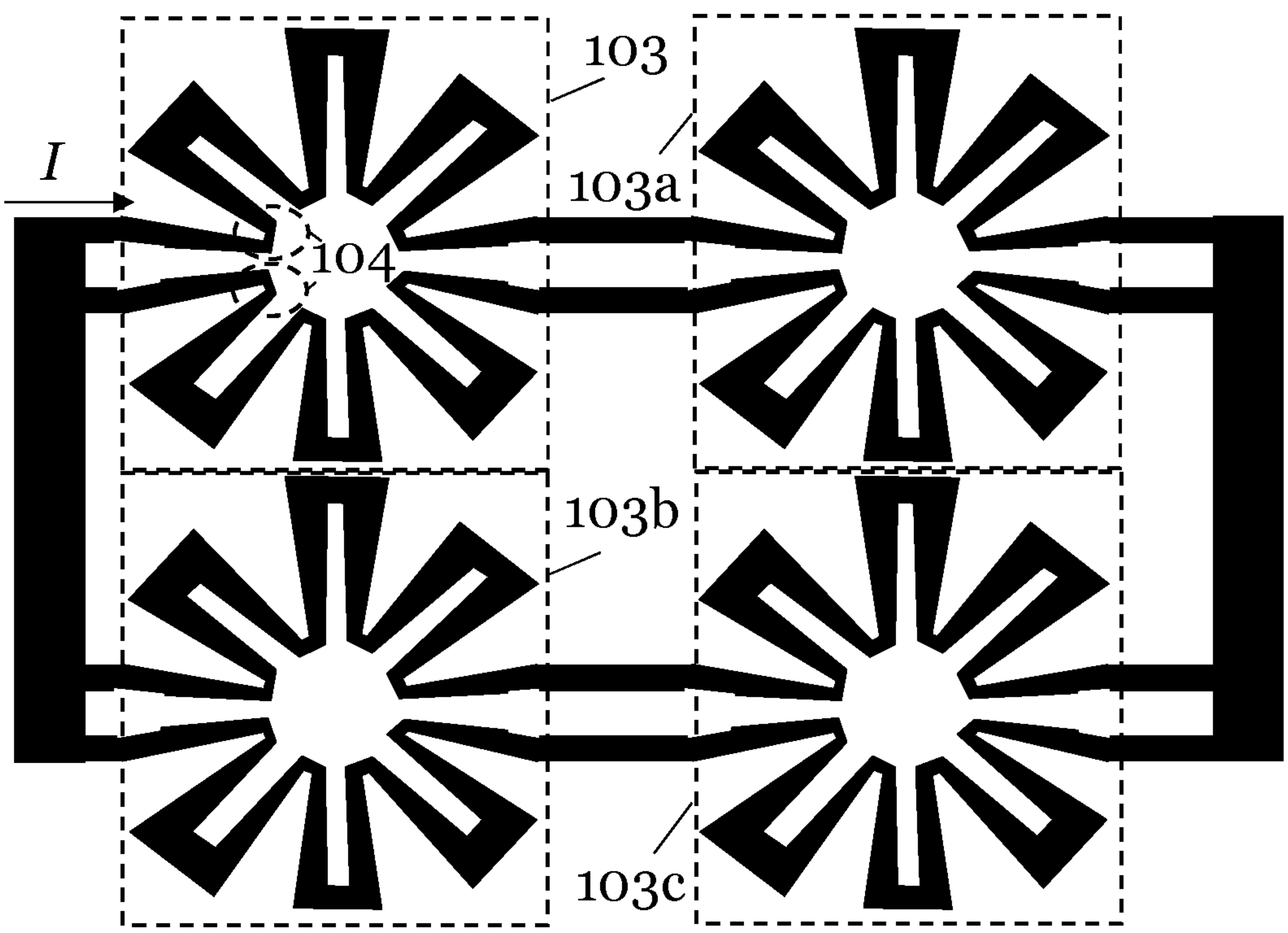


FIG 10

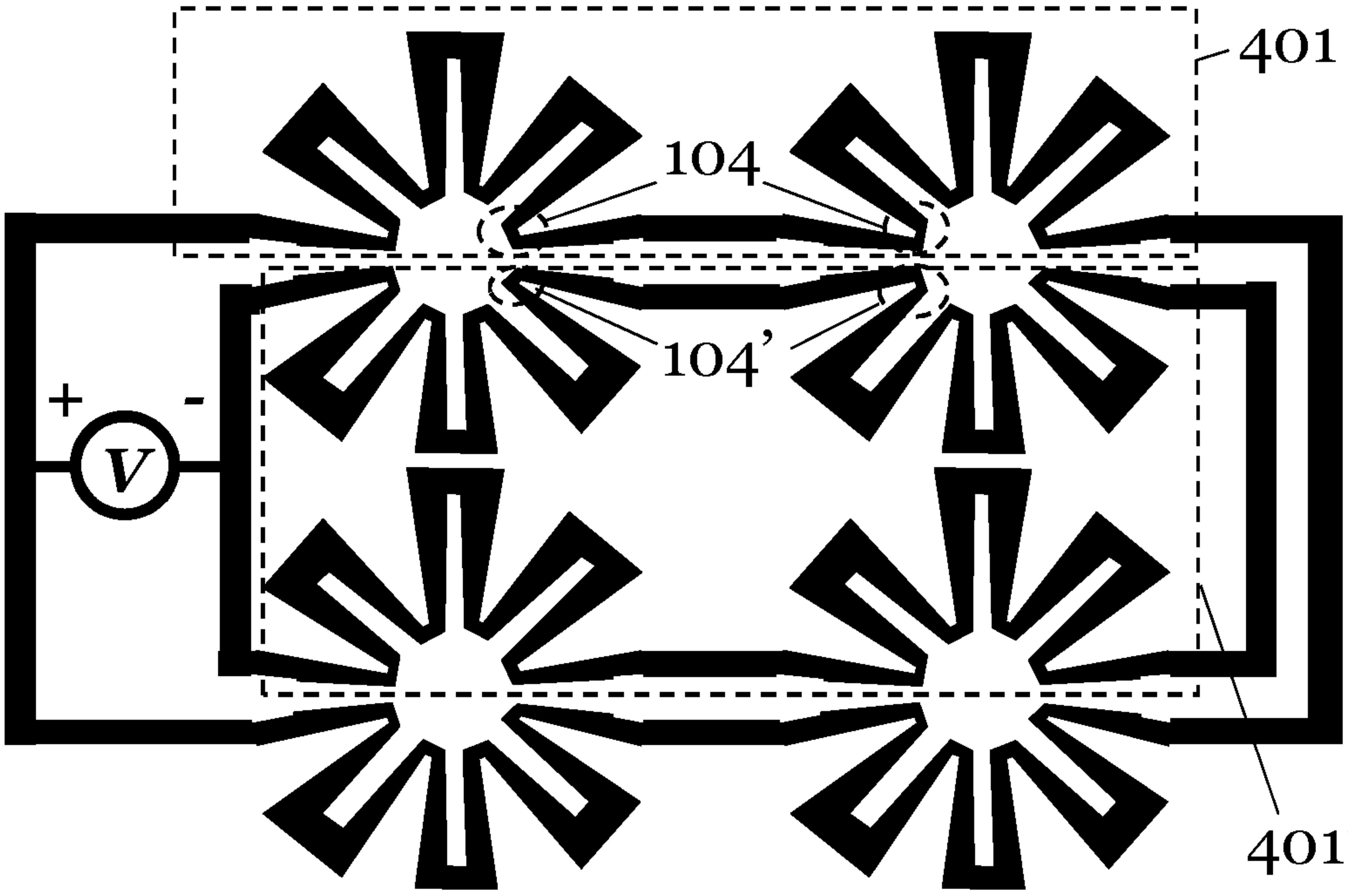


FIG 11

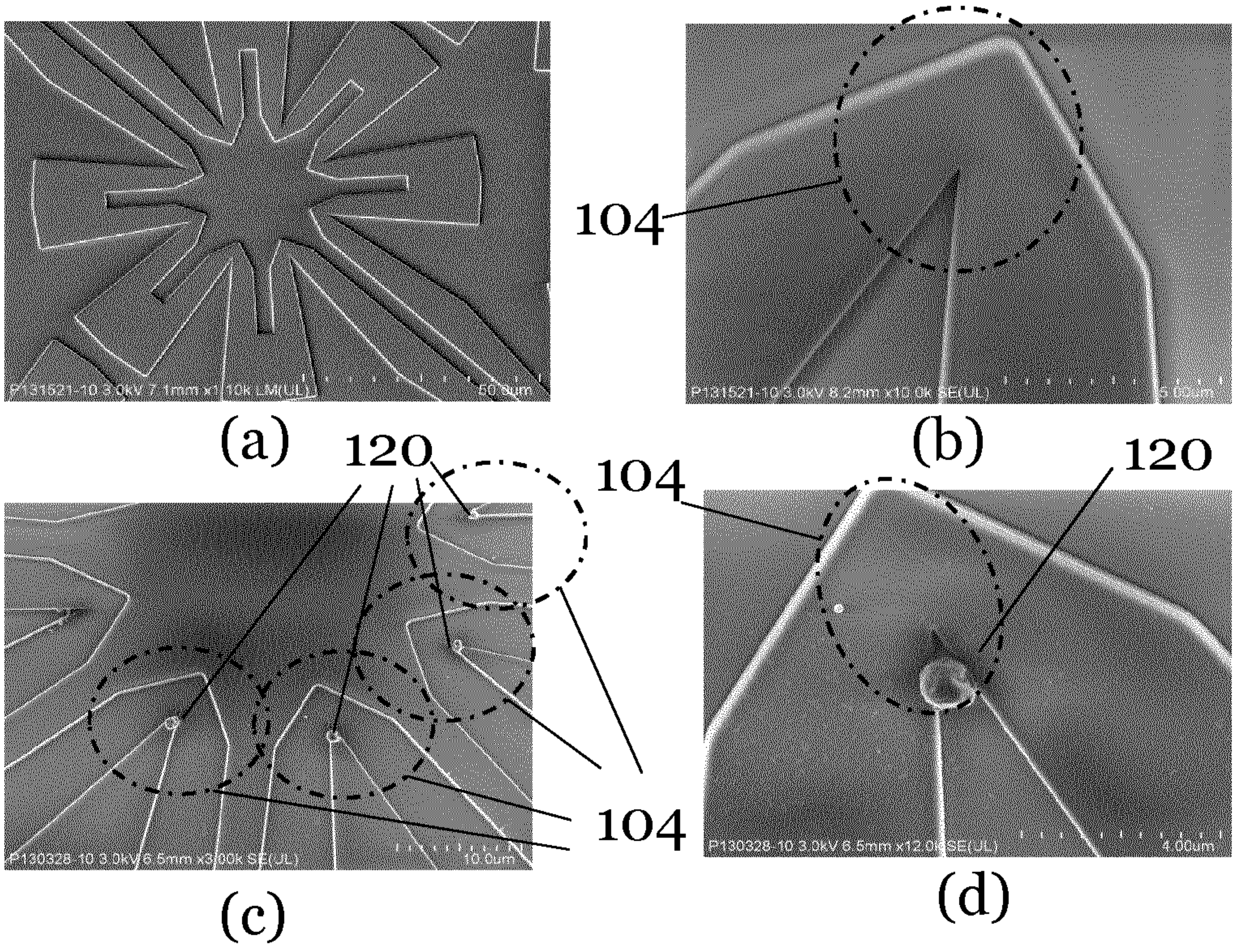


FIG 12

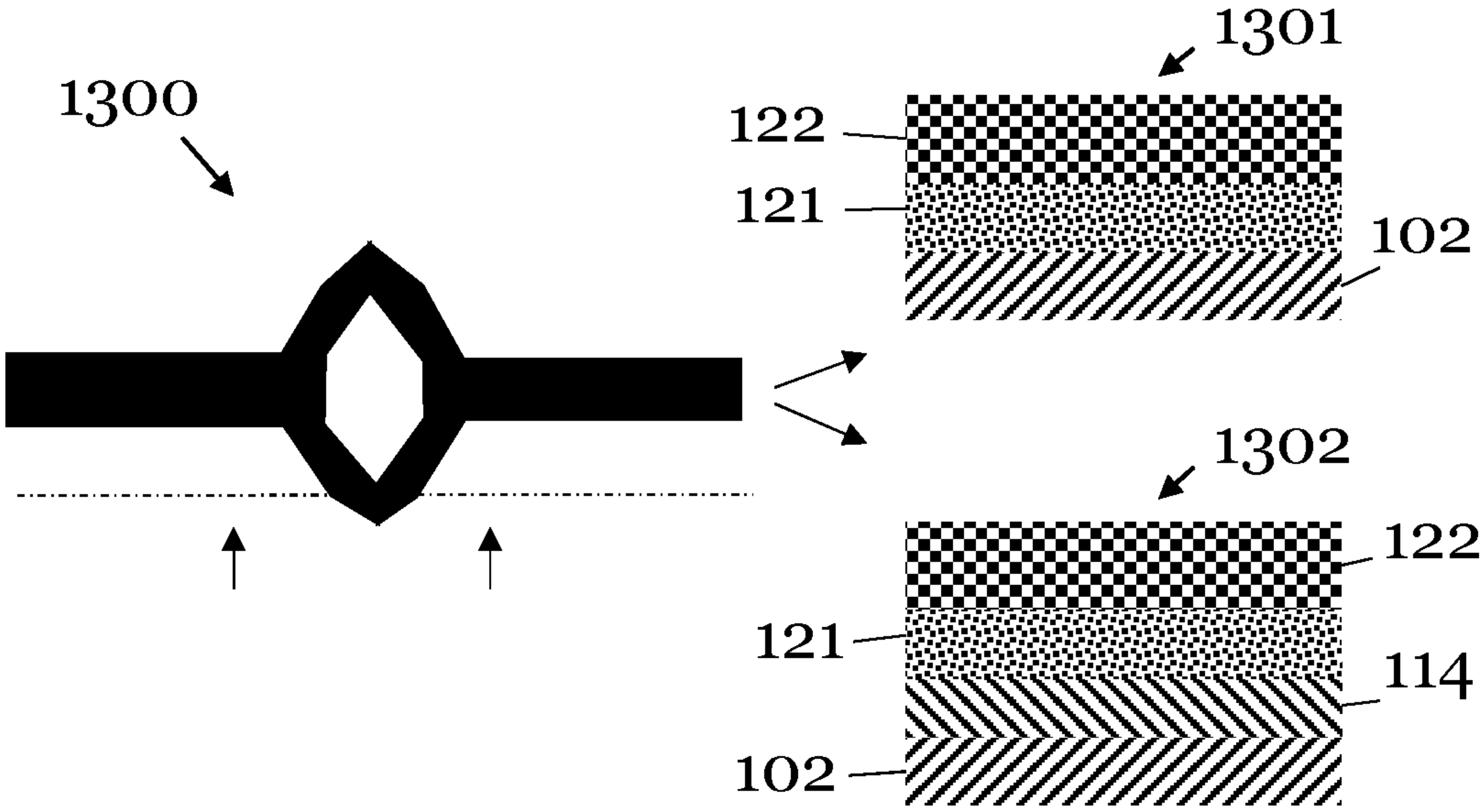


FIG 13

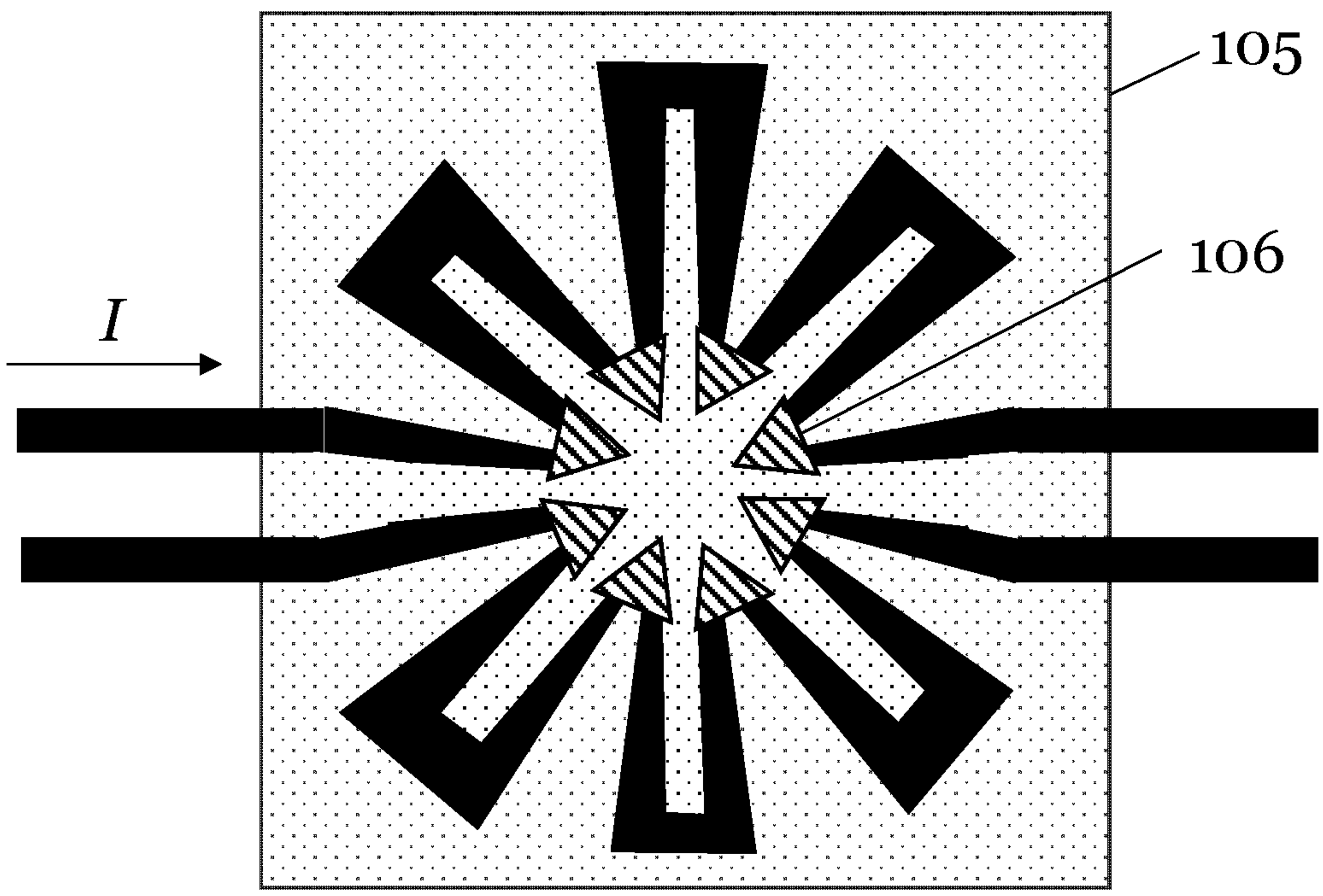


FIG 14

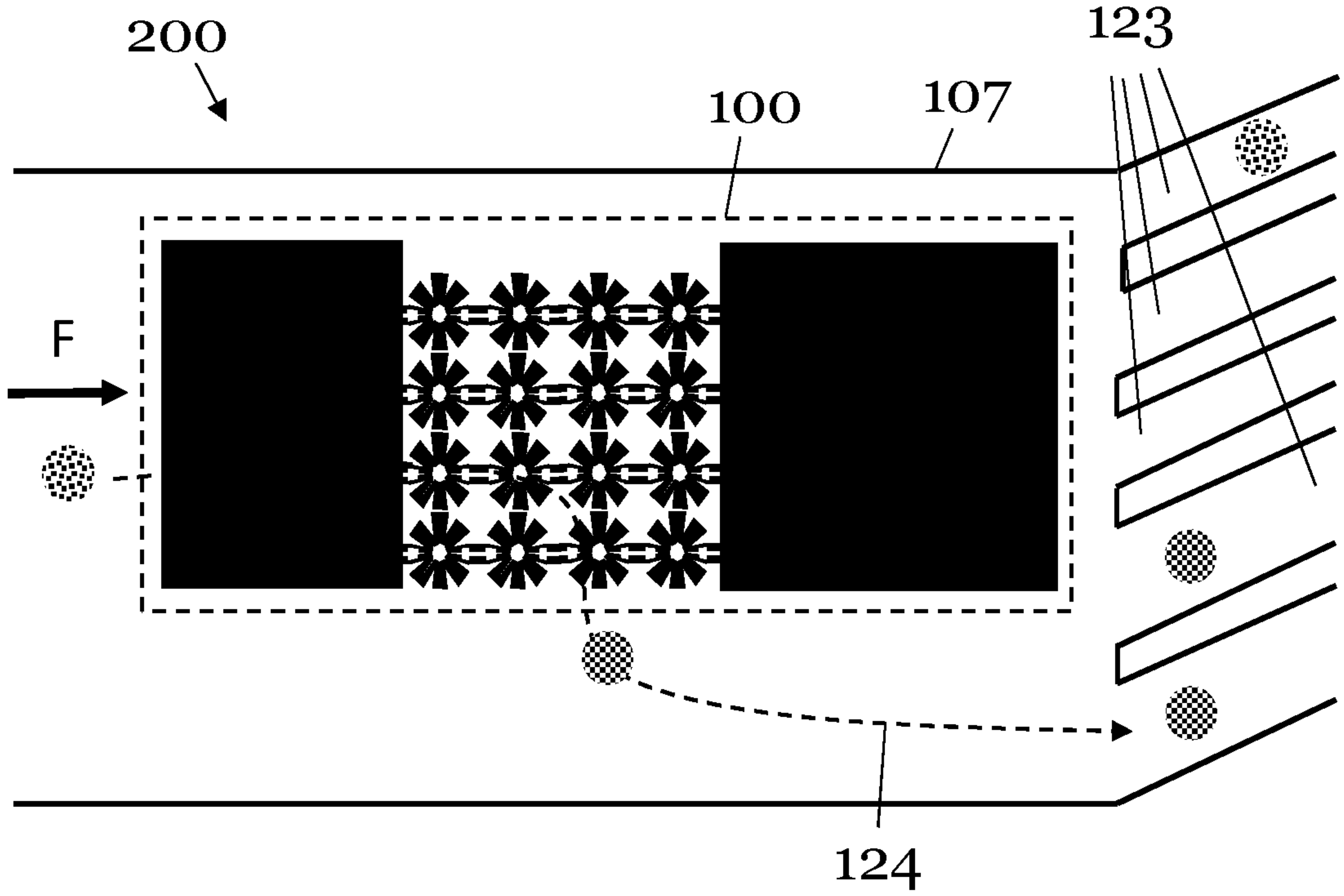


FIG 15

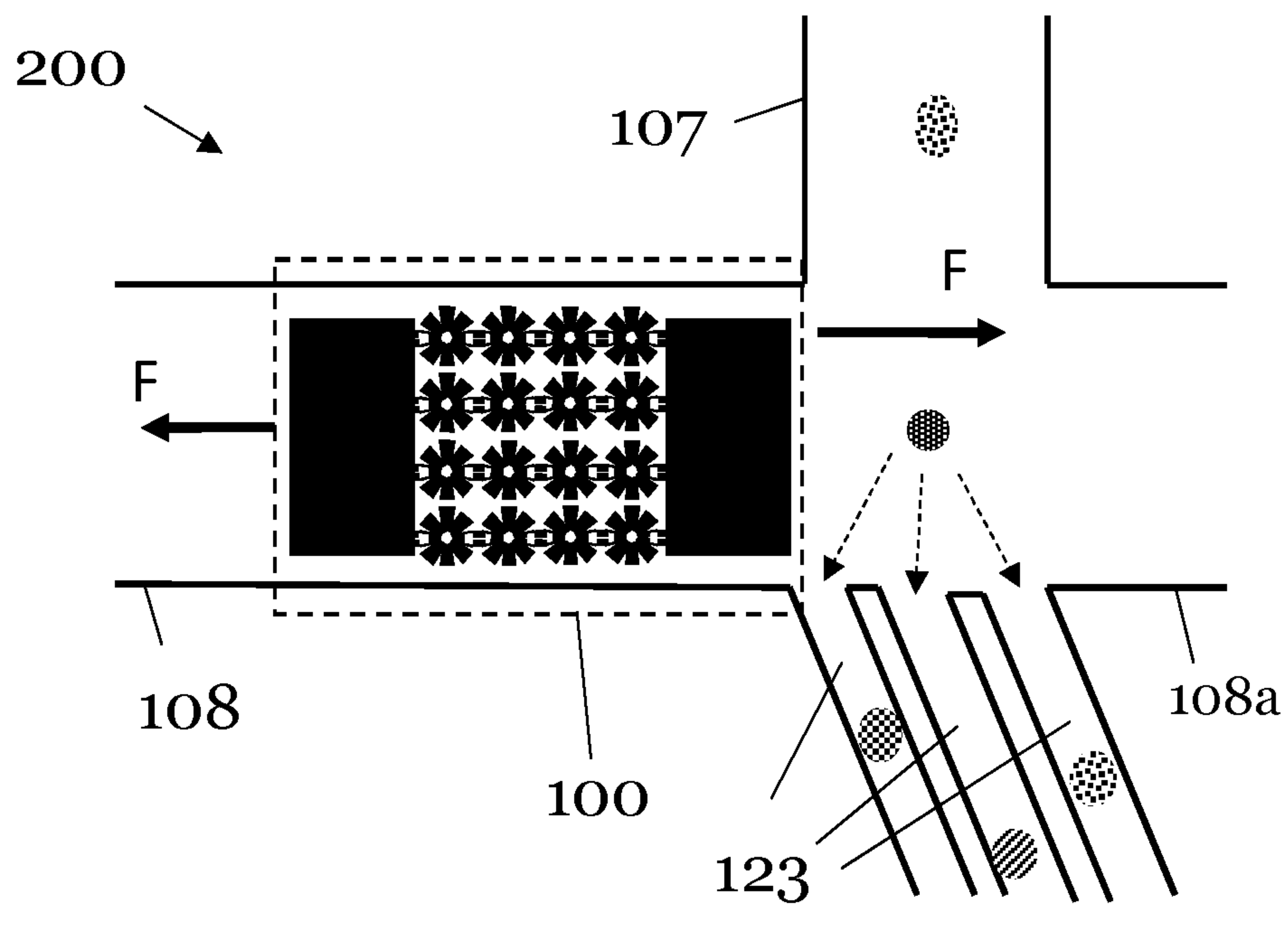


FIG 16

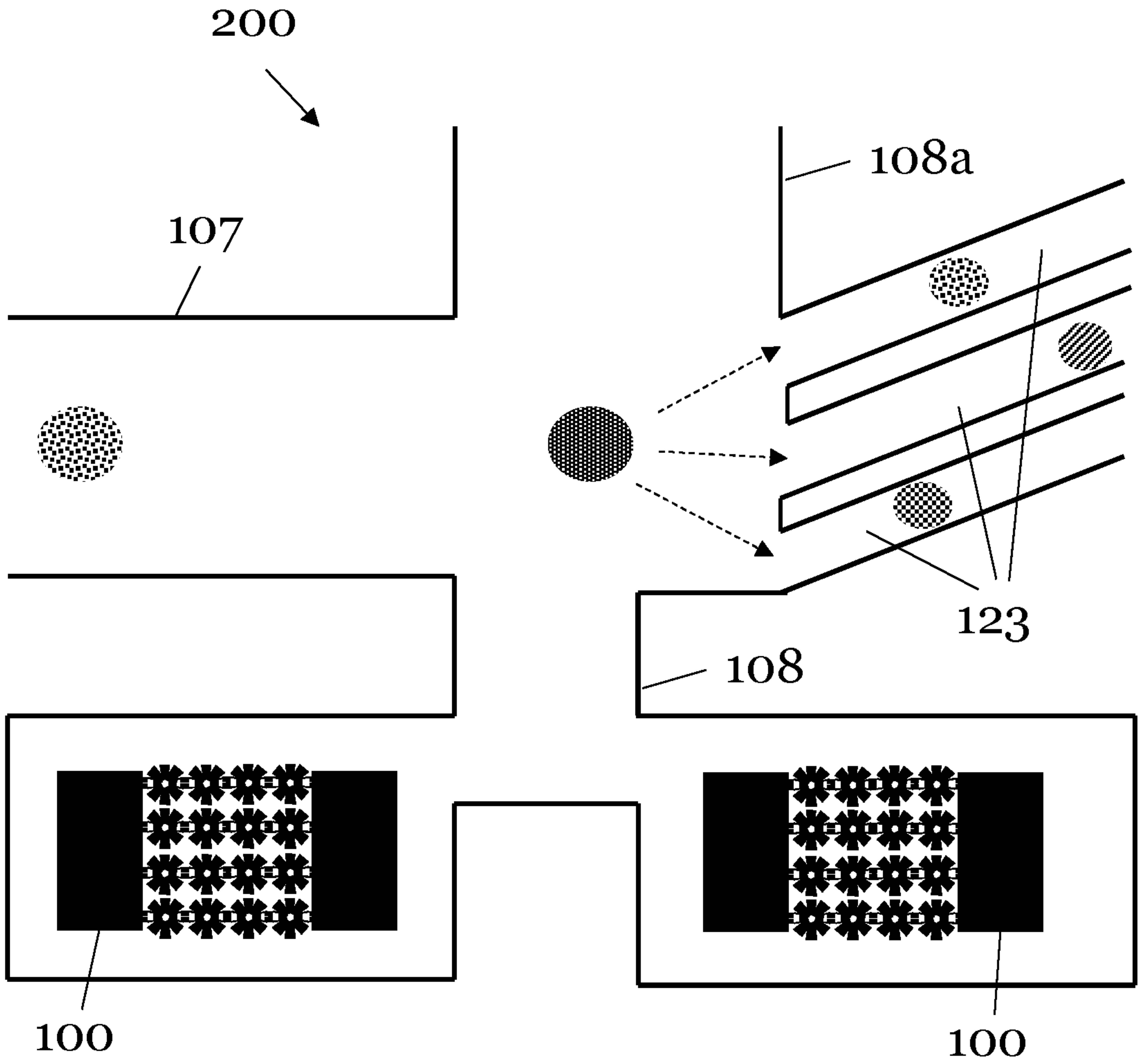


FIG 17

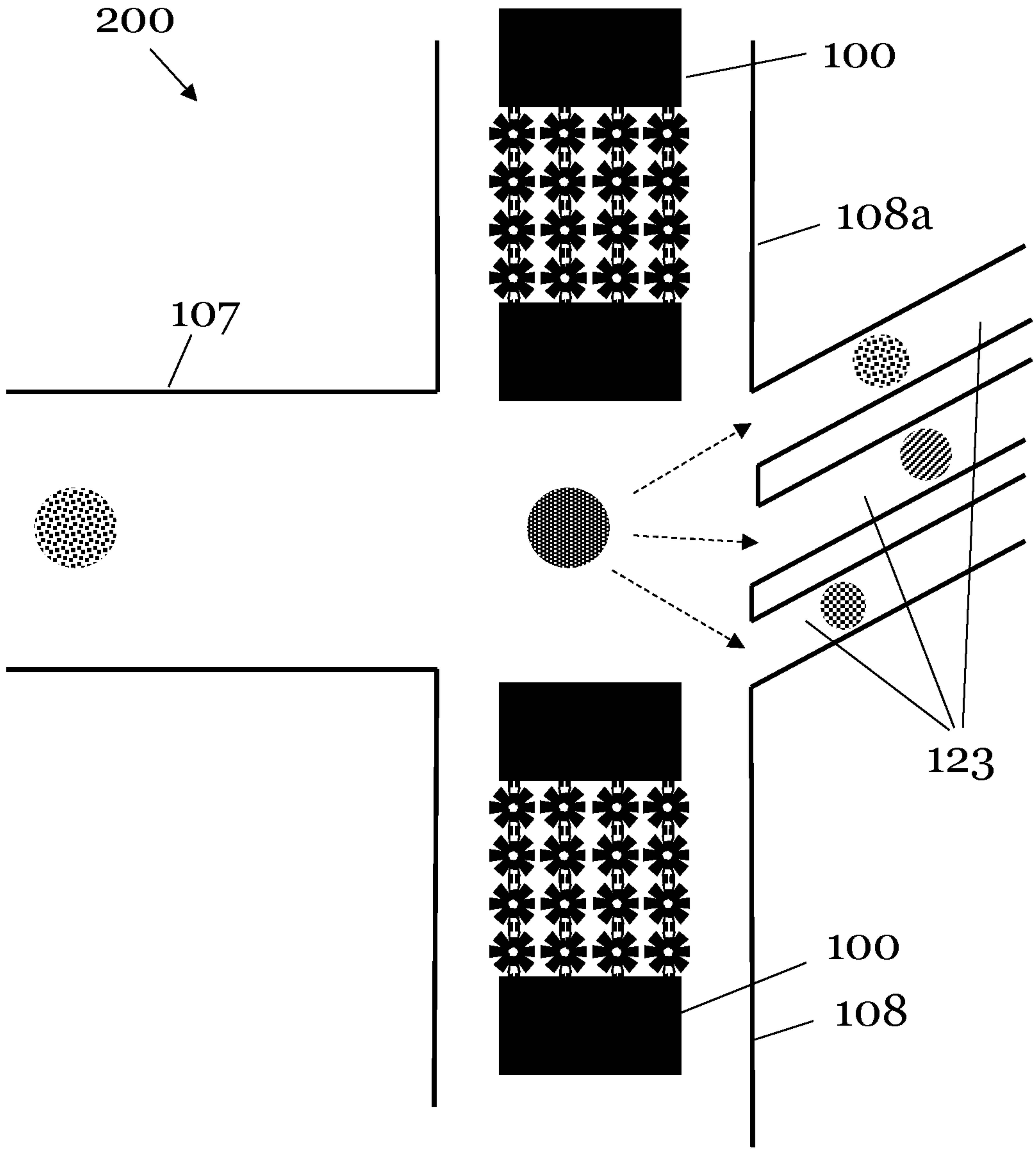


FIG 18

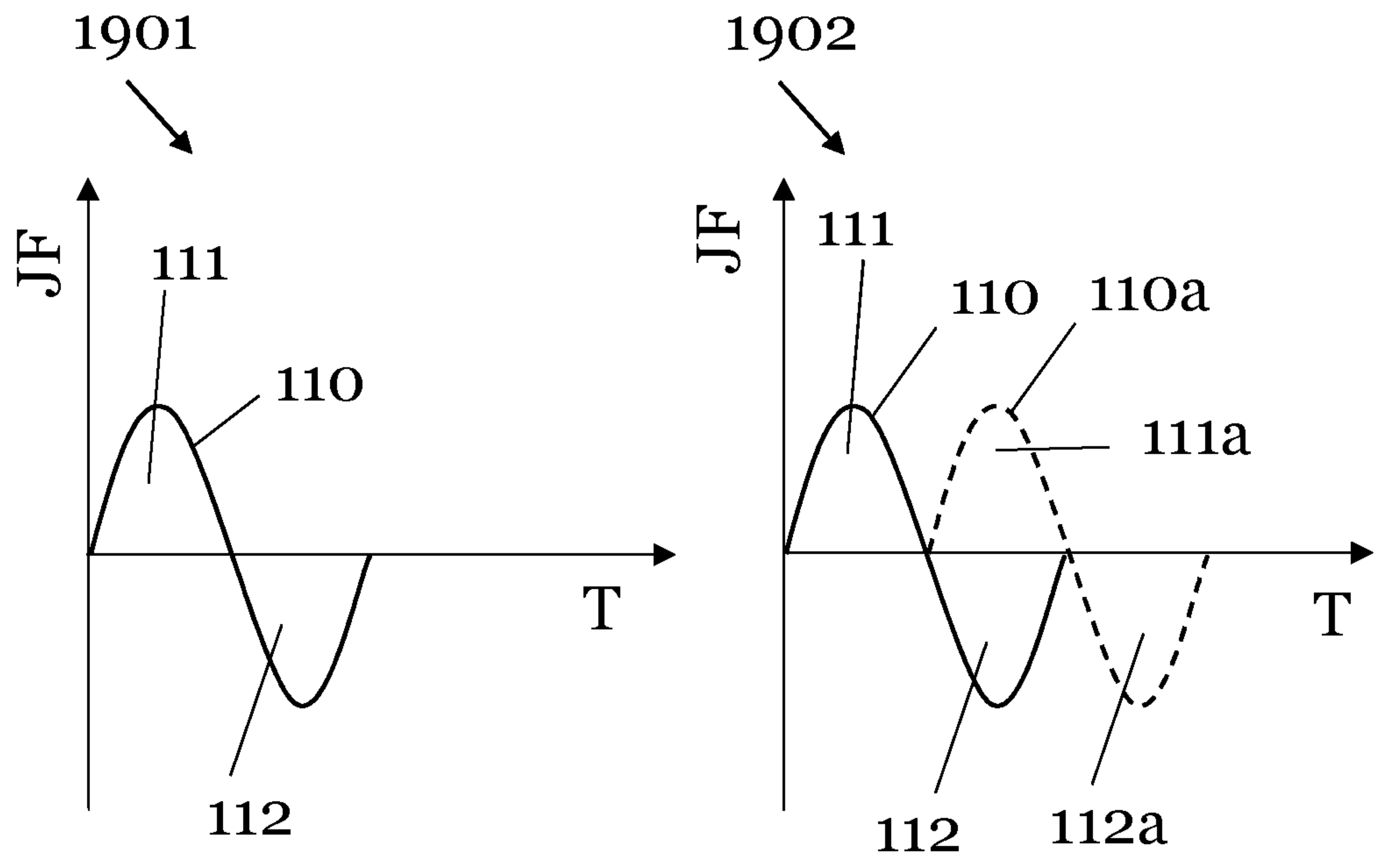
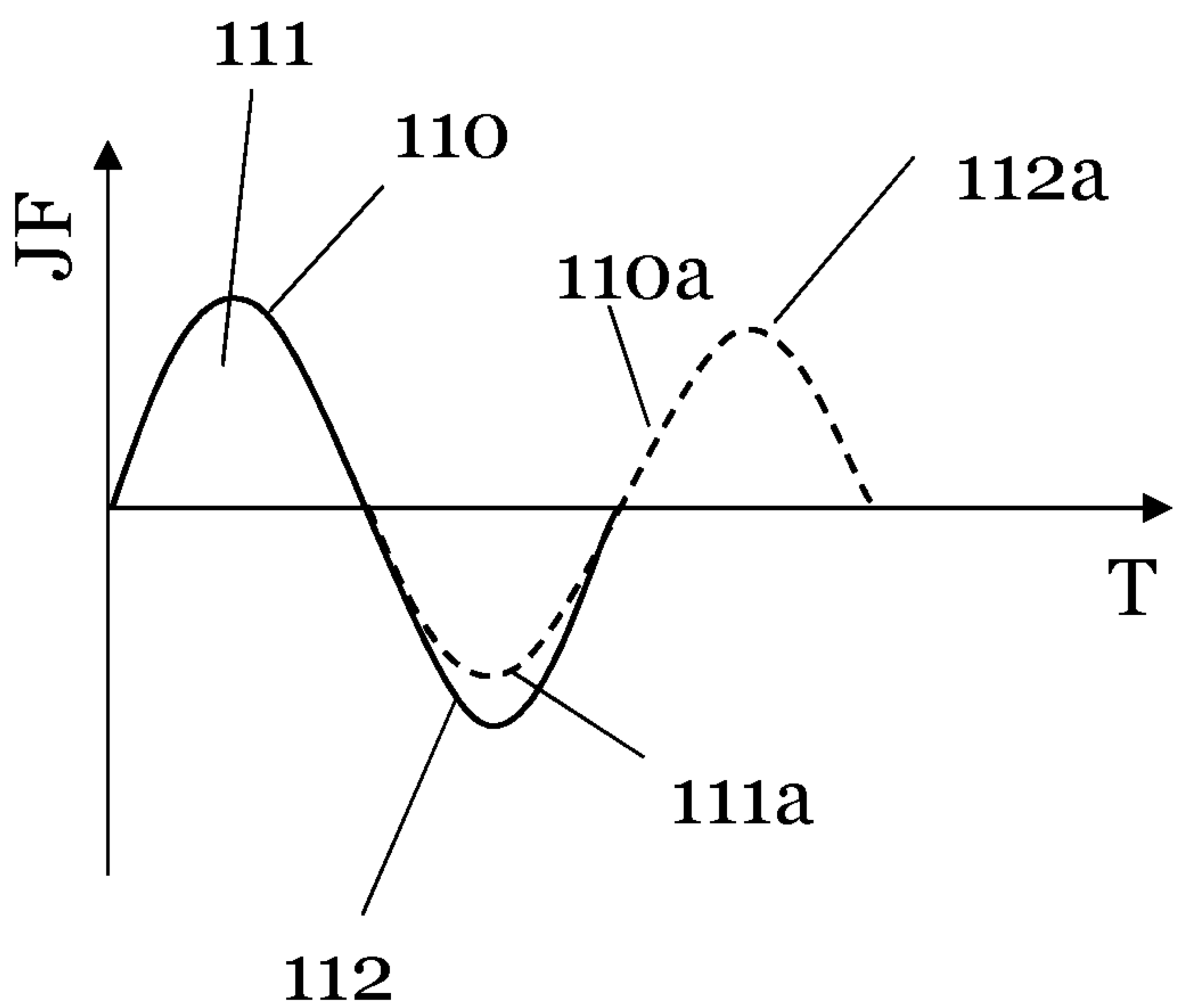

1901
JF
111
110
T
112
1902
JF
111
110
110a
111a
T
112
112a

FIG 19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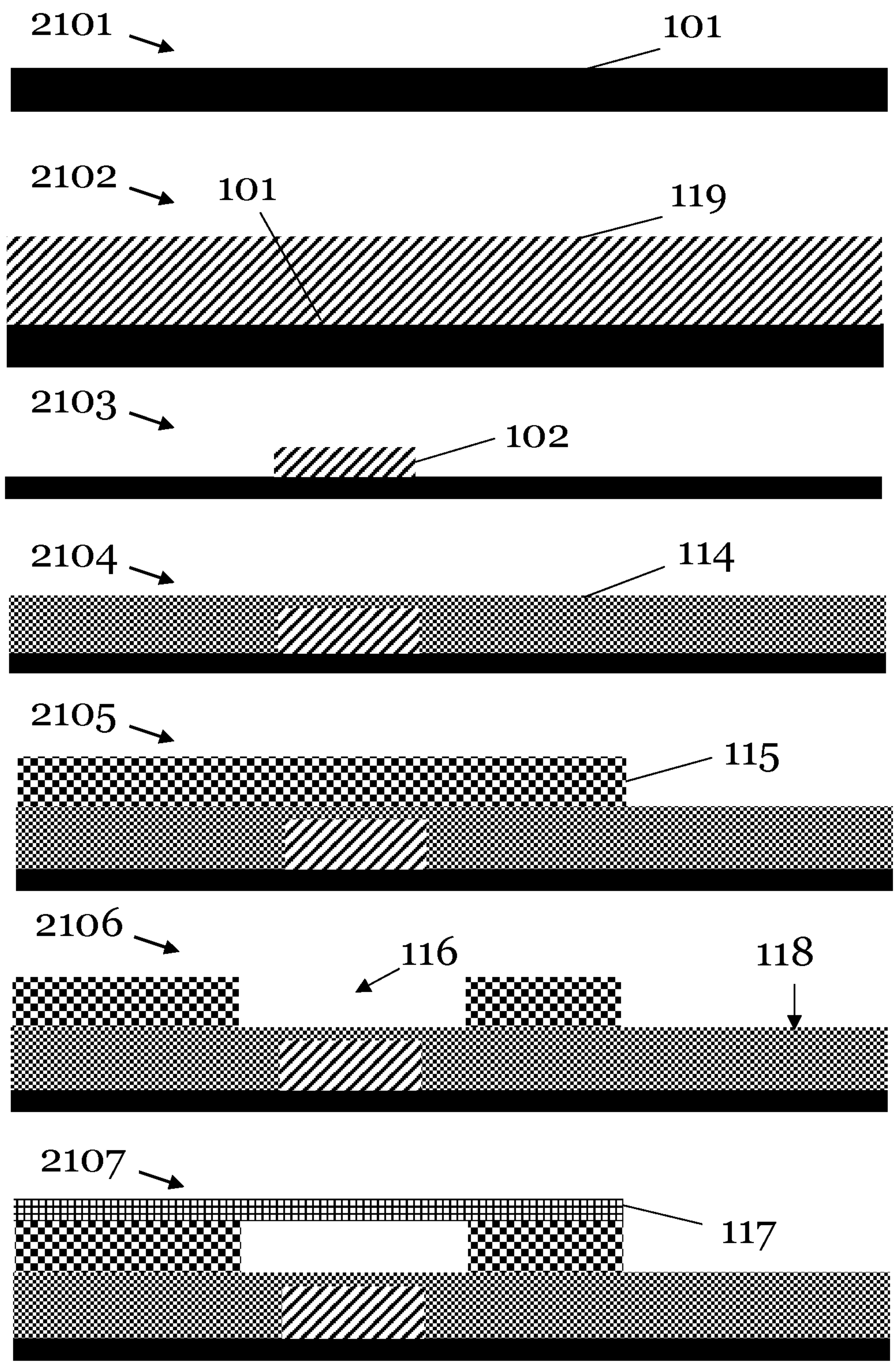
111a
112

FIG 20


2101
101
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101
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2103
102
2104
114
2105
115
2106
116
118
2107
117

FIG 21

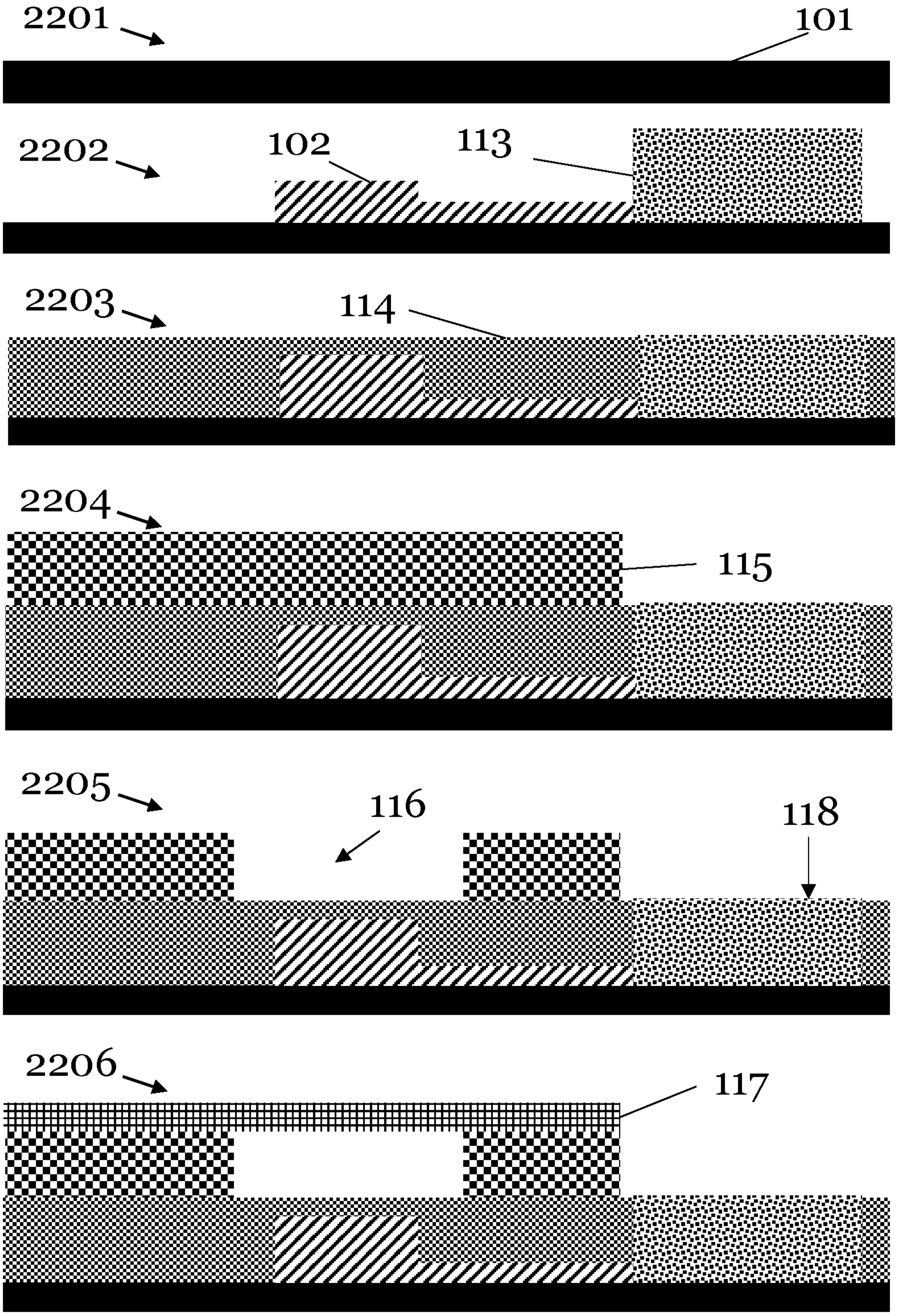

FIG 22

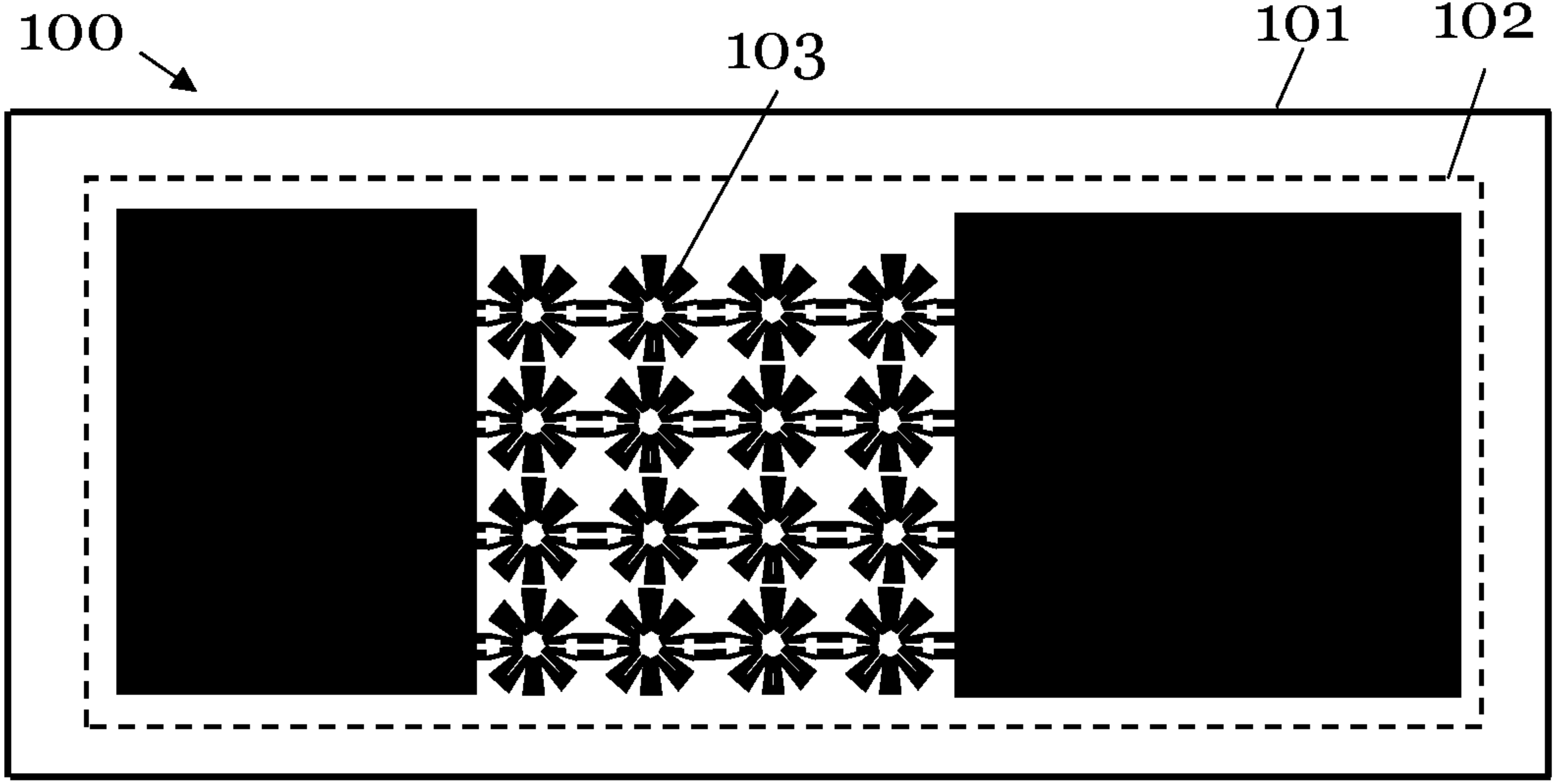

FIG 1