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Koser

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(54) **BACKGROUND DEFOCUSING AND CLEARING IN FERROFLUID-BASED CAPTURE ASSAYS**

(71) Applicant: **ANCERA, LLC.**, Branford, CT (US)

(72) Inventor: **Hur Koser**, Wallingford, CT (US)

(73) Assignee: **ANCERA, LLC**, Branford, CT (US)

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B03C 1/023 (2006.01)
B03C 1/32 (2006.01)

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See application file for complete search history.

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Primary Examiner — Michael A McCullogh
Assistant Examiner — Kalyanavenkateshware Kumar
(74) *Attorney, Agent, or Firm* — Cooley LLP

(57) **ABSTRACT**

Devices, methods, and systems are provided for extracting particles from a ferrofluid. Such methods may comprise receiving a flow of ferrofluid comprising target particles and background particles and generating a first, focusing magnetic field to focus the target particles towards a capture region. The capture region may capture the target particles and a plurality of background particles. A second, defocusing magnetic field may be configured to remove background particles from the capture region. A detector may be used to detect the target particles bound to the target region.

9 Claims, 10 Drawing Sheets

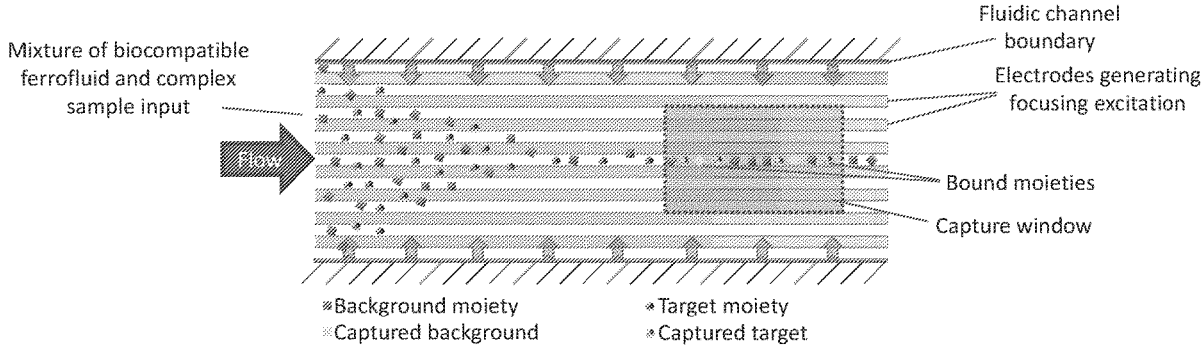

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FIGURE 1

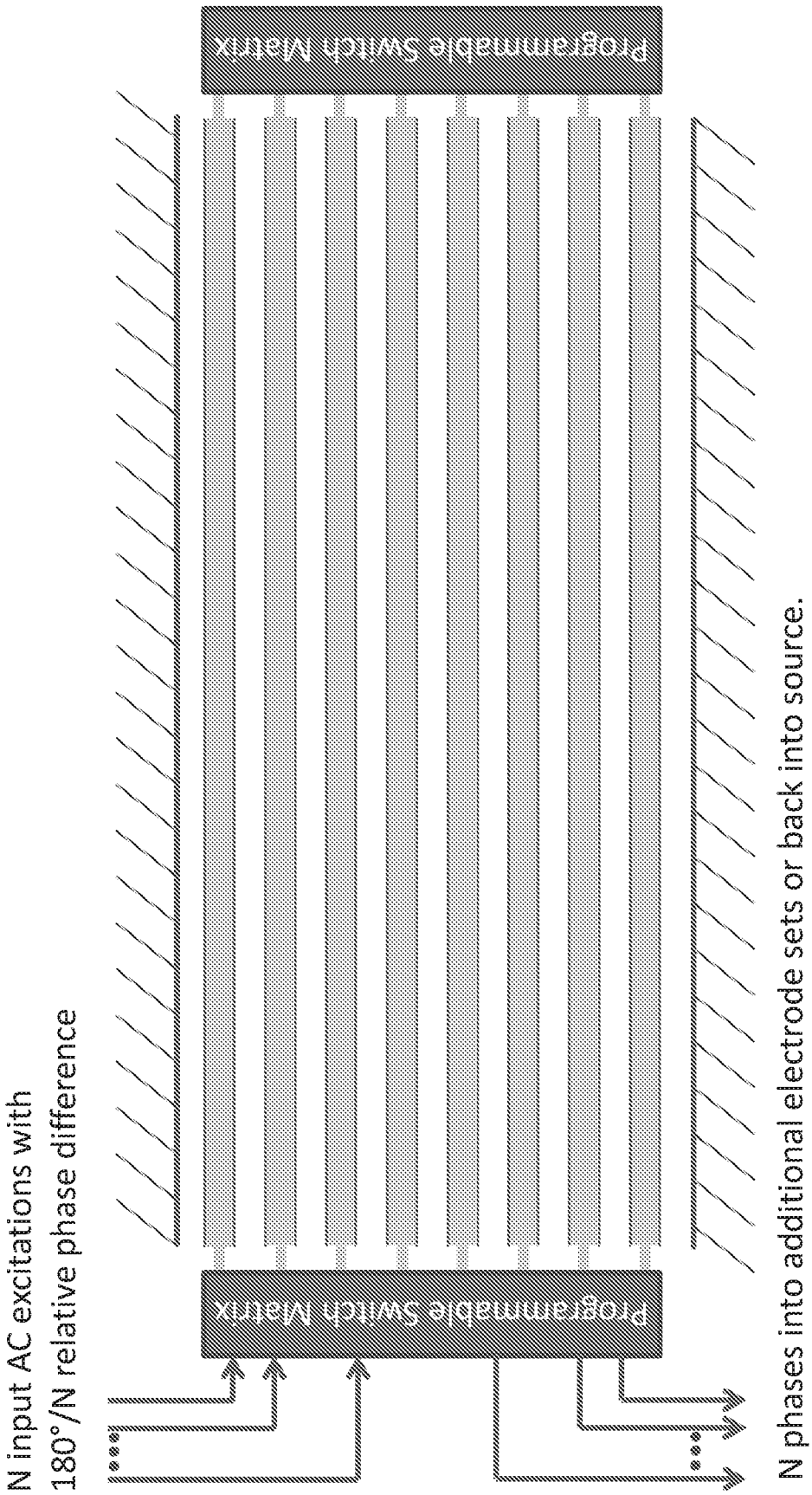

Programmable Switch Matrix
Programmable Switch Matrix
N input AC excitations with
180°/N relative phase difference
N phases into additional electrode sets or back into source.

FIGURE 2

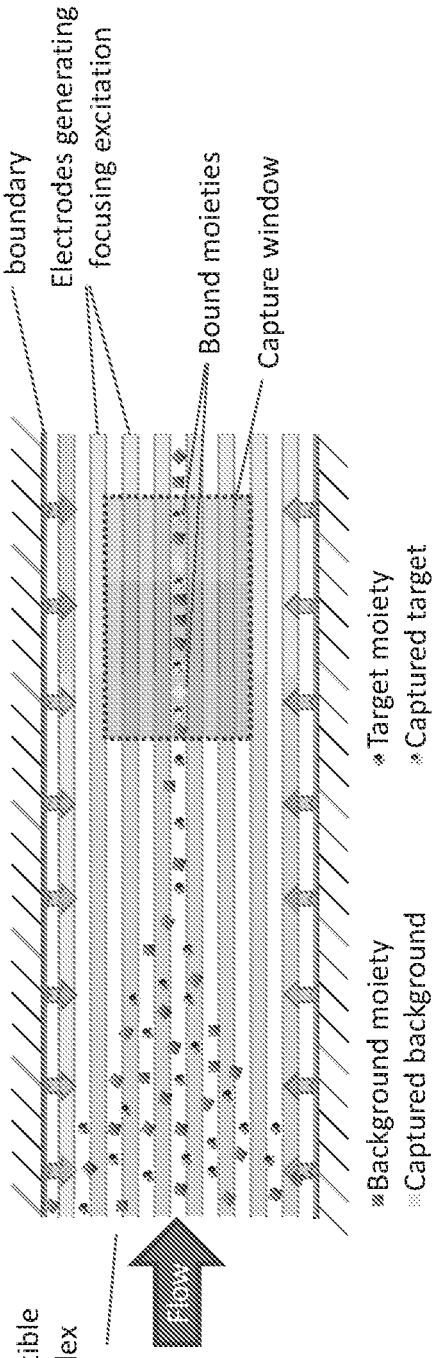

Fluidic channel boundary
Electrodes generating focusing excitation
Bound moieties
Capture window
Target moiety
Captured target
Background moiety
Captured background
Flow
Mixture of biocompatible ferrofluid and complex sample input

FIGURE 3

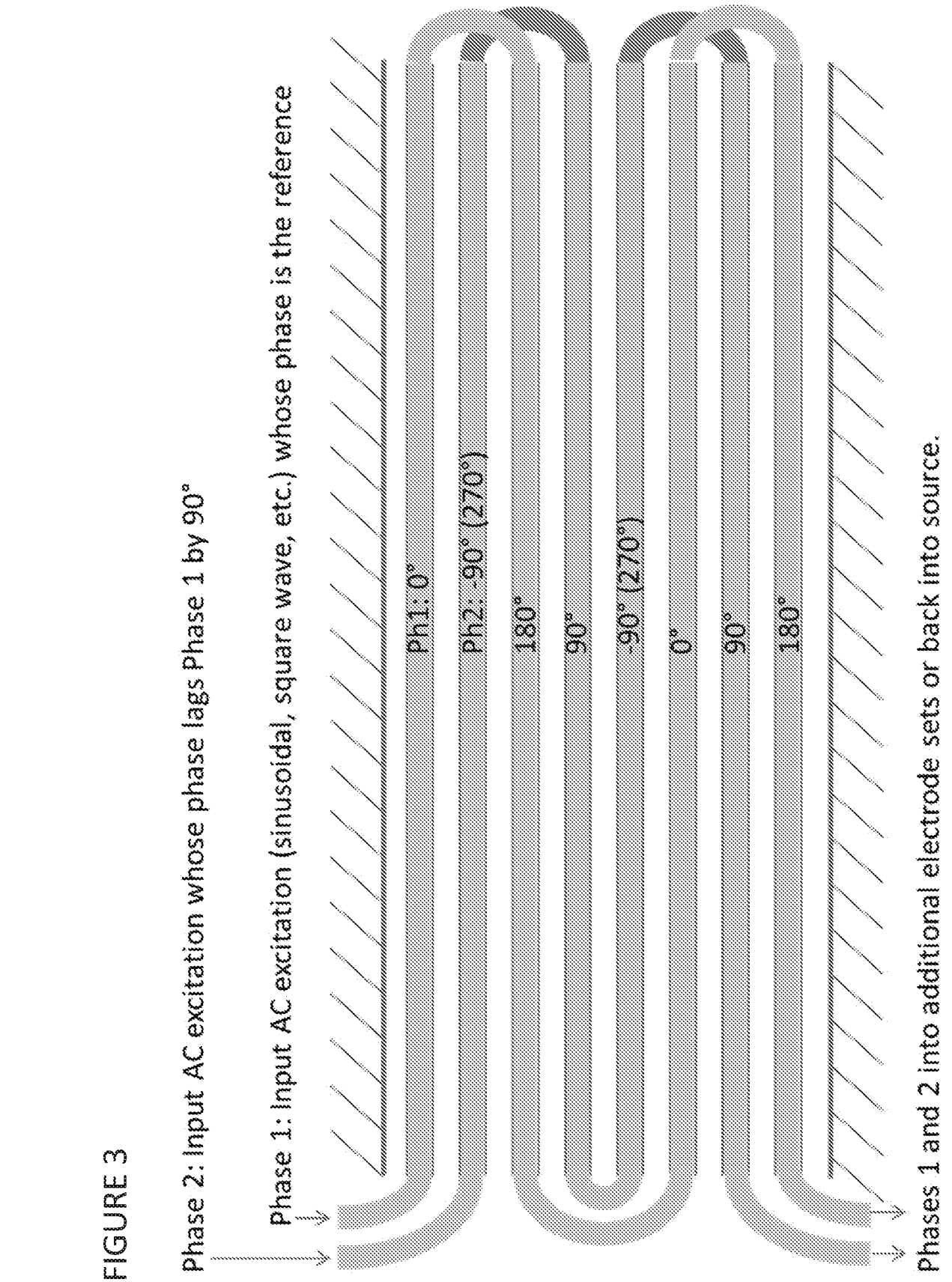

Phase 2: Input AC excitation whose phase lags Phase 1 by 90°
Phase 1: Input AC excitation (sinusoidal, square wave, etc.) whose phase is the reference
Ph1: 0°
Ph2: -90° (270°)
180°
90°
-90° (270°)
0°
90°
180°
Phases 1 and 2 into additional electrode sets or back into source.

FIGURE 4

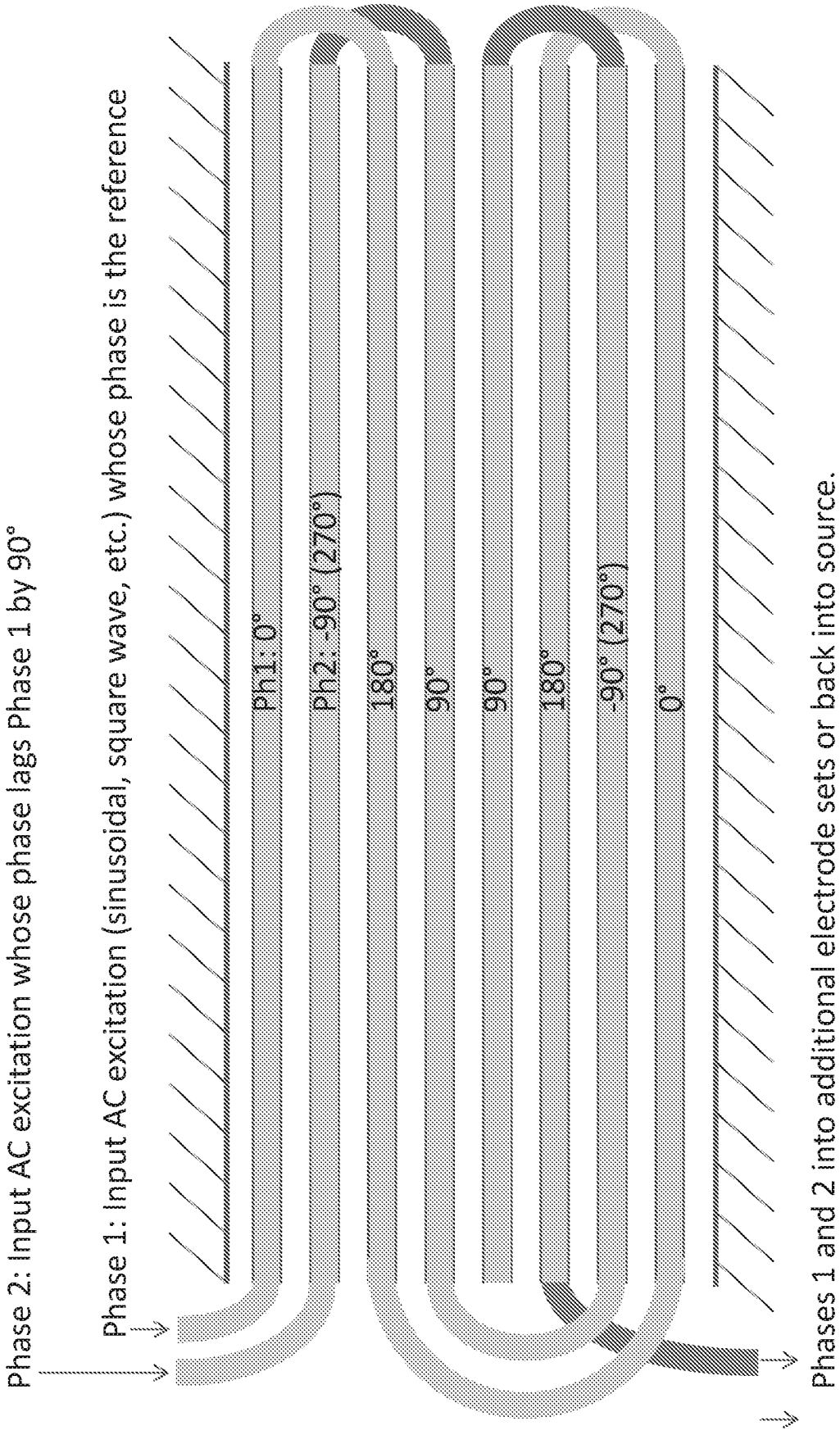
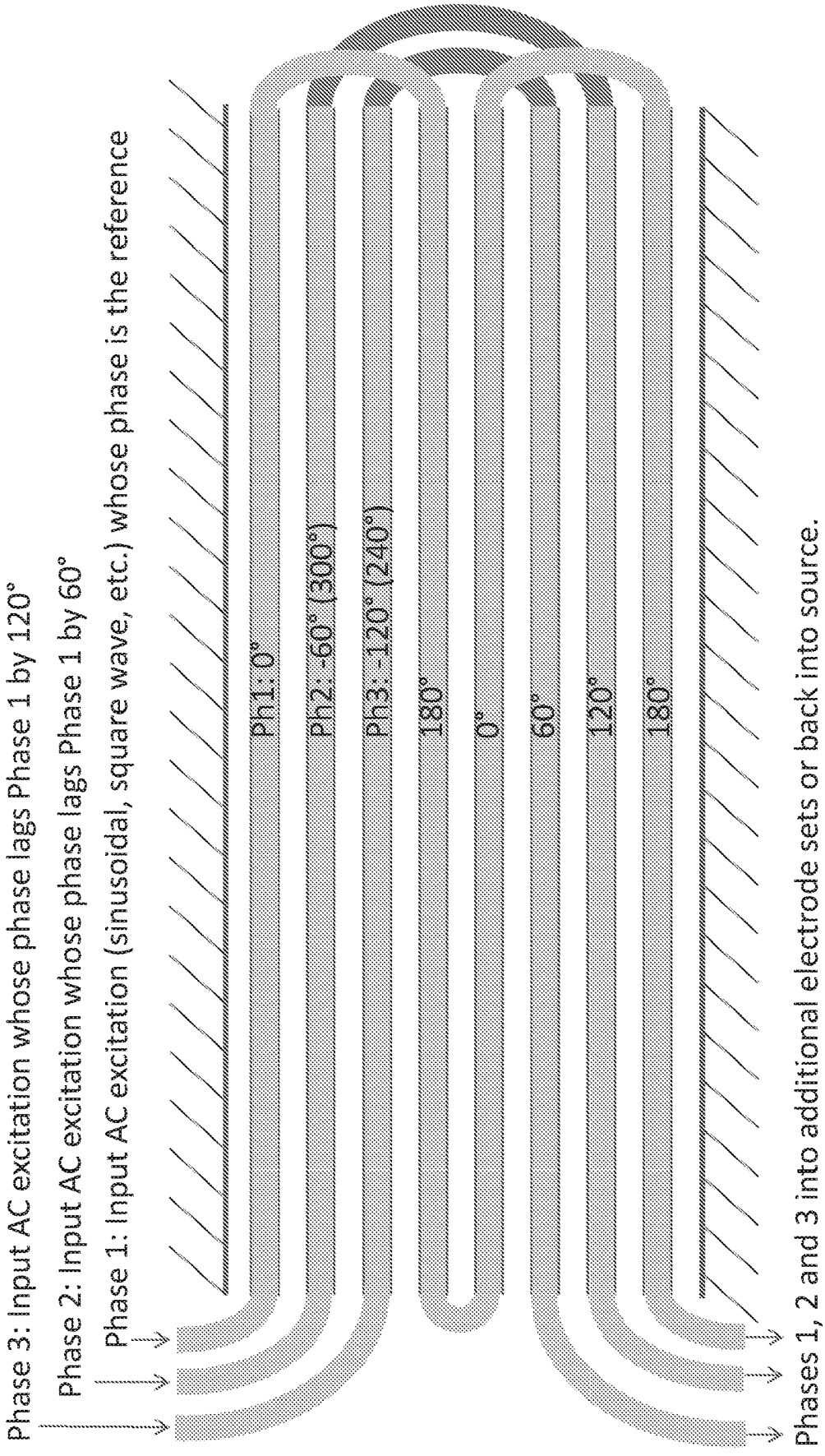

Phase 2: Input AC excitation whose phase lags Phase 1 by 90°
Phase 1: Input AC excitation (sinusoidal, square wave, etc.) whose phase is the reference
Ph1: 0°
Ph2: -90° (270°)
180°
90°
90°
180°
-90° (270°)
0°
Phases 1 and 2 into additional electrode sets or back into source.

FIGURE 5


Phase 3: Input AC excitation whose phase lags Phase 1 by 120°
Phase 2: Input AC excitation whose phase lags Phase 1 by 60°
Phase 1: Input AC excitation (sinusoidal, square wave, etc.) whose phase is the reference
Ph1: 0°
Ph2: -60° (300°)
Ph3: -120° (240°)
180°
0°
60°
120°
180°
Phases 1, 2 and 3 into additional electrode sets or back into source.

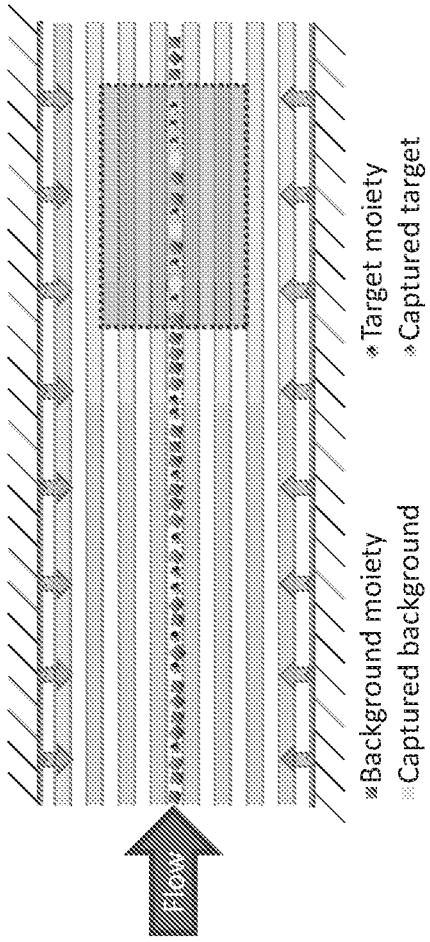

Flow
Background moiety
Captured background
Target moiety
Captured target

FIGURE 6

FIGURE 7

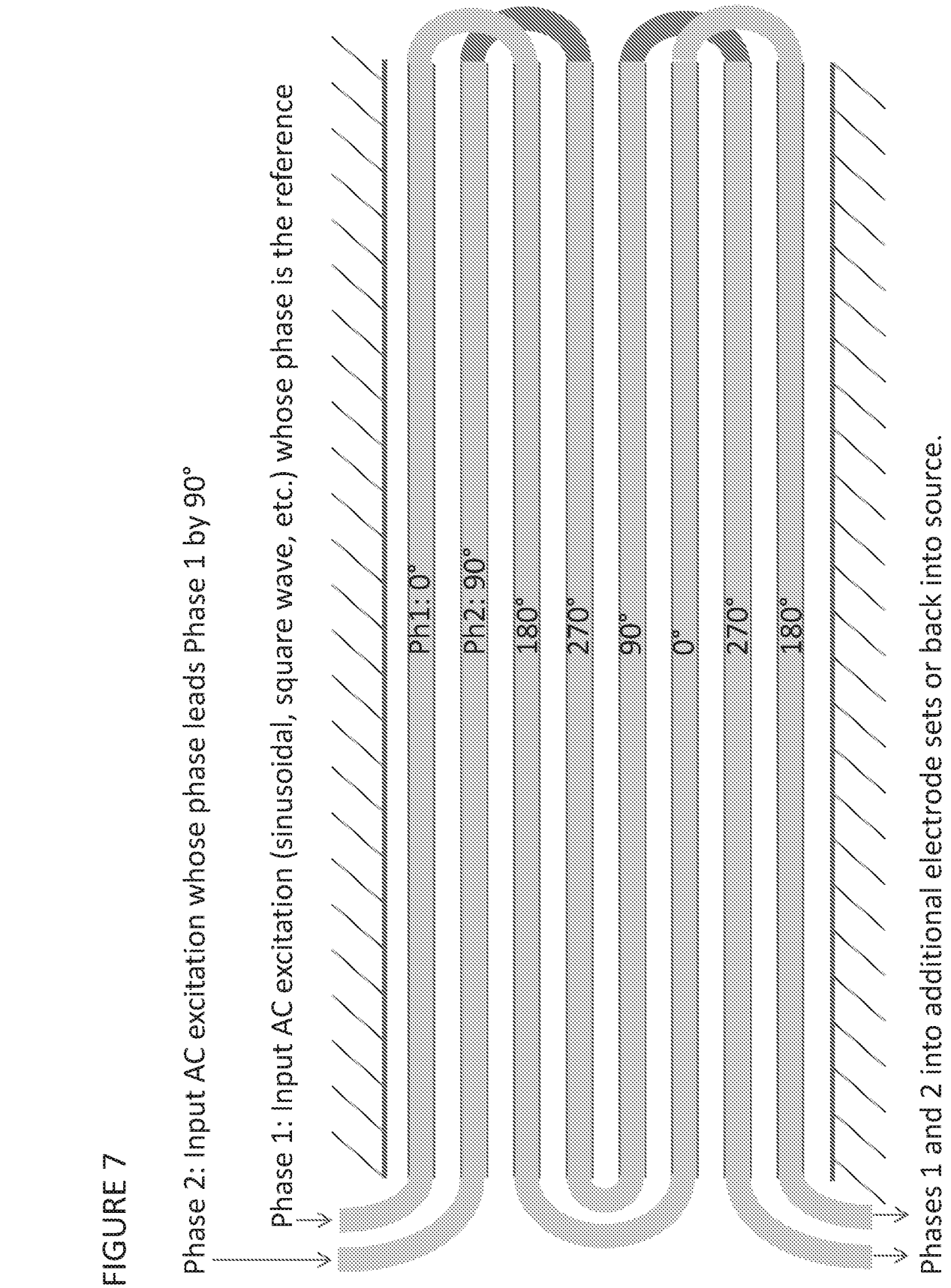

Phase 2: Input AC excitation whose phase leads Phase 1 by 90°
Phase 1: Input AC excitation (sinusoidal, square wave, etc.) whose phase is the reference
Ph1: 0°
Ph2: 90°
180°
270°
90°
0°
270°
180°
Phases 1 and 2 into additional electrode sets or back into source.

FIGURE 8

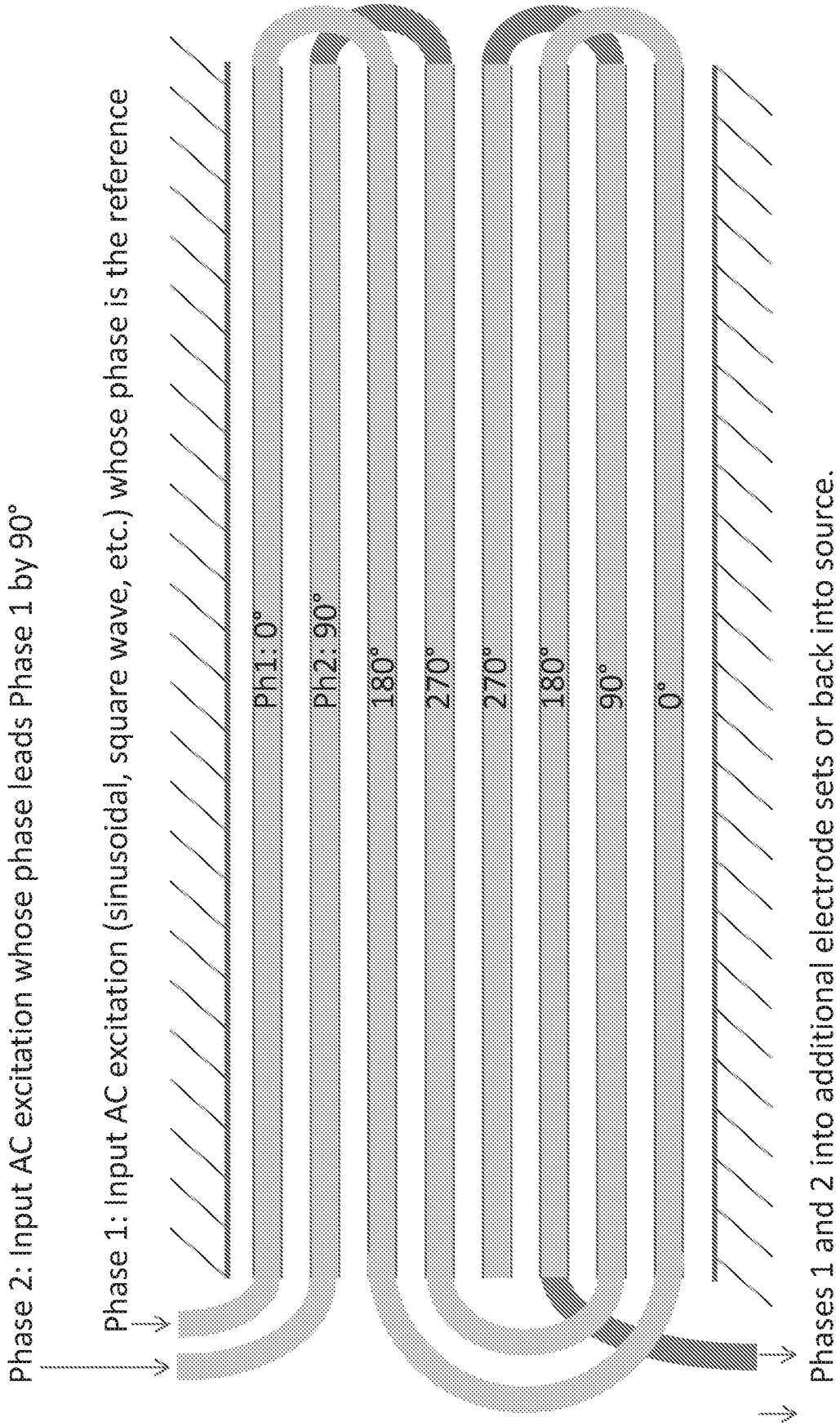

Phase 2: Input AC excitation whose phase leads Phase 1 by 90°
Phase 1: Input AC excitation (sinusoidal, square wave, etc.) whose phase is the reference
Ph1: 0°
Ph2: 90°
180°
270°
270°
180°
90°
0°
Phases 1 and 2 into additional electrode sets or back into source.

FIGURE 9

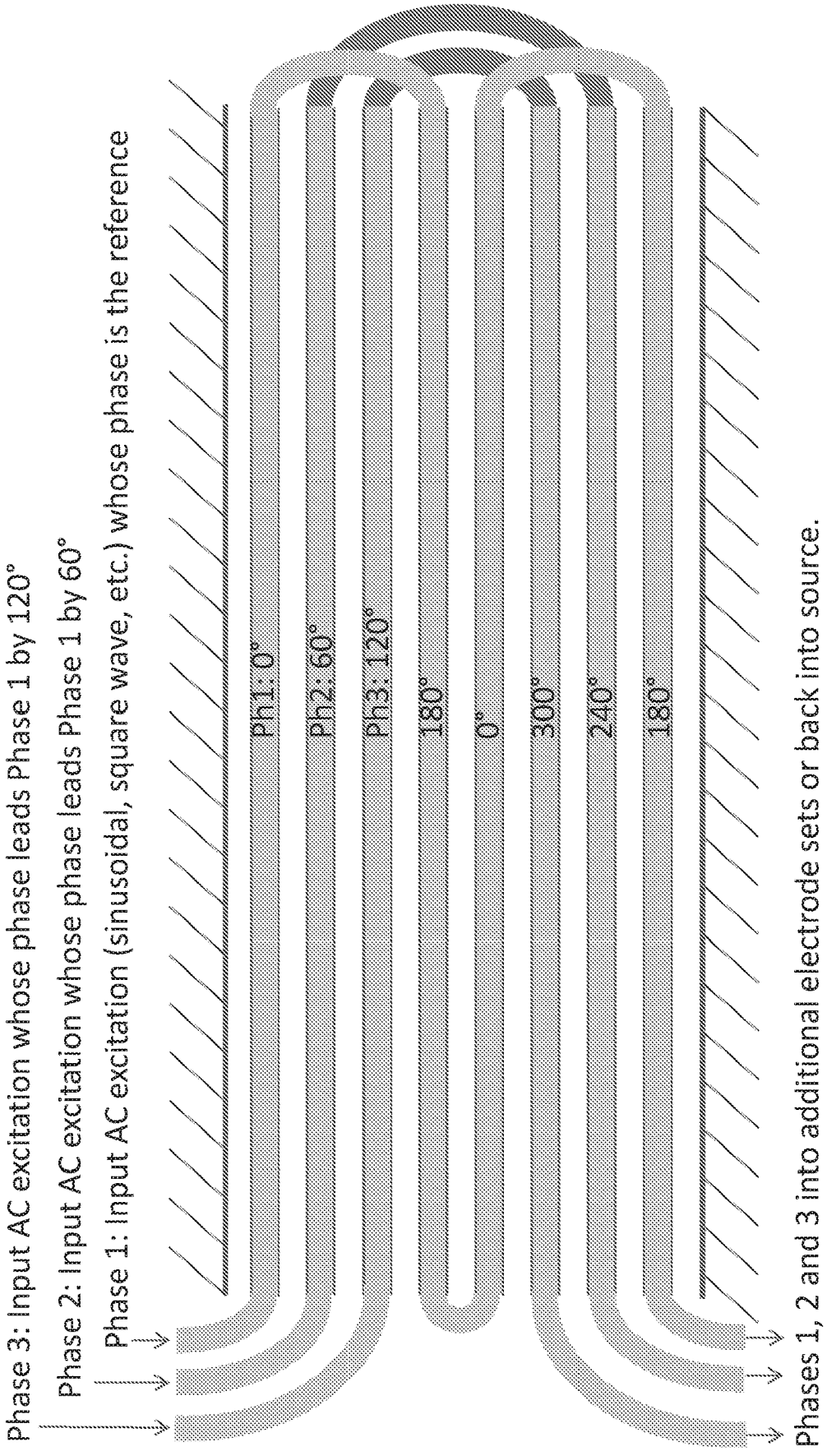

Phase 3: Input AC excitation whose phase leads Phase 1 by 120°
Phase 2: Input AC excitation whose phase leads Phase 1 by 60°
Phase 1: Input AC excitation (sinusoidal, square wave, etc.) whose phase is the reference
Ph1: 0°
Ph2: 60°
Ph3: 120°
180°
0°
300°
240°
180°
Phases 1, 2 and 3 into additional electrode sets or back into source.

FIGURE 10

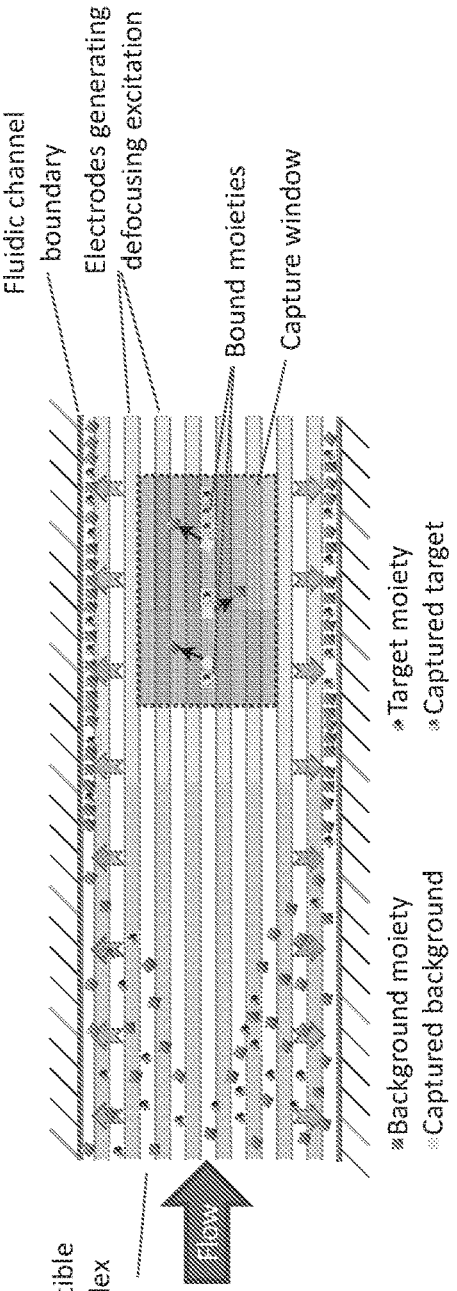
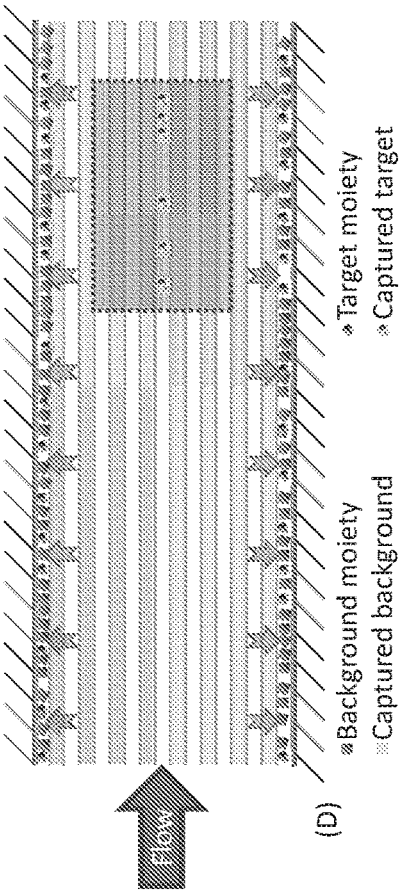


FIGURE 11



BACKGROUND DEFOCUSING AND CLEARING IN FERROFLUID-BASED CAPTURE ASSAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a national stage application of and claims priority to International Patent Application No. PCT/US2016/039394, filed Jun. 24, 2016, and entitled "Background Defocusing and Clearing in Ferrofluid-Based Capture Assays," which in turn claims priority to U.S. Provisional Patent Application No. 62/185,534, filed Jun. 26, 2015, and entitled "Background Defocusing and Clearing in Ferrofluid-Based Capture Assays." The present application incorporates herein by reference the disclosures of each of the above-referenced applications in their entireties.

FIELD OF THE DISCLOSURE

The present disclosure relates to methods and systems for extracting particles from ferrofluids and defocusing background particles from capture regions of assays.

BACKGROUND

WO2011/071912, WO2012/057878, and WO2014/144782 present systems and methods for separating microparticles or cells contained in a ferrofluid medium using magnetic forces. Magnetic field excitations can sort, separate, focus, and even capture cells and other microparticles.

Mechanical exclusion, via well-known filtration is, by its very nature, prone to clogging, and also subsequent increases in pressure drop across the filter as the filter becomes more and more clogged. Such filtration means rely on physically stopping a large enough target particle across a smaller opening on a surface. Additionally, diffusion on traditional assays is slowed by speed limitations. For example, in traditional immunoassays, multiple time-consuming and labor-intensive wash cycles are required between steps.

SUMMARY OF SOME OF THE EMBODIMENTS

Some embodiments of this disclosure present systems, methods and devices which remove background particles from a capture region of an assay.

Some embodiments of the subject disclosure present one or more additional features and/or functionality to methods, systems and devices presented in previous disclosures including, for example, PCT Publication Nos. WO2011/071912, WO2012/057878, and WO2014/144782, all of which are herein incorporated by reference in their entireties.

In some embodiments, methods for extracting target particles contained in a ferrofluid are provided. Such methods may comprise receiving a flow within a microchannel. The flow may comprise a plurality of target particles and background particles in a ferrofluid. A first magnetic field may be generated, and the first magnetic field may be a focusing excitation. At least two sets of electrodes arranged proximate to the microchannel may be used to generate the first magnetic field. The first set of electrodes may generate a first alternating current and the second set of electrodes may generate a second alternating current. The first and second alternating currents may be out of phase by a phase differential. In some embodiments, the focusing excitation may focus the flow of a plurality of target particles to a capture region, and the capture region may be functionalized with capture molecules that can each be configured to bind with a target particle. The capture region may capture a plurality of target particles by binding the target particles with the capture molecules.

In some embodiments, a plurality of unbound particles may also collect in the capture region. A second magnetic field that corresponds to a defocusing excitation may be generated by reversing the phase differential between the first alternating current and the second alternating current. The defocusing excitation may be configured to remove unbound particles from the capture region without removing target particles bound to the capture molecules. A detector may be used to detect the bound target molecules.

In some embodiments, a system for extracting target particles from a ferrofluid is provided and includes a microchannel configured to receive a flow comprising a plurality of target particles and background particles in a ferrofluid, and at least two sets of electrodes arranged proximate the microchannel, the at least two sets of electrodes configured to generate a first magnetic field and a second magnetic field. The first magnetic field corresponds to a focusing excitation and the second magnetic field corresponds to a defocusing excitation. The focusing excitation generated by a first of the at least two sets of electrodes generating a first alternating current and a second of the at least two sets of electrodes generating a second alternating current, where the first alternating current is out of phase with the second alternating current by a phase differential. The defocusing excitation is generated by reversing the phase differential of the focusing excitation. The system also includes a capture region functionalized with a plurality of capture molecules, each capture molecule configured to bind with one target particle type. The focusing excitation focuses the flow of target particles toward the capture region, wherein a plurality of the target particles bind with the capture molecules and a plurality of unbound background particles collect in the capture region, and the defocusing excitation removes the unbound background particles from the capture region without removing the target particles bound to the capture molecules. The system may also include a detector to detect the bound target particles.

In some embodiments, a system for extracting target particles from a ferrofluid is provided and includes a microchannel configured to receive a plurality of target particles and background particles in a ferrofluid, a plurality of electrodes arranged proximate the microchannel, the electrodes configured to generate a first magnetic field and a second magnetic field, wherein the first magnetic field corresponds to a focusing excitation and the second magnetic field corresponds to a defocusing excitation, and a capture region functionalized with a plurality of capture molecules, each capture molecule configured to bind with one target particle type.

In some embodiments, a method for extracting target particles from a ferrofluid is provided and includes receiving a plurality of target particles and background particles in a ferrofluid in a microchannel, generating a first magnetic field corresponding to a focusing excitation from a first set of electrodes, capturing a plurality of target particles in the capture region via the binding of the target particles with the capture molecules, where a plurality of unbound particles collect in the capture region, and generating a second magnetic field corresponding to a defocusing excitation to

remove unbound particles from the capture region without removing target particles bound to the capture molecules.

BRIEF DESCRIPTION OF SOME OF THE EMBODIMENTS

FIG. **1** is an illustration depicting structures of a fluidic channel and associated structures, including programmable switch matrices and electrodes, according to some embodiments.

FIG. **2** is an illustration depicting structures of a fluidic channel and associated structures containing a ferrofluid and a mixture of microparticles during a focusing excitation, according to some embodiments.

FIG. **3** is an illustration depicting structures of a fluidic channel and associated structures, including sets of electrodes and exemplary switch configurations, according to some embodiments.

FIG. **4** is an illustration depicting structures of a fluidic channel and associated structures, including sets of electrodes and exemplary switch configurations, according to some embodiments.

FIG. **5** is an illustration depicting structures of a fluidic channel and associated structures, including sets of electrodes and exemplary switch configurations, according to some embodiments.

FIG. **6** is an illustration depicting structures of a fluidic channel and associated structures containing a ferrofluid and a mixture of microparticles in a steady state during a focusing excitation, according to some embodiments.

FIG. **7** is an illustration depicting structures of a fluidic channel and associated structures, including sets of electrodes and exemplary switch configurations, according to some embodiments.

FIG. **8** is an illustration depicting structures of a fluidic channel and associated structures, including sets of electrodes and exemplary switch configurations, according to some embodiments.

FIG. **9** is an illustration depicting structures of a fluidic channel and associated structures, including sets of electrodes and exemplary switch configurations, according to some embodiments.

FIG. **10** is an illustration depicting structures of a fluidic channel and associated structures containing a ferrofluid and a mixture of microparticles during a defocusing excitation, according to some embodiments.

FIG. **11** is an illustration depicting structures of a fluidic channel and associated structures containing a ferrofluid and a mixture of microparticles in a steady state during a defocusing excitation, according to some embodiments.

DETAILED DESCRIPTION OF SOME OF THE EMBODIMENTS

In some embodiments, a fluidic channel may have multiple electrodes proximate thereto. A flow containing target and background particles may be introduced into the channel, and a capture region (also referred to herein as a "capture window") may be situated within the channel to capture the target particles contained in the flow. The multiple electrodes may be used to generate a magnetic field that focuses and defocuses the particles contained within the flow. Focused particles may form a condensed stream of particles, whereas defocused particles may move towards the side walls of the channel.

The electrodes may be spaced from each other by any amount of separation distance provided that contemporary technological and manufacturing capabilities allow the spacing of the electrodes by such separation distances. For example, the electrode separation distance maybe as small as manufacturing tolerances would allow (e.g., about 50 microns). Similarly, the separation distance may be as large as possible without negatively affecting the performance of the fluidic channel, i.e., while avoiding inefficiencies that accompany large electrode separations, such inefficiencies including fewer electrodes to generate the magnetic field for each unit area, diminished focusing and defocusing abilities (e.g., particles may collect along the surface of the fluidic channel (between the electrodes) instead of moving laterally across the electrodes), etc. As an example, the large electrode separation may be about 500 microns apart. As such, in some embodiments, the electrode separation distance may range from about 50 microns to about 500 microns, from about 100 microns to about 400 microns, from about 200 microns to about 300 microns, about 250 microns, and/or the like. In some embodiments, the separation distance may be less than about 50 microns. In some embodiments, the separation distance may be larger than about 500 microns. The separation distance may be a conveniently defined parameter to characterize the separation between electrodes. For example, for electrodes that are shaped as rectangular strips and aligned in a parallel configuration, the separation distance may be the distance between the closest longitudinal edges of neighboring electrodes. In some embodiments, the separation distance may not be constant, i.e., it may be changing, along the length of the fluidic device.

In some embodiments, the electrodes may be configured to form sets of electrodes, and the spacing between the sets of the electrodes may be determined by spacing of parallel flow channels in a disposable cartridge. The sets of electrodes may be programmable to generate one or more magnetic fields. In some embodiments, any number of sets of electrodes may be used where a set of electrodes can generate alternating current that may be out of phase with respect to alternating current generated by another set of electrodes. In some embodiments, these sets of electrodes may be configured to receive alternating current. For example, in some embodiments, two sets of electrodes may be used. A first set of electrodes can generate a first alternating current, and a second set of electrodes can generate a second alternating current that is out of phase with the first alternating current. In some embodiments, the first set of electrodes can receive a first alternating current and the second set of electrodes can receive a second alternating current. The sets of electrodes may be configured on printed circuit boards. The sets of electrodes may be parallel electrodes. The electrodes may be configured to generate the excitations.

In some embodiments, the set of electrodes may be configured in a variety of configurations. For example, the set of electrodes may be at least substantially parallel to each other or have major longitudinal axes that align with each other along the length of the fluidic channel. Further, the electrodes may have any shape, ranging from a rectangular strip to a completely irregular shape (albeit with a major axis running along and/or substantially parallel to the length of the fluidic channel). The width of the electrodes may also vary along the length of the fluidic channel. In some embodiments, the width may be substantially constant (for example, electrodes shaped as regular rectangular strips). The width of the electrodes may range from about 50 microns to about 1000 microns, from about 100 microns to about 800 microns, from about 200 microns to about 600 microns, from about 300 microns to about 500 microns,

from about 350 microns to about 450 microns, about several mms (e.g., 2 mm, 3 mm, 4 mm, 5 mm, etc.), and/or the like.

In some embodiments, the configuration of the electrodes (e.g., shape, electrode separation distance, size etc.) may be selected so as to facilitate the focusing and defocusing of particles in fluids in the fluidic channel. The fluids such as ferrofluids may contain or be configured to receive samples (e.g., cells, particles (e.g., microbeads), etc.) for focusing, defocusing, capturing, etc., along the fluidic channel. The configurations of the electrodes such as the separation distance between electrodes, the size (e.g., length, width, etc.) and shape of the electrodes, the number of electrodes in an electrode set and/or the fluidic channel, etc., may depend on the properties of the fluid and the sample cells or particles to be captured, such properties including shape, size, elasticity, density, etc., of the cells or particles, viscosity of the ferrofluid containing the sample, etc. Such configurations may be programmable.

FIG. **1** shows an exemplary configuration, wherein AC excitations are inputted with a relative phase difference. In some embodiments, the relative phase difference may be about +/−180°/n, where n is the number of sets of electrodes being used. Thus, for example, if two sets of electrodes are used, the relative phase difference would be about +/−ninety degrees (+/−90°), and if three sets of electrodes are used, the relative phase difference would be about +/−sixty degrees (+/−60°). In some embodiments the AC excitations may be periodic or substantially periodic excitations. For example, the excitations may be sinusoidal waves, square waves, rectangular waves, triangular waves, sawtooth waves, pulse waves, arbitrary periodic waves, and/or the like.

A programmable switch matrix may be used to control which electrodes are connected to form each set of electrodes at either side of the channel. As a result, the electrode configuration may be reconfigurable using the programmable switch matrices on either end of the electrodes. For example, a user may be able to enter a number of sets of electrodes and/or a configuration of the sets of electrodes into a programmable switch matrix. In some embodiments, the user may enter the number of sets of electrodes (s)he would like to use for a particular run, and the programmable switch matrix may determine an optimal configuration of the electrodes and may connect the electrodes according to the optimal configuration. In another embodiment, the user may enter a particular configuration and/or the number of sets of electrodes, and the programmable switch matrix will configure the connectors to connect the electrodes as instructed by the user. The configuration of the connectors that connect the electrodes may be controlled electronically or through software. The connectors may be reconfigured for each application, and in some embodiments, the configuration may be changed during the course of a focusing and/or defocusing.

After the AC excitations pass through the set(s) of electrodes, the output excitations may be inputted into additional electrode sets, may go back to the source, and/or may go to another output mechanism. For example, in some embodiments, multiple sets of electrodes could be used for multiple fluidic channels that are arranged in parallel or in series.

In an example with two sets of electrodes, the first alternating current and second alternating current may be out of phase by about +/−ninety degrees (+/−90°). A focusing excitation may be created by about a −90° phase difference (e.g., where the phase of the second alternating current lags the phase of the first alternating current by about 90°), while a defocusing excitation may be created by a about +90° phase difference (where the phase of the second alternating current leads the phase of the first alternating current by about 90°). In other embodiments, a different number of sets of electrodes (n) may be used, and the alternating currents may be out of phase by about +/−180/n degrees. For example, if there are three sets of electrodes, and the first alternating current, second alternating current, and third alternating current may be out of phase by about +/−sixty) (+/−60° degrees, and so on. In some embodiments, non-optimal phase differences may be used. A non-optimal phase difference may occur when the currents are out of phase by an amount other than about +/−180°/n.

When sets of electrodes are excited simultaneously, a traveling magnetic field may be created. The traveling magnetic field may spin particles flowing through the channel in a particular direction, which may focus or defocus the particles. In some embodiments, an ideal phase differential (about +/−180/n) may produce a high-intensity focusing or defocusing of the particles, while a non-optimal phase difference may modulate the intensity of the focusing or defocusing of the particles. In some embodiments, particle rotation may be maximized at ideal phase differences. In some embodiments, a non-optimal phase difference may be used to control the relative speed of particle rotation with respect to particle translation due to the magnetic forces. Non-optimal phase differences may also allow for size-based, shape-based, and/or elasticity-based separation of particles. In some embodiments, this separation may be achieved by changing excitation frequency, however this may also occur without changing the excitation frequency. In some embodiments, the focusing and defocusing of cells or particles can also be controlled by controlling the amplitude and/or the on/off duration of the AC waveform. For example, the magnetic field coupled to the flow channels can be varied by controlling the amplitude of the AC input waveform (e.g., the periodic or substantially periodic AC input) and/or modulating its on/off duration (i.e., a generalized pulse width modulation scheme), thereby affecting the focusing/defocusing of the cells/particles.

As shown in FIG. **2**, a flow may enter the channel, and the electrodes may generate a focusing excitation. The flow may comprise or be configured to receive both target particles/cells and background particles/cells suspended in biocompatible ferrofluid; one possible example of such flow includes rare circulating tumor cells in a large background of various different blood cells. In some embodiments, the flow may comprise a mixture of biocompatible ferrofluid and complex sample; one possible example of such flow consists of target bacterial cells in a complex food matrix. In some embodiments, the target particles may be a collection of microbeads functionalized with different ligands and suspended in a biocompatible ferrofluid; such embodiments would be able to run multiplex bead-based assays within the same flow by clearing from the capture region any beads that have not specifically bound their target antigen or cell.

As explained above, in some embodiments, the focusing excitation may be created by multiple sets of electrodes, such as two sets of electrodes having currents that are out of phase by about −90°. FIG. **3** shows a sample embodiment of the configuration of an exemplary focusing configuration with two sets of electrodes. In some embodiments, electrodes may extend the length of the channel. The electrodes may be connected in a specific configuration, or the configuration may be programmable. The connection of the electrodes may connect the individual electrodes to form the sets of electrodes. Thus, a current applied to a first electrode may travel through the first electrode and through the connector and back along another electrode. In some

embodiments, such as the embodiment shown in FIG. **3**, multiple electrodes and connectors are used to form each set of electrodes; here, there are four electrodes and three connectors used to form each set of electrodes.

In some embodiments, the electrodes and/or the connectors may be configured on separate connection layers such that the electrodes and/or connectors in one set do not touch electrodes and/or connectors of another set. In some embodiments, the connectors can be outside the plane of the electrodes. In embodiments where the electrodes are on printed circuit boards, the connectors may be wire bonds, and/or passive or active elements bonded externally to contact pads on the printed circuit board.

In some embodiments, a multi-level printed circuit board may be used, and the connectors may be internal traces on lower electrode layers on a multi-level printed circuit board. In such an embodiment, the internal electrode layers may also support additional sets of electrodes. This may allow for an augmented magnetic field to be generated when compared to the magnetic field generated by one layer of electrodes.

A first AC input excitation is inputted into and/or generated by a first set of electrodes. This first AC input may be a periodic or substantially periodic excitation such as but not limited to sinusoidal wave, a square wave, or a similar excitation. The phase of the first AC input in the first set of electrodes serves as the reference phase. A second AC input excitation is sent into a second set of electrodes. The phase of the second AC input excitation may be offset from the phase of the first AC excitation by about −90°. Thus, the phase of the second AC input excitation may lag the phase of the first AC excitation by about 90°, is a focusing excitation which results in the focusing of the particles.

As shown in FIG. **3**, Phase 1, which serves as the reference phase, may be referred to as a phase offset of about 0°. Because Phase 2 lags Phase 1 by about 90° in this embodiment, Phase 2 is shown as about −90°, which is also equivalent to about 270°. When the excitations loop back along the length of the channel through another electrode, the phase of Phase 1 becomes about 180°, while the phase of Phase 2 becomes about 90°. In some embodiments, the electrodes may loop down the side of the channel one or more additional times. For example, in the embodiment shown, the excitations may pass through four electrodes and three connectors. FIG. **4** shows an alternative embodiment with two sets of electrodes in a focusing configuration.

FIG. **5** shows an embodiment with three sets of electrodes in a focusing configuration. Here, the phase difference between the phase of the AC excitation in the first set of electrodes (about 0°) lags the phase of Phase 2 in the second set of electrodes by about 60° and Phase 3 in the third set of electrodes by about 120°.

When the focusing excitation is applied, the particles may be focused towards the center of the microchannel, as shown in FIG. **2**. In some embodiments, the focusing excitation may create a traveling magnetic field that may cause the particles to rotate in a particular direction. This rotation of the particles may result in particles that are focused into a concentrated stream in the flow within the channel. FIG. **6** shows the channel in a steady state wherein the focusing excitation is applied and the particles are concentrated into a stream. In some embodiments, such as those depicted in FIGS. **2** and **6**, the particles may be tightly focused (e.g., to the center of the channel). In some embodiments, the focusing may be partial where some particles may be focused into a streamlined flow while others may be traveling through the channel in a diffuse manner. In any case, the capturing of some or all of the focused as well as the partially focused particles may be accomplished over the capture window. In some embodiments, the electrodes and their associated properties (size, shape, electrode separation, etc.), the AC excitations (e.g., amplitude, periodicity, on/off duration, etc.), etc., may be selected so as to control the amount of focusing (e.g., streamlined or merely diffuse but within the capture window, etc.) of the particles in the flow to facilitate the capturing of the particles over the capture window.

The focused stream of FIG. **2** and/or FIG. **6** may travel towards a capture window. The capture window may be part of a fluidic device, which, in some embodiments, may be a disposable cartridge. The capture region may have capture molecules configured to bind with the target particles. In some embodiments, the capture molecules may specifically bind with target particles. While some background particles may pass through the capture window, the capture window may immobilize at least some background particles. These immobilized particles may not specifically bind with the capture molecules in the capture region.

In some embodiments, a defocusing excitation may be applied to the channel, such as by changing the phase differential between the alternating currents. In some embodiments, the phase differential for the defocusing excitation may be determined by inverting the phase differential used for the focusing excitation. For example, two sets of electrodes may generate a defocusing excitation by reversing the phase differential used in the focusing excitation, such as two sets of electrodes having currents that are out of phase by about +90°.

FIG. **7** shows an exemplary embodiment with two sets of electrodes. This defocusing excitation is configured similarly as compared to the focusing excitation shown in FIG. **3**, but here Phase 2 leads Phase 1 by about 90°. Phase 1, which has input AC excitation comprising a periodic or substantially periodic excitation such as sinusoidal excitation, square wave excitation, and/or other similar excitation, serves as the reference phase (0°), and Phase 2, the phase of the second AC excitation, is offset by about +90°. This phase difference may be a defocusing excitation that results in the defocusing of the particles.

As shown in FIG. **7**, Phase 1, the reference phase, has on offset of about 0°. Phase 2, which leads Phase 1 by about 90°, is therefore about +90°. When the excitations loop back along the length of the channel through a second electrode, the phase of Phase 1 becomes about 180°, while the phase of Phase 2 is about 270°. The excitations may loop back down the length of the channel one or more additional times. For example, in the embodiment shown in FIG. **7**, the excitations may travel through four electrodes and three connectors. FIG. **8** shows an alternative embodiment of the defocusing configuration of the electrodes in another embodiment with two sets of electrodes.

FIG. **9** shows an embodiment with three sets of electrodes in a defocusing configuration. As explained above, the defocusing configuration may be generated using multiple ("n") sets of electrodes with alternating currents out of phase by about +180°/n, such that the phase of the second and third sets of electrodes lead the first set of electrodes. Thus, an ideal configuration for a three-electrode defocusing embodiment may be a about +60° phase differential between the first and second sets of electrodes and a about +60° phase differential between the second and third sets of electrodes. Here, the phase difference between Phase 1, the phase of the AC excitation in the first set of electrodes (about 0°) leads the phase of Phase 2 in the second set of electrodes by about

60° and Phase 3 in the third set of electrodes by about 120°. As shown, the first set of electrodes may be configured to traverse the length of the channel four times, and the second and third set of electrodes may traverse the length of the channel twice. This creates a about 60° phase differential between Phase 1 and Phase 2, Phase 2 and Phase 3, and Phase 3 and Phase 1 in the second electrode as the current traverses the opposite direction along the length of the channel. A similar about 60° differential is created between the third traversal of Phase 2, the second traversal of Phase 2 and Phase 3, and the fourth traversal of Phase 1.

As shown in FIG. **10**, the defocusing excitation may change the direction of the spin of the particles, resulting in the particles moving towards the side walls of the channel. In some embodiments, the defocusing excitation may stop movement of the particles toward the capture window. The defocusing excitation may remove the immobilized background particles from the capture window. Background particles may not be specifically bound to the capture molecules, and may therefore release from the capture window and move and/or spin towards the channel wall. Meanwhile, target particles that are specifically bound to the capture molecules may remain on the capture region.

In FIG. **11**, this process has reached a steady state. At least some of the background particles that were within the capture window may have been displaced to the side wall of the channel, while at least some bound target particles may remain in the capture window. In some embodiments, all background particles may be removed from the capture window, and in some embodiments, a majority or at least a certain percentage of background particles may be removed from the capture window. In some embodiments, all target particles may remain in the capture window, and in some embodiments, a majority of target particles may remain in the capture window.

A detector may be used to determine whether the background particles, or at least some of the background particles, have been removed from the capture region. For example, the detector may determine that the amount of background particles on the capture region is over a threshold percentage or threshold number of background particles. A detector may also be used to determine that at least some target particles, or at least a certain amount (number or percentage) of target particles, have been captured by the capture region. In some embodiments, the detector may be an automated scanning microscope, a sensitive mass balance, an electrochemical sensor and/or the like. A sensitive mass balance may be a quartz crystal mass-balance; an electrochemical sensor may respond to the presence of live cells metabolizing over a surface of the capture region.

In some embodiments, once a capture region is determined to have at least a threshold (number of percentage) of target particles and/or determined to have below a certain threshold (number or percentage) of background particles, the capture region may be removed from the channel. In some embodiments, the removed capture region may be replaced with a new capture window.

In some embodiments, if a capture region is determined not to have at least a threshold of target particles, another focusing excitation may be applied, followed by another defocusing excitation. The detector may perform another test, and this process may continue until the detector senses that a sufficient amount (number or percentage) of target particles have been captured by the capture window.

In some embodiments, if a capture region is determined to have over a certain threshold of background particles, another defocusing excitation may be applied to remove the background particles from the capture window. The detector may perform an additional test, and this process may continue until the detector senses that a sufficient amount of background particles have been removed.

Any and all references to publications or other documents, including but not limited to, patents, patent applications, articles, webpages, books, etc., presented in the present application, are herein incorporated by reference in their entirety.

Example embodiments of the devices, systems and methods have been described herein. As noted elsewhere, these embodiments have been described for illustrative purposes only and are not limiting. Other embodiments are possible and are covered by the disclosure, which will be apparent from the teachings contained herein. Thus, the breadth and scope of the disclosure should not be limited by any of the above-described embodiments but should be defined only in accordance with claims supported by the present disclosure and their equivalents. Moreover, embodiments of the subject disclosure may include methods, systems and devices which may further include any and all elements from any other disclosed methods, systems, and devices, including any and all elements corresponding to target particle separation, focusing/concentration. In other words, elements from one or another disclosed embodiments may be interchangeable with elements from other disclosed embodiments. In addition, one or more features/elements of disclosed embodiments may be removed and still result in patentable subject matter (and thus, resulting in yet more embodiments of the subject disclosure). Correspondingly, some embodiments of the present disclosure may be patentably distinct from one and/or another reference by specifically lacking one or more elements/features. In other words, claims to certain embodiments may contain negative limitation to specifically exclude one or more elements/features resulting in embodiments which are patentably distinct from the prior art which include such features/elements.

What is claimed is:

1. A method for extracting target particles from a ferrofluid, the method comprising:

receiving a flow within a microchannel, the flow comprising a plurality of target particles and background particles in a ferrofluid;

generating a first magnetic field corresponding to a focusing excitation, the first magnetic field generated by at least two sets of electrodes arranged proximate the microchannel,

wherein

a first of the at least two sets of electrodes generates a first alternating current and

a second of the at least two sets of electrodes generates a second alternating current, wherein

the first alternating current is out of phase with the second alternating current by a phase differential;

the focusing excitation is configured to focus the flow of a plurality of target particles to a surface of a capture region, and

the surface of the capture region is functionalized with capture molecules each configured to bind with a target particle;

capturing a plurality of target particles on the surface of the capture region via the binding of the target particles with the capture molecules, wherein a plurality of unbound particles collect in the capture region;

generating a second magnetic field, different from the first magnetic field and after generation of the first magnetic field, the second magnetic field corresponding to a defocusing excitation,

wherein

the second magnetic field is generated by reversing the phase differential between the first alternating current and the second alternating current, and

the defocusing excitation is configured to remove unbound particles from the capture region without removing target particles bound to the capture molecules; and

detecting the bound target particles via a detector.

2. The method of claim **1**, wherein the detector is one of: an automated scanning microscope, a sensitive mass balance, and an electrochemical sensor.

3. The method of claim **1**, wherein the phase differential between the first alternating current and the second alternating current is 90°.

4. The method of claim **3**, wherein the focusing excitation caused by the first magnetic field rotates the particles in a particular direction.

5. The method of claim **4**, wherein the rotation of the particles in the particular direction causes the particles to focus.

6. The method of claim **3**, wherein the reverse phase differential between the first alternating current and the second alternating current is −90°.

7. The method of claim **6**, wherein the defocusing excitation caused by the second magnetic field rotates the particles in a second particular direction, wherein the rotation in the second particular direction causes the particles to defocus.

8. The method of claim **1**, wherein the phase differential is determined using a total number of sets of electrodes used, such that the phase differential is +180 divided by the number of sets of electrodes and the reverse phase differential is −180 divided by the number of sets of electrodes.

9. A method for extracting target particles from a ferrofluid, the method comprising:

receiving a plurality of target particles and background particles in a ferrofluid in a microchannel;

generating a first magnetic field corresponding to a focusing excitation from a first set of electrodes;

capturing a plurality of target particles on a surface of a capture region, the surface of the capture region being functionalized with capture molecules, wherein:

the capture molecules bind with the target particles, and a plurality of unbound particles collect in the capture region;

and

generating a second magnetic field corresponding to a defocusing excitation to remove unbound particles from the capture region without removing target particles bound to the capture molecules.

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