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(54) **Title:** MICROFLUIDIC ENRICHMENT OF SELECTED CELL POPULATIONS

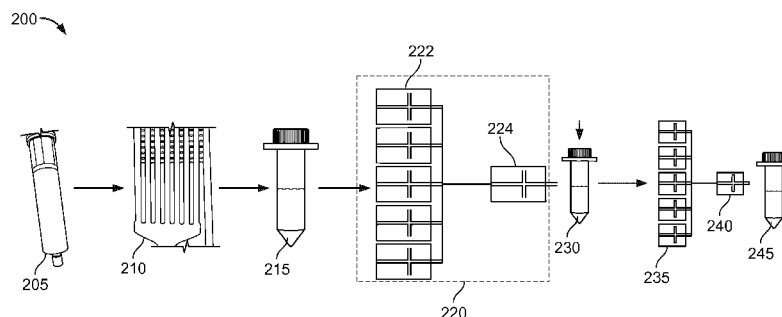


FIG. 2

(57) **Abstract:** Methods, devices, and systems for microfluidic enrichment of selected cell populations are described. Methods of labeling white blood cells include obtaining whole blood drawn from a patient, separating the white blood cells from the whole blood by flowing the whole blood through a microfluidic device that includes one or more arrays of obstacles configured to deflect the white blood cells in a direction away from a remaining components of the whole blood, and collecting the separated white blood cells. A buffer containing a labeling agent with an affinity for white blood cells is mixed with the white blood cells either before or after separating the white blood cells from the whole blood resulting in labeled white blood cells.

MICROFLUIDIC ENRICHMENT OF SELECTED CELL POPULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/318,065, entitled "Microfluidic Enrichment of Selected Cell Populations, filed on March 26, 2010, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD

This document describes technologies related to manipulation of cells, for example, cells obtained from whole blood.

BACKGROUND

Current clinical standard of practice for imaging in-vivo infections involves enrichment of white blood cells from peripheral blood draw, labeling of the enriched leukocyte population with radioactive or fluorogenic dyes, and re-infusion of labeled cells into patient. The dye tagged leukocytes migrate to sites of infection, wherein they can be imaged with PET-scan and other whole body imaging modalities. The autologous dye-tagged leukocytes serve as beacons for localizing infection in-vivo. Imaging of infections in prosthetic joints & vascular grafts, diagnosis of pedal osteomyelitis, detection of inflammatory bowel disease, and detection of indolent infections are some of the varied clinical indications for labeled leukocytes. The current standard of clinical care constitutes sterile withdrawal of 50 ml of peripheral blood into an anticoagulant containing syringe, time based sedimentation through hydroxyethyl starch to deplete red blood cells, washing white blood cells to remove residual plasma, incubating enriched white blood cells with tracer dye, washing the cell suspension repeatedly to remove unbound tracer dye, and measuring an aliquot to ensure labeling performance prior to re-infusion back into the patient.

SUMMARY

The invention relates, at least in part, to an automated, continuous, stand-alone processing unit that resides at point of care and can be used to process blood drawn from

a patient (e.g., into a Vacutainer® or a syringe) through a microfluidic device to provide an output of a labeled population of white cells, with very low carryover of red cells, platelets and plasma for reinfusion back into the patient.

In general, one aspect of the subject matter described herein is methods of
5 labeling white blood cells by obtaining whole blood drawn from a patient, separating the white blood cells from the whole blood by flowing the whole blood through a microfluidic device that includes one or more arrays of obstacles configured to deflect the white blood cells in a direction away from the direction of remaining components of the whole blood, and collecting the separated white blood cells. A buffer containing a
10 labeling agent with an affinity for white blood cells is mixed with the white blood cells either before or after separating the white blood cells from the whole blood resulting in labeled white blood cells.

This, and other aspects, can include one or more of the following features.
Flowing the whole blood through the microfluidic device can include flowing the whole
15 blood through the microfluidic device that includes a first channel extending from one or more inlets to one or more outlets. The first channel can be bounded by a first side wall and a second side wall located opposite from the first side wall. A first array of rows of obstacles can be disposed within the first channel and can be bounded by the first side wall and a first array boundary formed by ends of the rows of obstacles in the first array.
20 A second array of rows of obstacles can be disposed within the first channel and bounded by the second side wall and a second array boundary formed by ends of the rows of obstacles in the second array. A bypass channel can be defined by a region devoid of obstacles between the first array boundary and the second array boundary. The first array and the second array can be configured to deflect the white blood cells toward the bypass
25 channel and separate the white blood cells from remaining components of the whole blood.

The methods can further include infusing the labeled white blood cells into the patient. The labeling agent can include a radioactive material. The collected labeled white blood cells in the buffer can be flowed through a microfluidic concentration device
30 that includes at least one channel at a flow rate that focuses the labeled white blood cells

in the at least one channel into one or more localized stream lines. Each stream line can define a width that is substantially equal to or greater than a size of the focused labeled white blood cells to increase a concentration of the labeled white blood cells in the buffer relative to a concentration of the labeled white blood cells in the buffer collected from the outlet of the bypass channel. The labeling agent can be mixed with the white blood cells before or after the white blood cells are flowed through the microfluidic concentration device.

The microfluidic concentration device can include a second channel that includes an inlet coupled to the outlet of the bypass channel to receive the white blood cells, e.g., labeled white blood cells, in the buffer, a first outlet through which substantially all of the focused white blood cells in the buffer are flowed, and a second outlet and a third outlet through which buffer that is not flowed through the first outlet is flowed. The labeled white blood cells in the buffer can be flowed through the first inlet at the flow rate, and the labeled white blood cells of the higher concentration can be collected from the first outlet.

The microfluidic concentration device can include a spiral channel that includes a first inlet coupled to the outlet of the bypass channel to receive the labeled white blood cells in the buffer, a first outlet through which substantially all of the focused labeled white blood cells in the buffer in the buffer are flowed, and a second outlet through which buffer that is not flowed through the first outlet is flowed. The labeled white blood cells in the buffer can be flowed through the first inlet at the flow rate, and the labeled white blood cells of the higher concentration can be collected from the first outlet.

The microfluidic concentration device can include a second channel arranged in parallel with the first channel. The second channel can include an inlet coupled to the outlet of the bypass channel to receive the labeled white blood cells in the buffer, a first outlet through which substantially all of the focused labeled white blood cells in the buffer are flowed, and a second outlet and a third outlet through which buffer that is not flowed through the first outlet is flowed. The labeled white blood cells in the buffer can be flowed simultaneously through the second channel, and the labeled white blood cells

of the higher concentration can be collected from the first outlet of the second channel. The labeled white blood cells of the higher concentration can be infused into the patient.

A first portion of the whole blood mixed with the buffer and the labeling agent can be flowed through a first inlet located closer to the first side wall than to the bypass
5 channel and through the first array of obstacles. A remaining portion of the whole blood mixed with the buffer and the labeling agent can be flowed through a second inlet located closer to the second side wall than to the bypass channel and through the second array of obstacles. The first portion and the remaining portion can be flowed simultaneously. Flowing the first portion of the whole blood mixed with the buffer and the labeling agent
10 through the first inlet can include flowing the first portion at an angle relative to the first array such that white blood cells bound to the labeling agent in the first portion are displaced out of flow streamlines upon encountering an obstacle in the first array.

The labeling agent not bound to white blood cells in the buffer can be separated from the labeled white blood cells in the buffer collected from the outlet of the bypass
15 channel. This can be done by flowing the labeled white blood cells in the buffer through a third array of obstacles disposed within the first channel between the first side wall and the second side wall, wherein the third array is configured to deflect the labeling agent not bound to the white blood cells in the buffer in one direction and the labeled white blood cells in the buffer in another direction.

The first array of obstacles can include multiple first obstacles that form a network of gaps and includes at least first and second rows of the obstacles. The second row can be displaced relative to the first row so that a fluid passing through a gap in the first row is divided unequally when flowing around an obstacle and into two gaps on either side of the obstacle in the second row. The second row can be displaced laterally
20 relative to the first row.

Other aspects of the subject matter described herein are devices to perform the methods described herein. Yet other aspects of the subject matter described here are methods for separating monocytes from neutrophils and lymphocytes.

Implementations of the subject matter described in this document can provide one
30 or more of the following potential advantages over current standard of clinical care. The

devices and methods described here can decrease (and nearly eliminate) the loss of white blood cells that are separated from whole blood. Consequently, less patient blood needs be used for imaging and opens up wider adoption of the test in pediatric applications. The time required to obtain labeled white blood cells for reinfusion in a patient can be
5 decreased. Consequently, both the quality of cells and quality of clinical care for the patient is positively impacted. The labeled white blood cells can be of higher quality than those obtained by standard processes described above. Consequently, the in-vivo imaging can have better diagnostic quality. The devices can enable using large blood volumes in high throughput devices. The devices and methods can be implemented for
10 all patient populations served by current clinical test, and in addition enable new patient populations (e.g., sickle cell anemia patients) that need a point-of-care device that can separate white blood cells without contamination (e.g., by red blood cells).

The devices and methods described can provide a fully automated process in which whole blood is provided to the device as input and white blood cells in plasma-free
15 cell-friendly medium are obtained as output. The white blood cells can be bound to a labeling agent, concentrated, and provided for re-infusion to the patient in injectable saline. Use of the devices and methods can reduce labor required relative to other approaches which can require up to several (e.g., 21) discrete steps of manual sample manipulation to obtain a similar output. The devices and methods do not require
20 centrifugation, reduce sample handling, and can reduce cell clumping that can result from multiple centrifugation steps and that can result in focal accumulation of radioactivity in lungs which lead to false positive results.

The devices and methods can provide very low red blood cell carryover. For example, maximum red blood cell carryover can be limited to 0.001% (~5 million from
25 50 ml of processed blood) in comparison to centrifugation-based methods that typically have 1% carryover. Lower red blood cell carryover reduces red blood cell and hemolysis product contamination. The devices and methods can retain white blood cells with high efficiency (e.g., more than 99%) decreasing losses in white blood cells in the whole blood. These loss levels are lower relative to centrifugation methods in which the white
30 blood cell loss can be range between 20% and 40%, and which can require, for example,

larger volumes of whole blood to compensate for the white blood cell loss. Consequently, the amount of whole blood required when implementing the methods described here can be decreased.

The devices and methods can isolate white blood cells or sub-populations of the white blood cells in a physiological shear stress environment without exposure to chemicals (e.g., Ficoll®, hydroxyethyl starch, and the like), thereby providing high quality cells that can be labeled more effectively with a labeling agent. Such labeled cells can migrate to site(s) of infection. The devices and methods can deplete plasma, along with the red cells and platelets to reduce interference in downstream labeling from carryover of plasma.

Some implementations of the devices and methods described here can enrich monocytes relative to lymphocytes and neutrophils in whole blood. In such implementations, the monocytes can be retained with high efficiency, and as such, the relative percentage of the total monocyte population can be increased.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a flowchart of a process for manipulating whole blood to obtain labeled white blood cells.

FIG. 2 is a schematic of one embodiment of a system to manipulate whole blood to obtain labeled white blood cells.

FIG. 3A is a schematic that shows an example of a multiplex microfluidic device.

FIGs. 3B-E are schematics of scanning electron microscope images that show microfluidic channels and arrays of obstacles through which fluid is flowed.

FIGs. 4A-E are schematic representations of an example of a multiplex microfluidic device.

FIG. 5A is a schematic that shows an example of a microfluidic concentration device.

FIG. 5B is a schematic that shows an enlarged portion of an end of one of the channels shown in FIG. 5A.

FIG. 5C is a graph that shows a line cut image of a focused stream created using the focusing device of FIG. 5A.

FIG. 6A is a schematic that shows an example of a microfluidic focusing device including multiple straight focusing channels arranged in parallel.

FIG. 6B is a schematic that shows an enlarged portion of an end of one of the channels shown in FIG. 6A.

FIG. 7A is a schematic that shows an example of a first stage of a two-stage microfluidic separation device.

FIG. 7B is a schematic that shows an example of a second stage of a two-stage microfluidic separation device.

FIG. 7C is a schematic that shows an example of the two-stage microfluidic separation device.

FIG. 8 is a schematic that shows the different elements of a system to manipulate whole blood to obtain labeled white blood cells.

FIG. 9 is a schematic that shows an example of a microfluidic separation device including a spiral channel for concentrating the cells in the sample fluid.

FIGs. 10A and 10B are schematics that show two different multiplex microfluidic devices to separate leukocytes from blood (FIG. 10A) and to preferentially separate monocytes from lymphocytes and neutrophils (FIG. 10B).

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

A microfluidic-based approach is implemented to separate leukocytes from the other components of human whole blood (red blood cells, platelets and serum components) with high efficiency. The microfluidic devices described with reference to the following figures are automated, continuous, stand-alone processing units that can process whole blood drawn from a patient (e.g., in a Vacutainer®) to provide an output of white blood cells, with very low carryover of red cells. The separated white blood cells can be collected in a cell-friendly medium and concentrated using a microfluidic concentration device described below. The white blood cells can be bound to a labeling agent resulting in labeled white blood cells, and can be further processed using another microfluidic device to remove any labeling agent that is unbound to the white blood cells. The labeled white blood cells can then be provided for re-infusion into the patient to image infection sites to which the labeled cells migrate.

General Methodology

FIG. 1 is a flowchart of a process 100 for manipulating whole blood to obtain labeled white blood cells. Whole blood drawn from a patient is obtained (105). For example, 40 mL of blood is aseptically drawn into sterile 50 mL syringes containing 10 mL of acid citrate dextrose anticoagulant (22.0 g/L trisodium citrate, 8.0 g/L citric acid, 24.5 g/L dextrose) with the use of a 20G needle. Samples are drawn and transported immediately to the laboratory for processing.

The whole blood is flowed through a microfluidic device that separates white blood cells from remaining portions of the whole blood (110). Such a microfluidic device can be implemented as a multiplex device including multiple arrays of obstacles, each of which operates under the principles of deterministic lateral displacement described in U.S. Published Application No. 2007/0026381 (entitled “Devices and Methods for Enrichment and Alteration of Cells and Other Particles,” the entire contents of which are incorporated herein by reference). The device has been developed and

validated with over 1000 blood samples and used to isolate leukocytes from maternal blood for prenatal diagnosis.

In such a device, whole blood is introduced into an array of obstacles (for example, microposts) under laminar flow conditions. The large white blood cells are displaced out of the flow streamlines upon encountering an obstacle. The enucleated components of the blood, on the other hand, remain unperturbed by the array and follow the flow streamlines. As the whole blood flows through an array, the displacement of the white blood cells out of the flow streamlines separates these cells from remaining components of the whole blood. As described below, within a microfluidic device, multiple arrays of obstacles are arranged with bypass channels that are devoid of obstacles to increase a throughput of white blood cells separated from whole blood. The separated white blood cells can then be collected (e.g., in a Vacutainer®) and provided to a microfluidic concentration device configured to increase the concentration of the white blood cells in solution.

For example, the fluidic pathway of the device and associated buffer solutions will be sterile, endotoxin free components. The container in which the separated white blood cells will be collected is filled with a collection buffer (e.g., injectable saline solution) and sealed. Next, the required connections (fluidic and pneumatic) are made between the microfluidic device and the various reservoirs and containers. Then, a synthetic blocking buffer (1% Pluronic® F68) is loaded into a temporary sample reservoir and used to create fluidic connections between the microfluidic device and the sample and buffer reservoirs. This blocking buffer passivates the blood-contacting surfaces to minimize protein adsorption and associated leukocyte accumulation/activation. In the final step prior to sample processing, the sample reservoir is replaced with a fresh reservoir and injectable grade saline is flushed through the system to removed excess Pluronic® F68.

After the desired sample volume has been processed, the product container is decoupled from the system and available for radiolabeling. All consumables are then disposed of using standard universal precautions. Exposed instrument surfaces are decontaminated using 70% ethanol or equivalent.

The separated white blood cells can be flowed through a microfluidic concentration device that increases a concentration of white blood cells relative to concentration of white blood cells collected from an outlet of the microfluidic device (115). The microfluidic concentration device can be implemented as a flow-based
5 focusing device that operates under the principles of inertial focusing described in U.S. Published Application No. 2009/0014360 (entitled “Systems and Methods for Particle Focusing in Microchannels,” the entire contents of which are incorporated herein by reference).

In some implementations, the microfluidic concentration device can include a
10 straight channel through which the white blood cells in solution (for example, buffer or labeling agent solution) can be flowed at flow rates that cause inertial forces to act on and focus the white blood cells into one or more stream lines. The straight channel can include multiple outlets (e.g., three outlets) – one to collect the focused white blood cells, and the others to collect waste (i.e., the remaining solution). In some implementations,
15 the microfluidic concentration device can be implemented in a spiral channel having one or more inlets and one or more outlets, as described below. In some implementations, multiple concentration devices can be arranged in series or in parallel or both. The dimensions of the concentration devices can be same or different. The decrease in the solution in which the white blood cells are flowed results in an increase in white blood
20 cell concentration. Examples of microfluidic concentration devices that yield 4X or higher increase in concentration are described below.

As described previously, applications of the microfluidic devices described here include re-infusion of labeled white blood cells (i.e., white blood cells bound to a labeling agent) into a patient so that the labeled white blood cells can be imaged upon migration to
25 an infection site. An agent that has an affinity for a selected cell population (i.e., white blood cells) can be chosen. In some implementations, the labeling agent includes a radio-labeling agent, such as, e.g., Tc-HMPAO (Ceretec, GE Healthcare), Indium 111 Oxyquinoline solution (Indium Oxine, GE Healthcare), or an equivalent. The labeling agents can be conjugated to lipophilic moieties, e.g., Tc-HMPAO and Oxyquinoline
30 respectively, to enable uptake by cells. Other examples of labeling agents include FDG

(used to image primary tumors) by active uptake through glucose transport receptors. In some situations, the white blood cells can be labeled with nanoparticles for imaging by Magnetic Resonance Imaging (MRI). In general, any in-vivo imaging agent can be used as is or conjugated to cellphillic agents for cellular uptake as a labeling agent for labeling
5 the white blood cells. In some implementations, imaging reagents that are not conducive to modification for cellular uptake can be electroporated into the white blood cells.

To this end, the labeling agent can be mixed with the white blood cells (113) at one of multiple instances. For example, the labeling agent can be mixed with the whole blood drawn from the patient and incubated for a duration that is sufficient to result in
10 labeled white blood cells. Alternatively, the labeling agent can be mixed with the white blood cells that have been separated using the multiplex device. In another alternative, the labeling agent can be mixed with the white blood cells that have been concentrated using the microfluidic concentration device. The labeled white blood cells can be separated from the remaining components of the blood using the multiplex device and
15 then concentrated using the microfluidic concentration device. Also, as described previously, the labeled white blood cells can further be processed to separate unbound labeling agent from the labeled white blood cells (120).

To do so, in some implementations, the labeled white blood cells can be flowed through another microfluidic separation device that includes one or more arrays of
20 obstacles arranged in series, each of which is similar to one of the arrays of obstacles in the multiplex device. The microfluidic separation device can be configured to separate labeled white blood cells from unbound labeling agent, for example, by the principles of deterministic lateral displacement. Alternatively, the labeled white blood cells can be flowed through one or more microfluidic concentration devices under flow conditions
25 that further concentrate and separate the labeled white blood cells from the unbound labeling agent. The labeled white blood cells thus separated can be collected in a suitable container, e.g., a syringe, and provided for re-infusion in a patient (125).

System Overview

FIG. 2 shows a schematic to implement a system 200 to manipulate whole blood to obtain labeled white blood cells. The system 200 includes the microfluidic devices described above. Whole blood 205 obtained from a patient is flowed through a multiplex
5 microfluidic device 210 described herein with reference to FIGS. 3A to 4E that separates white blood cells from remaining components of the whole blood. The separated white blood cells can be collected, for example, in a container 215 and provided as input to a microfluidic concentration device 220.

System 200 shows a microfluidic concentration device 220 that includes multiple
10 concentration devices 222 arranged in series and coupled to another concentration device 224 in series, e.g., as described with reference to FIGS. 5A to 9. The concentrated white blood cells can be collected in another container 230 and provided as input to microfluidic separation devices 235 and 240 configured to separate unbound labeling agent from the white blood cells. The separation devices 235 and 240 can be similar to
15 either the multiplex device 210 or the concentration devices 222 or 224 or combinations of them.

As described previously, the white blood cells can be mixed with a labeling agent that binds to the white blood cells at one of several stages, for example, before the whole blood 205 is flowed through the multiplex microfluidic device 210, before the white
20 blood cells separated by the multiplex microfluidic device 210 are flowed through the microfluidic concentration device 220, or after the white blood cells have been concentrated using the concentration device 220.

The system 200 is configured to process 60 mL of whole blood 205. The multiplex microfluidic device 210 can harvest 99% of white blood cells with less than
25 0.1% carryover of red blood cells and nearly no platelets or proteins. The white blood cells separated by the multiplex microfluidic device 210 can be collected in 60 mL of PBS in the container 215. The microfluidic concentration device 220 can include a five-channel concentration device 222 arranged in parallel to have a throughput of 2.5 mL/min coupled in series to a one-channel concentration device 224. The five-channel
30 concentration device 222 and the one-channel concentration device can result in a 5X

concentration of white blood cells. The concentrated white blood cells can be collected in 2.5 mL of PBS in the container 230. The labeling agent can be added to the container 230. After an incubation period (e.g., 15 minutes), the labeled white blood cells can be flowed through another 5-channel microfluidic separation device 235 coupled to a one-channel microfluidic separation device 240 that separates unbound agent from the labeled white blood cells. The entire process can span approximately 3.5 hours (2 hrs to separate white blood cells from whole blood using the multiplex microfluidic device 210 can span approximately two hours, 30 minutes to concentrate the separated white blood cells, 30 minutes to label the white blood cells, and 30 minutes to separate unbound agent from the labeled white blood cells).

FIG. 3A shows an example of a multiplex microfluidic device 210. FIGS. 3B-D show scanning electron micrographs of arrays of obstacles included in the device 210. The device 210 includes one or more arrays of obstacles that allow deterministic lateral displacement of components of whole blood. The array includes rows and columns of obstacles (e.g., microposts) arranged to separate particles according to size in a network of gaps formed between the obstacles. A fluid passing through a gap is divided unequally into subsequent gaps. The array includes a network of gaps arranged such that fluid passing through a gap is divided unequally, even though the gaps may be identical in dimensions. The gaps are formed by an array of obstacles that deterministically direct particles having a hydrodynamic size above a critical size in one direction and particles having a hydrodynamic size below the critical size in a different direction.

In some implementations, the spacing between any two adjacent obstacles in the same row can be equal (e.g., 21 μm from center-to-center). Further, each row of obstacles can be shifted horizontally with respect to a previous row by a distance that is a fraction of a center-to-center distance between the posts (e.g., $1/60^{\text{th}}$ the center-to-center distance between two adjacent obstacles in the same row). The ratio of the fraction to the center-to-center distance determines the ratio of flow bifurcated to the left of the next obstacle. In some implementations, flow bifurcation can be achieved based on a cross-section of each obstacle in the array. For example, as shown in FIG. 3D, each obstacle can have a substantially elliptical (e.g., tear-drop) cross-section in which a curvature of a

first edge of the obstacle is different from a curvature of a second edge that is opposite the first edge in a direction transverse to fluid flow. In some implementations, flow bifurcation can be obtained by flowing fluid at an angle to a first row of the array of obstacles. In such implementations, the rows need not be horizontally offset from one another, and the obstacles can be of any cross-sectional shape (e.g., cylindrical, teardrop).

For example, whole blood can be flowed at a small angle ($\sim 1^\circ$) relative to the array, such that the white blood cells, which are large with respect to the flow streamlines, are displaced out of the streamline when they encounter an obstacle (e.g., a post). By contrast, the enucleated components of the blood follow the streamlines predicted by laminar flow theory, unperturbed by the array. This process repeats itself throughout the length of the array, acting as a size filter, where the effective pore size of the array is dictated by the width of the flow stream rather than the spacing between the posts (gap size). The width of the flow streams can be engineered to be much smaller than the gap size permitting separation of white blood cells with a gap size much larger ($> 20 \mu\text{m}$) than their actual size ($\leq 10 \mu\text{m}$). This large physical gap size, relative to cell size, causes the cells to experience low shear stress (e.g., at or below physiological shear stress) and prevents large volumes of cells from clogging the microfluidic device. Consequently, processing times of whole blood is reduced.

With reference to FIG. 3E, the critical cutoff diameter (D_c , above which cells are deflected and below which cells pass through unperturbed), is defined by the following relationship:

$$D_c = 2\eta g \epsilon,$$

where ϵ is the row shift fraction (shift of each row relative to the obstacle spacing), g is the gap between obstacles, and η is a variable parameter that corrects for flow non-uniformity in the gap. For non-ideal particles, size can be thought of more as a “hydrodynamic size,” a combination of size, shape, and flexibility. For example, erythrocytes, which have a discoid shape, separate based on their small axis ($\sim 2 \mu\text{m}$) even though their widest dimension ($4\text{--}6 \mu\text{m}$) overlaps with typical leukocyte dimensions. Accordingly, deterministic lateral displacement can be used to effectively distinguish leukocytes from erythrocytes as described above.

Referring to FIGS. 4A-4E, the multiplex microfluidic device 401 is a multi-stage multiplex device in which multiple multiplexes are arranged in series. For example, the device 401 can be a silicon device multiplexing fourteen 3-stage duplex arrays. The dimensions of this particular device are 90 mm x 34 mm x 1 mm. The array design
5 includes 3 stages, gap size = 18, 12, and 8 μm for the first, second and third stages, respectively, and a bifurcation ratio of 1/10. Each array is a duplex array with a single bypass channel and includes flow resistors for flow stability. In some embodiments the device arrays and channels can be fabricated in silicon using standard photolithography and deep silicon reactive etching techniques with an etch depth of 150 μm . Through
10 holes for fluid access can be made, e.g., using KOH wet etching. The silicon substrate can be sealed on the etched face to form enclosed fluidic channels, e.g., using a blood compatible pressure sensitive adhesive (9795, 3M, St Paul, Minn.). The device can be mechanically mated to a manifold, e.g., of plastic, with external fluidic reservoirs to deliver blood and buffer to the device and extract the generated fractions. An external
15 pressure source can be used to apply a pressure, e.g., in a range of 1 to 35 psi, e.g., a pressure of about 2.4 psi, to the buffer and blood reservoirs to modulate fluidic delivery and extraction from the packaged device.

The multiplex microfluidic device 210 shown in FIG. 4B includes multiple duplex arrays (FIGS. 4C-E) that are connected by a common inlet 400 and a common outlet 402.
20 As shown in FIG. 4C, each duplex array includes a channel 403 extending from one of the inlets 400 to one of the outlets 475, and is bounded by a first side wall 404 and a second side wall 406 located opposite the first side wall 404. Within the channel, a first array of obstacles 405 is disposed and bounded by a first array boundary formed by ends of obstacles in the first array 405 and the first side wall 404. Also, a second array of
25 obstacles 410 is disposed within the channel, and bounded by a second array boundary formed by ends of obstacles in the second array 410 and the second side wall 406. A bypass channel 415 defined by a region devoid of obstacles is formed between the first array boundary and the second array boundary. The first array 405 and the second array 410 are configured to deflect the labeled white blood cells in the buffer toward the bypass
30 channel 415. In some implementations, the first array 405 and the second array 410 are

formed as mirror-images of each array other so that each deflects white blood cells in the direction of the other resulting in the white blood cells being collected in the bypass channel 415. The collected white blood cells flow toward the outlet of the bypass channel.

5 In some implementations, the obstacles in the array may continue up to and run into the side wall. Alternatively, another region devoid of obstacles can be formed immediately adjacent the side wall and the array boundary. In such implementations, the physical boundary of the bypass channel may be defined by the array boundary on one side and a sidewall on the other side. If the array were infinitely large, the flow
10 distribution would be the same at every gap. In practice, the boundaries of the array perturb this infinite flow pattern. Portions of the boundaries of arrays may be designed to generate the flow pattern of an infinite array. Boundaries may be flow-feeding, i.e., the boundary injects fluid into the array, or flow-extracting, i.e., the boundary extracts fluid from the array. A flow-extracting boundary widens gradually to extract flux from each
15 gap at the boundary. For example, the distance between the array and the sidewall gradually increases to allow for the addition of flux to the boundary from each gap along that boundary. The flow pattern inside this array is not affected by the bypass channel because of the boundary design. A flow-feeding boundary narrows gradually to feed flux into each gap at the boundary. For example, the distance between the array and the
20 sidewall gradually decreases to allow for the addition of flux to each gap along the boundary from that boundary. Again, the flow pattern inside this array is not affected by the bypass channel because of the boundary design. A flow-feeding boundary may also be as wide as or wider than the gaps of an array. A wide boundary may be desired if the boundary serves as a bypass channel, e.g., to allow for collection of particles. A
25 boundary may be employed that uses part of its entire flow to feed the array and feeds flux into each gap at the boundary.

Multiple bypass channels can be designed, in conjunction with an array to maintain constant flux through a device. In this example, bypass channels are designed to remove an amount of flow so the flow in the array is not perturbed, i.e., substantially
30 constant. Such a design may also be employed with an array duplex. In this design, the

center bypass channel may be shared between the two arrays in the duplex. For example, FIG. 4D shows a third array of obstacles 420 and a fourth array of obstacles 425 formed within the channel in series with the first array of obstacles 405 and the second array of obstacles 410, respectively. A bypass channel 430 devoid of obstacles is formed between the third array 420 and the fourth array 425. The outlet of the bypass channel 415 is connected to an inlet of the bypass channel 430. Spacing between the obstacles in the first array 405 and the second array 410 can be the same as each other, and can be different from spacing between the obstacles in the third array 420 and the fourth array 425. Similarly, a fifth array of obstacles 435 and a sixth array of obstacles 440 can be disposed within the channel in series with the third array 420 and the fourth array 425, respectively. A bypass channel 445 devoid of obstacles can be formed between the fifth and sixth arrays, and connected to the bypass channel 430.

The device described with reference to FIGS. 4C-D represents a three-stage duplex microfluidic device. Several such duplex devices can be coupled in parallel (FIG. 4B) to form the multiplex microfluidic device 210. Table 1 shows an effectiveness of a multiplex microfluidic device 210.

Table 1: Clinical performance (> 1000 samples)

	Mean	S.E.M.	S.D.
% Nucleated cell retention	98%	0.2	3
% RBC removal	99.5%	0.03	0.4
% Platelet removal	99	0.2	2
% Viability	99	0.5	1

The multiplex microfluidic device 210 can support a flow throughput ranging to 30 mL/hr (within physiological shear stress) to separate white blood cells from red blood cells, platelets, and plasma with a reliability of greater than 97%.

The bio-burden of product obtained by separating particles in the multiplex microfluidic device 210 were tested based on endotoxin levels assayed via a Limulus Amoebocyte Lysate (LAL) test. To do so, a surrogate medium (e.g., 1x iDPBS) was

flowed through the device 210 to generate a baseline value for device sterility. The endotoxin levels were measured in three components flowed through the device 210 – input buffer, waste output, and product output. The bio-burden of the product output was assayed by measuring the Total Aerobic Microbial Count of the product. The assay results showed that each of the input buffer, the waste output, and the product output had less than 0.05 EU/mL mean endotoxin value indicating no microbial growth.

Further, white blood cells separated from whole blood using the multiplex microfluidic device 210 were enriched (as observed by Wright-Giemsa Staining), viable (as observed by 1 μ g/mL Calcein AM cell Staining and 1.5 μ g/mL CellTracker® Orange Cell Staining), and motile (average neutrophil velocity of 18 ± 3 μ m/min). When compared to known processes of centrifugation and sedimentation that offer white blood cell retention of 57%, red blood cell retention of 0.24%, and platelet retention of 33%, white blood cell separation using the multiplex microfluidic device 210 offers a white blood retention of 98%, red blood cell retention of 0.02%, with no platelets retained.

Human white blood cells obtained from the multiplex microfluidic device 210 were bound with In-111-Oxyquiline and re-infused in the patient to image *E. coli* muscle infection and *Pseudomonas* muscle infection. Similarly obtained human white blood cells were bound with Tc99-HMPAO and used to image cecal ligation. Thus, the human white blood cells enriched with the multiplex microfluidic device 210 and labeled with In-111-Oxy migrate to site of infection. Such labeled white blood cells are absent from inflammation control (injury model without infection) demonstrating that localization is specific to presence of infection. Human white blood cells enriched with the multiplex microfluidic device 210 and labeled with Tc99-HMPAO migrate to site of infection as demonstrated by cecal puncture and infection in rats. Such labeled white blood cells are absent in cecum of control rates (no cecal ligation).

As described above, white blood cells in whole blood can be separated using the multiplex microfluidic device 210. The cells can be collected in a buffer (e.g., PBS) and optionally mixed with a labeling agent. The concentration of the cells in the buffer can then be increased using the microfluidic separation device 220.

Microfluidic Cell Separation and Concentration Devices

FIG. 5A is a schematic that shows an example of a microfluidic concentration device 220 into which the white blood cells separated from whole blood can be flowed to concentrate the white blood cells. The concentration device 220 can include a straight channel 500 formed in a substrate (e.g., silicon substrate) and can have an inlet through which the white blood cells (labeled or unlabeled) are flowed through the buffer solution. The flow rate can be selected such that inertial forces established in the flowing fluid act upon and focus the white blood cells into one or more localized stream lines. Each stream line can define a width that is substantially equal to or greater than a size of the focused labeled white blood cells. Thus, a stream line can have a width that is lesser than the cross-sectional width of the channel 500 and can include a white blood cell within.

Channel 500 can have various geometries and cross-sections for focusing the white blood cells suspended within the buffer. For example, the channel 500 can have a rectangular cross-section with an aspect ratio of substantially 1 to 1. White blood cells flowing within such a channel geometry will be separated, ordered, and focused into four streamlines corresponding to four equilibrium points or potential minimums at a distance from each face of the four channel walls. Alternatively, the channel 500 can have a rectangular cross-section with an aspect ratio of substantially 2 to 1. White blood cells flowing within such a channel geometry can be separated, ordered, and focused into two focused streamlines corresponding to two equilibrium points or potential minimums along top and bottom walls across the width of the channel. In some implementations, an aspect ratio of 1 to 2 can also be used. Further, the channel can have a circular, triangular, diamond-shaped, or hemispherical cross-section. For example, in a straight channel having a circular or hemispherical cross-section, an annulus or arc of focused particles can be formed within the channel. In a straight channel having a triangular or diamond cross-section, particles can be focused into streamlines corresponding to equilibrium positions at a distance from the flat faces of each wall in the geometry.

There is a range of substantially non-1-to-1 aspect ratios for which these effects may be observed. The focusing effects may be seen regardless of whether it is the width

or the height that is twice the other dimension. In addition to ratios of about 1 to 2, this reduction in symmetry can be observed in rectangular channels having dimensional ratios of approximately 15 to 50, 3 to 5, and 4 to 5.

The channel 500 can have a hydraulic diameter and a ratio of a size of the white blood cells to the hydraulic diameter that is greater than or equal to about 0.07. The ratio of white blood cell size to hydraulic diameter can be less than or equal to about 0.5. In some implementations, a Reynolds Number of the fluid flow during focusing can be greater than or equal to about 1 and less than or equal to about 250. Further, a particle Reynolds number for the whole blood moving through the channel can be greater than or equal to about 0.2. The one or more focused stream lines can have a width that is less than or equal to about five times, four times, three times, two times, and/or 1.05 times a size of the focused particles.

The whole blood flow through the channel 500 can be laminar. Focusing can produce a localized flux of enriched white blood cells or another whole blood component based on the component size. A white blood cell diameter divided by a hydraulic diameter of the channel can be greater than or equal to about 0.07 and the white blood cell diameter divided by the hydraulic diameter of the channel can be less than or equal to about 0.5. In implementations in which the channel has a rectangular cross-section, a height, a width, a hydraulic diameter is equal to $2 \cdot \text{height} \cdot \text{width} / (\text{width} + \text{height})$. In such implementations, the rectangular cross-section can have an aspect ratio of between approximately 0.3 and 0.8 and/or approximately 1 to 2.

Two dimensionless Reynolds numbers can be defined to describe the flow of particles in closed channel systems: the channel Reynolds number (R_c), which describes the unperturbed channel flow, and the particle Reynolds number (R_p), which includes parameters describing both the particle and the channel through which it is translating.

$$R_c = \frac{U_m D_h}{\nu}$$

$$R_p = R_c \frac{a^2}{D_h^2} = \frac{U_m a^2}{\nu D}$$

In the above equations for channel Reynolds number (R_c) and particle Reynolds number (R_p), " U_m " is the maximum channel velocity, " ν " is the kinematic viscosity of the whole blood cells, " a " is the white blood cell diameter, " D_h " is the hydraulic diameter of the channel 500. Additional details related to the equations are found in U.S. Published
5 Application No. 2009/0014360, which, as noted above, is incorporated herein by reference.

FIG. 5B is a schematic that shows an enlarged portion of an end of one of the channels shown in FIG. 5A. In some implementations, the stream of white blood cells can be flowed through the channel 500 at volumetric flow rates of approximately 100
10 $\mu\text{L}/\text{min}$. The channel 500 can include three outlet branches – one to receive the focused stream of white blood cells and the other two to receive the buffer that is not in the focused stream. In some implementations, each of the outlets can be coupled to an inlet of a corresponding channel to transport the white blood cells and the buffer that is not in the focused stream. Such channels can be sized to transport the white blood cells at a
15 volumetric flow rate of 20 $\mu\text{L}/\text{min}$, and to transport the separated fluid at flow rates of 40 $\mu\text{L}/\text{min}$ each. In some implementations, the channel that transports the focused stream of white blood cells can be longitudinally coupled to the channel 500, and the channels that transport the separated buffer can be coupled transversely to the channel 500.

In some implementations, the channel 500 can be 80 μm wide, 30 μm tall, and
20 support a throughput of 100 $\mu\text{L}/\text{min}$. In such a channel, 3.22 M WBC/mL in 6.08 mL were flowed. The focused stream of white blood cells that were collected included 12.7 M WBC/mL in 1.33 mL resulting in an overall yield of 87% representing a 4.6X reduction in volume and approximately 4X increase in concentration. As shown in FIG. 5C, the white blood cells were focused within a stream spanning approximately 30 μm
25 within the 80 μm width of the channel 500.

FIG. 6 shows an example of a microfluidic concentration device 600 including multiple channels arranged in parallel. In one implementation, ten straight focusing channels (similar to channel 500), each having a width of 80 μm , a height of 30 μm , and supporting a throughput of 1 mL/min were coupled in parallel. The channels were
30 arranged to have a common inlet for the white blood cells in the buffer and a common

outlet for the focused stream of white blood cells. Two outlets were formed for each channel to separate the buffer from the focused stream. The concentrated white blood cells obtained using the microfluidic concentration device 600 shown in FIG. 6 are presented in Table 2.

Table 2

	I	II	III	IV	Avg.
Product	86%	95%	91%	85%	89%
Waste	6%	5%	9%	8%	7%
System Loss	8%	0%	0%	7%	3.8%
Vol. Reduction	4.66X	4.66X	4.48X	5.15X	4.7X
White Blood Cell conc.	4.02X	4.53X	4.19X	4.37X	4.3X

Table 2 shows that, on average 89% of the white blood cells flowed into the microfluidic separation device 220 were recovered, 7% of white blood cells were not focused, 3.8% were lost (due to experimental errors – CBC counting, white blood cells in dead volume, white blood cells retained in input syringe), resulting in an average volumetric reduction of 4.7X and an average white blood cell concentration of 4.3X.

FIGS. 7A and B are schematics of an example of a two-stage microfluidic concentration device 700 that includes a first concentration device coupled in series to a second concentration device. The first concentration device (FIG. 7A) is similar to the device described with reference to FIG. 6B, and includes five straight focusing channels, each having a width of 80 μm and a height of 30 μm . The focused stream of white blood cells from the first separation device is provided as input to a second concentration device (FIG. 7B) that includes a single straight channel having a width of 80 μm and a height of 30 μm . The throughput of the two-stage microfluidic separation device (FIG. 7C) 220 is 500 $\mu\text{L}/\text{min}$. In this device, 72.9% of white blood cells were focused and collected with 18.5% lost in the first separation device and 3.8% lost in the second separation device. Using this device, a volumetric reduction of 17.75X and a white blood cell concentration of 13.55X were achieved.

Returning to FIG. 2, the focused white blood cells can be collected in a container 230. If the labeling agent has been mixed with the white blood cells prior to flowing the cells through the microfluidic separation device 220, then the container 230 contains labeled white blood cells. If not, then the labeling agent can be mixed with the white blood cells in the container resulting in labeled white blood cells. The labeled white blood cells can then be flowed through the microfluidic separation devices 235 and 240 to separate the labeled cells from unbound agent. As described previously, the microfluidic separation devices 235 and 240 can be similar to the multiplex microfluidic device 210 or the microfluidic concentration device 220 or combinations of them. For example, the microfluidic separation device can include a single array of obstacles configured to flow unbound agent in an axial direction and to deflect labeled white blood cells in a direction lateral to the axial direction.

FIG. 8 shows a schematic to implement a system 800 to manipulate whole blood to obtain labeled white blood cells. The system 800 is similar to the system 200 described with reference to FIG. 2, and includes a multiplex microfluidic device 210 through which whole blood is flowed to separate white blood cells collected in a container 215. The system 800 includes a microfluidic concentration device 805 that includes a spiral channel including one or more inlets and at least two outlets. The separated white blood cells (labeled with a labeling agent or unlabeled) are flowed through the inlet with a buffer. The spiral channel of the concentration device 805 is configured to separate the white blood cells from the buffer, thereby increasing a concentration of the white blood cells.

FIG. 9 shows an example of a microfluidic separation device 805 including a spiral channel. The spiral channel can be an expanding spiral shaped channel having a rectangular cross-section with an aspect ratio of substantially 2 to 1, although the aspect ratio can vary. For example, the width of the spiral channel can range from about 2000 μm to 772 μm , and the height can be about 50 μm . The input volumetric flow rate can be about 3 mL/min. The focused white blood cells can be collected through two outlets, and the fluid that is not in the focused stream lines can be collected through a waste outlet. In such implementations, the white blood cells are focused into a single stream line a

distance away from an inner wall of the channel corresponding to a single equilibrium point or potential minimum within the channel. A reduction in volume of between about 4X and 10X and an increase in concentration of between about 4X and 6X were measured.

5 The location of the focused stream of white blood cells within the channel can depend upon inertial forces and Dean drag forces acting on the cells. The location can further depend upon centrifugal forces acting on the cells. A Dean number for flow through the channel can be less than or equal to about 20. The curvature in the spiral channel introduces Dean drag that will push the white blood cells in different transversal
10 directions depending on position. For example, a particle located in the center will be pushed towards the outer wall and recirculated through the outer edge roof or bottom until the particles reach the equilibrium position near the inner wall where the Dean forces are superimposed to the inertial forces from the inner walls. Thus, the main forces impacting the white blood cell focusing in the channel height direction may be inertial lift
15 forces, while the Dean forces have a strong influence on the lateral positioning of cells. A cell can remain focused as long as the Dean force trying to push the particle away from the inner wall is balanced by the inertia lift force from the inner wall trying to push the cell towards the inner wall. This results in cells focusing in single-stream lateral positions in two parallel symmetric streams along the height of the channel. In addition
20 to focusing, the cells are ordered in uniform spacing in the direction of the flow.

Microfluidic Devices to Separate Specific White Blood Cells

FIGs. 10A and 10B are schematics that show two different multiplex microfluidic devices to separate leukocytes from blood (FIG. 10A) and to preferentially separate
25 monocytes from lymphocytes and neutrophils (FIG. 10B). In some implementations, the system 200 can be configured to separate monocytes from lymphocytes and neutrophils. As described above with reference to FIG. 3E, the critical cutoff diameter (D_c , above which cells are deflected and below which cells pass through unperturbed), is defined by the following relationship:

$$D_c = 2\eta g \varepsilon,$$

where ϵ is the row shift fraction (shift of each row relative to the obstacle spacing), g is the gap between obstacles, and η is a variable parameter that corrects for flow non-uniformity in the gap. Large flexible cells like neutrophils are able to deform enough to behave like effectively smaller cells. This phenomenon can be exploited to enrich monocytes relative to lymphocytes and neutrophils. To test this hypothesis, two devices with the same basic device architecture were produced, each having a different D_c (Table 3).

Table 3

Design	λ (μm)	ϵ	g (μm)	η	D_c (μm)
Type "P"	35	1/60	20	4.6	3.1
Type "O"	56	1/60	32	4.6	4.9

The shift in D_c resulted in a loss of small lymphocytes and a portion of the neutrophils, presumably due to their flexibility. Monocytes were retained with high efficiency in the higher D_c cut off design, and as such, their relative percentage of the total population nearly doubled. For example, white blood cells having a population distribution of 69% neutrophils, 7% monocytes, and 25% lymphocytes were flowed through each of the Type "P" and Type "O" multiplex microfluidic devices. The output of the Type "P" multiplex microfluidic device was substantially identical to the input (neutrophils – 68%; monocytes – 7%; lymphocytes – 25%). The output of the Type "O" chip, on the other hand, showed a near doubling of the monocyte concentration (neutrophils – 55%; monocytes – 12%; lymphocytes – 33%). The critical cutoff diameter can be further optimized to identify the maximal possible monocyte enrichment allowable. The concentrated white blood cells obtained using the microfluidic separation device 805 shown in FIG. 9 are presented in Table 4.

Table 4

	I	II	III	IV	Avg.
Product	75%	69%	83%	83%	78%
Vol. Red.	9.1X	7.1X	8.1X	7.1X	7.9X
WBC conc.	7.0X	3.7X	4.2X	3.7X	4.7X

OTHER EMBODIMENTS

5 In some implementations, the whole blood can first be flowed through a microfluidic concentration device under laminar flow conditions in which the white blood cells are focused in one or more localized stream lines. The whole blood with focused white blood cells can be flowed into a multiplex microfluidic device to separate white blood cells from remaining components of the whole blood. In such

10 implementations, the microfluidic concentration device can focus the white blood cells into streams that include some red blood cells (e.g., 10%). The multiplex microfluidic device can remove the red blood cells from the white blood cells, which can then be incubated with the labeling agent. In some situations, the labeled white blood cells can be flowed through a second microfluidic concentration device to separate unlabeled

15 labeling agent from the labeled white blood cells.

 In this manner, the microfluidic concentration device can be used upstream of the multiplex microfluidic device. Because the upstream concentration device provides concentrated white blood cells from which the multiplex microfluidic device can remove residual red blood cells (and other whole blood components), the white blood cells can be

20 separated from a small volume of whole blood (e.g., 30 mL) and labeled in a short duration (e.g., one hour). Also, the number of interactions that the multiplex microfluidic device needs to deflect the white blood cells away from the residual red blood cells is decreased as the microfluidic concentration device has already concentrated the white blood cells into localized streams. Consequently, the throughput of the multiplex

25 microfluidic device can increase while its size can decrease.

In some implementations, after the microfluidic concentration device has focused the white blood cells, the cells can be mixed with the labeling agent, and flowed through the multiplex microfluidic device. In addition to separating residual red blood cells (and other whole blood components) from the focused white blood cells, the multiplex
5 microfluidic device can additionally separate unlabeled labeling agent from the labeled white blood cells. Such a device can further increase the throughput, e.g., to process 30 mL in 30 minutes.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate
10 and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of labeling white blood cells, the method comprising:
obtaining whole blood drawn from a patient;
separating the white blood cells from the whole blood by flowing the whole blood
5 through a microfluidic device that includes one or more arrays of obstacles configured to
deflect the white blood cells in a direction away from a direction of remaining
components of the whole blood; and
collecting the separated white blood cells,
wherein a buffer containing a labeling agent with an affinity for white blood cells
10 is mixed with the white blood cells either before or after separating the white blood cells
from the whole blood resulting in labeled white blood cells.

2. The method of claim 1, wherein flowing the whole blood through the microfluidic
device includes flowing the whole blood through the microfluidic device that includes:

a first channel extending from one or more inlets to one or more outlets,
15 wherein the first channel is bounded by a first side wall and a second side wall located
opposite from the first side wall;

a first array of rows of obstacles disposed within the first channel and
bounded by the first side wall and a first array boundary formed by ends of the rows of
obstacles in the first array;

20 a second array of rows of obstacles disposed within the first channel and
bounded by the second side wall and a second array boundary formed by ends of the rows
of obstacles in the second array; and

a bypass channel defined by a region devoid of obstacles between the first
array boundary and the second array boundary,

25 wherein the first array and the second array are configured to deflect the
white blood cells toward the bypass channel and separate the white blood cells from
remaining components of the whole blood.

3. The method of claim 1, further comprising infusing the labeled white blood cells into the patient.

4. The method of claim 1, wherein the labeling agent comprises a radioactive material.

5. The method of claim 1, further comprising flowing the collected labeled white blood cells in the buffer through a microfluidic concentration device that includes at least one channel at a flow rate that focuses the labeled white blood cells in the at least one channel into one or more localized stream lines, wherein each stream line defines a width that is substantially equal to or greater than a size of the focused labeled white blood cells to increase a concentration of the labeled white blood cells in the buffer relative to a concentration of the labeled white blood cells in the buffer collected from the outlet of the bypass channel.

6. The method of claim 5, wherein the labeling agent is mixed with the white blood cells after the collected labeled white blood cells are flowed through the microfluidic concentration device.

7. The method of claim 5, wherein the microfluidic concentration device includes a second channel comprising:

an inlet coupled to the outlet of the bypass channel to receive the labeled white blood cells in the buffer;

a first outlet through which substantially all of the focused labeled white blood cells in the buffer are flowed; and

a second outlet and a third outlet through which buffer that is not flowed through the first outlet is flowed,

the method further comprising:

flowing the labeled white blood cells in the buffer through the first inlet at the flow rate; and

collecting the labeled white blood cells of the higher concentration from the first outlet.

8. The method of claim 5, wherein the microfluidic concentration device includes a spiral channel that includes:

a first inlet coupled to the outlet of the bypass channel to receive the labeled white blood cells in the buffer;

5 a first outlet through which substantially all of the focused labeled white blood cells in the buffer in the buffer are flowed; and

a second outlet through which buffer that is not flowed through the first outlet is flowed,

the method further comprising:

10 flowing the labeled white blood cells in the buffer through the first inlet at the flow rate; and

collecting the labeled white blood cells of the higher concentration from the first outlet.

9. The method of claim 5, wherein the microfluidic concentration device comprises
15 a second channel arranged in parallel with the first channel, wherein the second channel includes:

an inlet coupled to the outlet of the bypass channel to receive the labeled white blood cells in the buffer;

20 a first outlet through which substantially all of the focused labeled white blood cells in the buffer are flowed; and

a second outlet and a third outlet through which buffer that is not flowed through the first outlet is flowed,

the method further comprising:

25 flowing the labeled white blood cells in the buffer simultaneously through the second channel; and

collecting the labeled white blood cells of the higher concentration from the first outlet of the second channel.

10. The method of claim 9, further comprising flowing the labeled white blood cells of higher concentration collected from the first outlet of each of the plurality of second

channels through another microfluidic concentration device that includes at least one additional channel at a flow rate that focuses the labeled white blood cells of higher concentration in the at least one additional channel into one or more localized stream lines, wherein each stream line defines a width that is substantially equal to or greater
5 than a size of the focused labeled white blood cells to increase a concentration of the labeled white blood cells in the buffer relative to a concentration of the labeled white blood cells in the buffer collected from the first outlet of each of the plurality of second channels.

11. The method of claim 5, further comprising infusing the labeled white blood cells
10 of the higher concentration into the patient.

12. The method of claim 1, further comprising:

flowing a first portion of the whole blood mixed with the buffer and the labeling agent through a first inlet located closer to the first side wall than to the bypass channel and through the first array of obstacles; and

15 flowing a remaining portion of the whole blood mixed with the buffer and the labeling agent through a second inlet located closer to the second side wall than to the bypass channel and through the second array of obstacles, wherein the first portion and the remaining portion are flowed simultaneously.

13. The method of claim 12, wherein flowing the first portion of the whole blood
20 mixed with the buffer and the labeling agent through the first inlet comprises flowing the first portion at an angle relative to the first array such that white blood cells bound to the labeling agent in the first portion are displaced out of flow streamlines upon encountering an obstacle in the first array.

14. The method of claim 1, further comprising separating labeling agent not bound to
25 white blood cells in the buffer from the labeled white blood cells in the buffer collected from the outlet of the bypass channel.

15. The method of claim 14, wherein separating the labeling agent not bound to white blood cells in the buffer from the labeled white blood cells in the buffer collected from the outlet of the bypass channel comprises flowing the labeled white blood cells in the buffer through a third array of obstacles disposed within the first channel between the first side wall and the second side wall, wherein the third array is configured to deflect the labeling agent not bound to the white blood cells in the buffer in one direction and the labeled white blood cells in the buffer in another direction.

16. The method of claim 1, wherein the first array of obstacles comprises a plurality of first obstacles that form a network of gaps and comprises at least first and second rows of the obstacles, wherein the second row is displaced relative to the first row so that a fluid passing through a gap in the first row is divided unequally when flowing around an obstacle and into two gaps on either side of the obstacle in the second row.

17. The method of claim 16, wherein the second row is displaced laterally relative to the first row.

18. A microfluidic device comprising:

a first substrate;

a first channel formed on the first substrate and bound by a first side wall and a second side wall located opposite from the first side wall, wherein the first channel extends from one or more inlets to one or more outlets;

at least three stages of arrays of obstacles disposed in series within the first channel, wherein each stage includes:

a first array of rows of obstacles bounded by the first side wall and a first array boundary formed by ends of the rows of obstacles in the first array,

a second array of rows of obstacles bounded by the second side wall and a second array boundary formed by ends of the rows of obstacles in the second array; and

a bypass channel defined by a region devoid of obstacles and spanning a length of the first channel between at least one inlet and at least one outlet, wherein the bypass channel is formed between each first array boundary and each second array boundary of

each stage of the at least three stages of arrays of obstacles,

wherein each first array and each second array are configured to deflect white blood cells in whole blood toward the bypass channel to separate labeled white blood cells from remaining components of the whole blood.

5 19. The device of claim 18, further comprising a microfluidic concentration device to concentrate the labeled white blood cells by focusing the labeled white blood cells into one or more localized stream lines.

20. The device of claim 19, wherein the microfluidic concentration device includes:
a second substrate;

10 an inlet formed in the second substrate and coupled to the outlet of the bypass channel to receive the labeled white blood cells;

a first outlet through which the focused labeled white blood cells are flowed; and
a second outlet and a third outlet through which fluid that is not flowed through the first outlet is flowed.

15 21. The device of claim 20, wherein the first substrate and the second substrate are portions of the same substrate.

22. The device of claim 19, wherein the microfluidic concentration device includes:
a second substrate;

20 a spiral channel formed in the second substrate, wherein the spiral channel includes:

a first inlet coupled to the outlet of the bypass channel to receive the labeled white blood cells in the buffer;

a first outlet through which substantially all of the focused labeled white blood cells in the buffer in the buffer are flowed; and

25 a second outlet through which buffer that is not flowed through the first outlet is flowed.

23. A method of separating monocytes from neutrophils and lymphocytes, the method comprising:

flowing a mixture comprising monocytes, neutrophils, and lymphocytes through a microfluidic device that includes:

5 a first channel extending from one or more inlets to one or more outlets, wherein the first channel is bounded by a first side wall and a second side wall located opposite from the first side wall,

a first array of rows of obstacles disposed within the first channel and bounded by the first side wall and a first array boundary formed by ends the rows of obstacles in the first array,

10 a second array of rows of obstacles disposed within the first channel and bounded by the second side wall and a second array boundary formed by ends of the rows of obstacles in the second array,

a bypass channel defined by a region devoid of obstacles between the first array boundary and the second array boundary,

15 wherein the first array and the second array are configured to deflect monocytes in the mixture toward the bypass channel and

collecting the mixture through an outlet of the microfluidic device.

24. A microfluidic method for aseptic isolation and labeling of white blood cells, the method comprising:

20 obtaining a sample of greater than 15 mL of whole blood from a patient;

aseptically isolating the white blood cells from the whole blood sample by flowing the sample through a microfluidic device that includes one or more arrays of obstacles configured to deflect the white blood cells in a direction away from a direction of remaining components of the whole blood; and

25 collecting the separated white blood cells, wherein greater than 99% of the white blood cells in the whole blood sample are collected and greater than 99% of remaining components of the whole blood sample are removed from the white blood cells,

wherein a buffer containing a labeling agent with an affinity for white blood cells

is mixed with the white blood cells either before or after separating the white blood cells from the whole blood sample resulting in labeled white blood cells for re-infusing the labeled white blood cells for in-vivo imaging.

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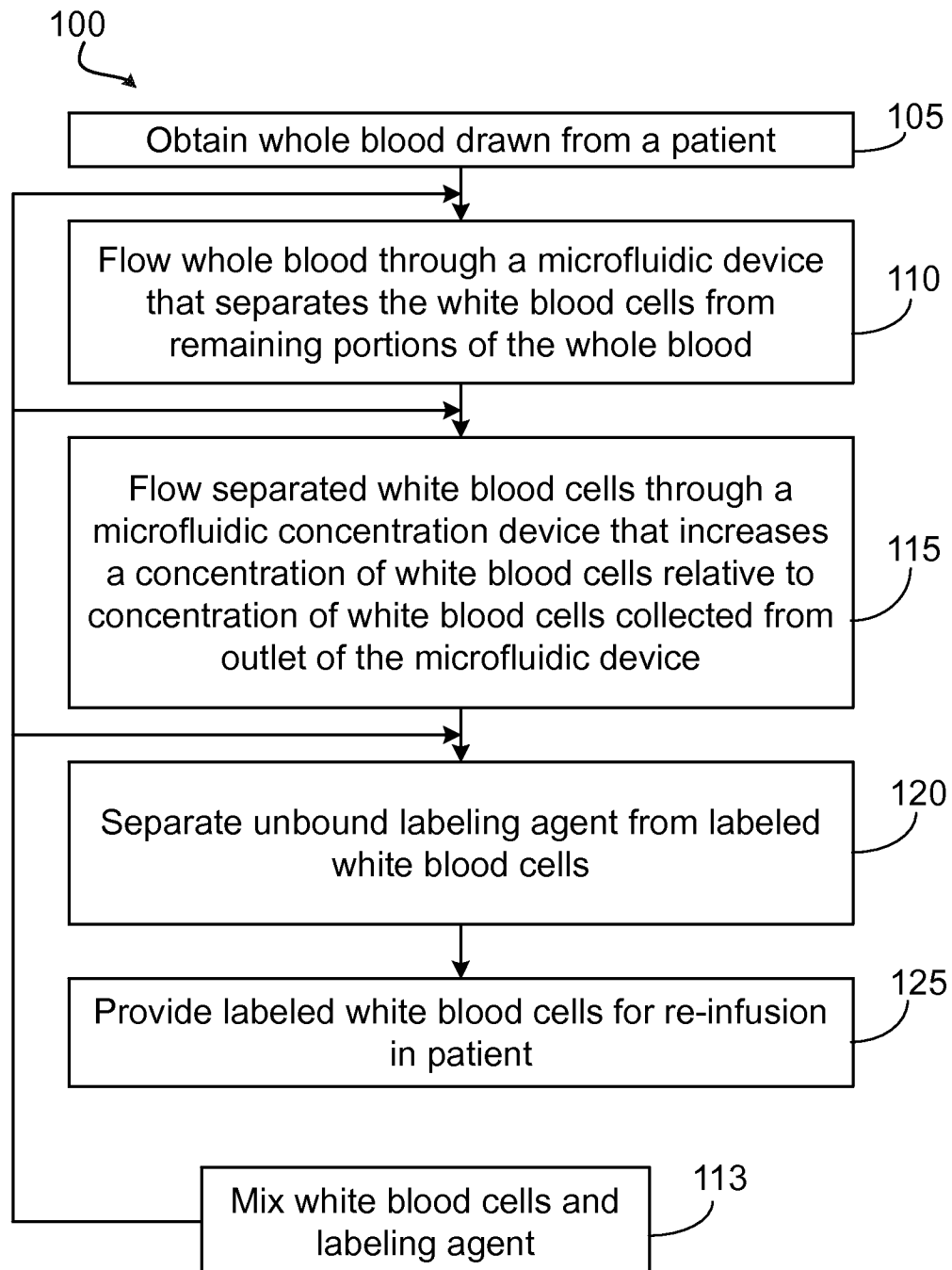


FIG. 1

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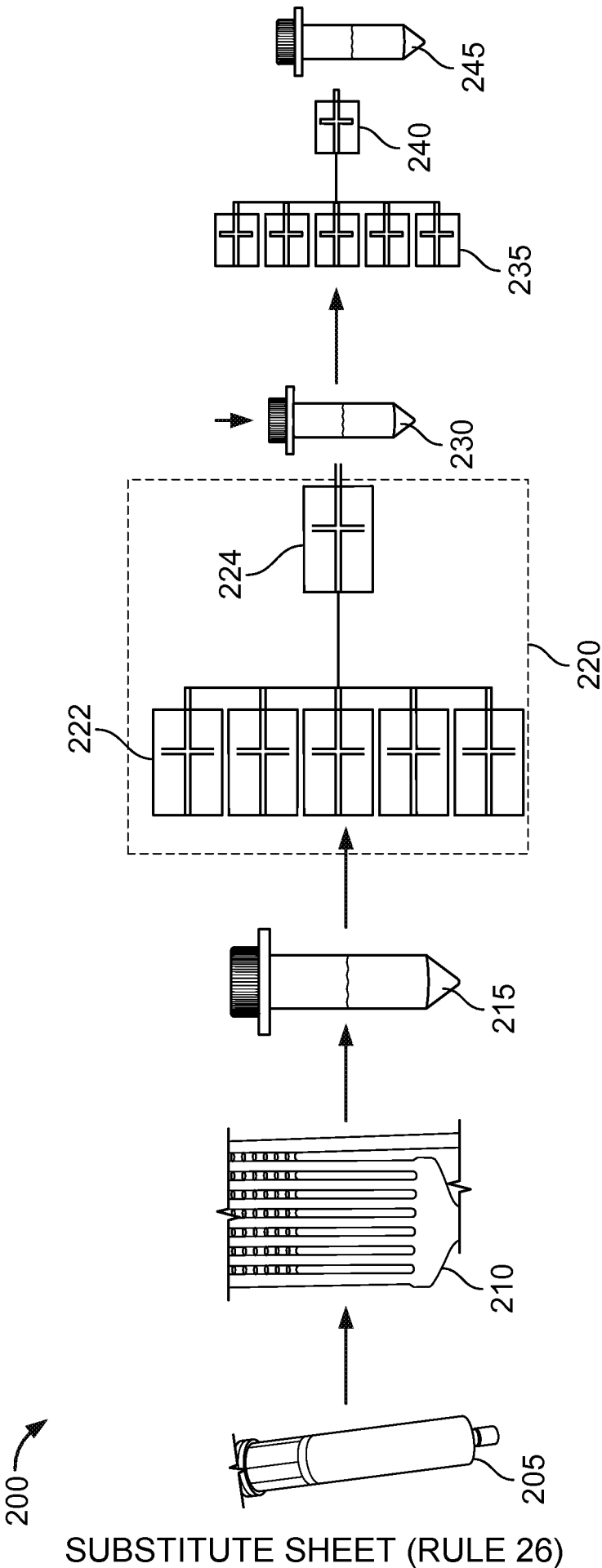


FIG. 2

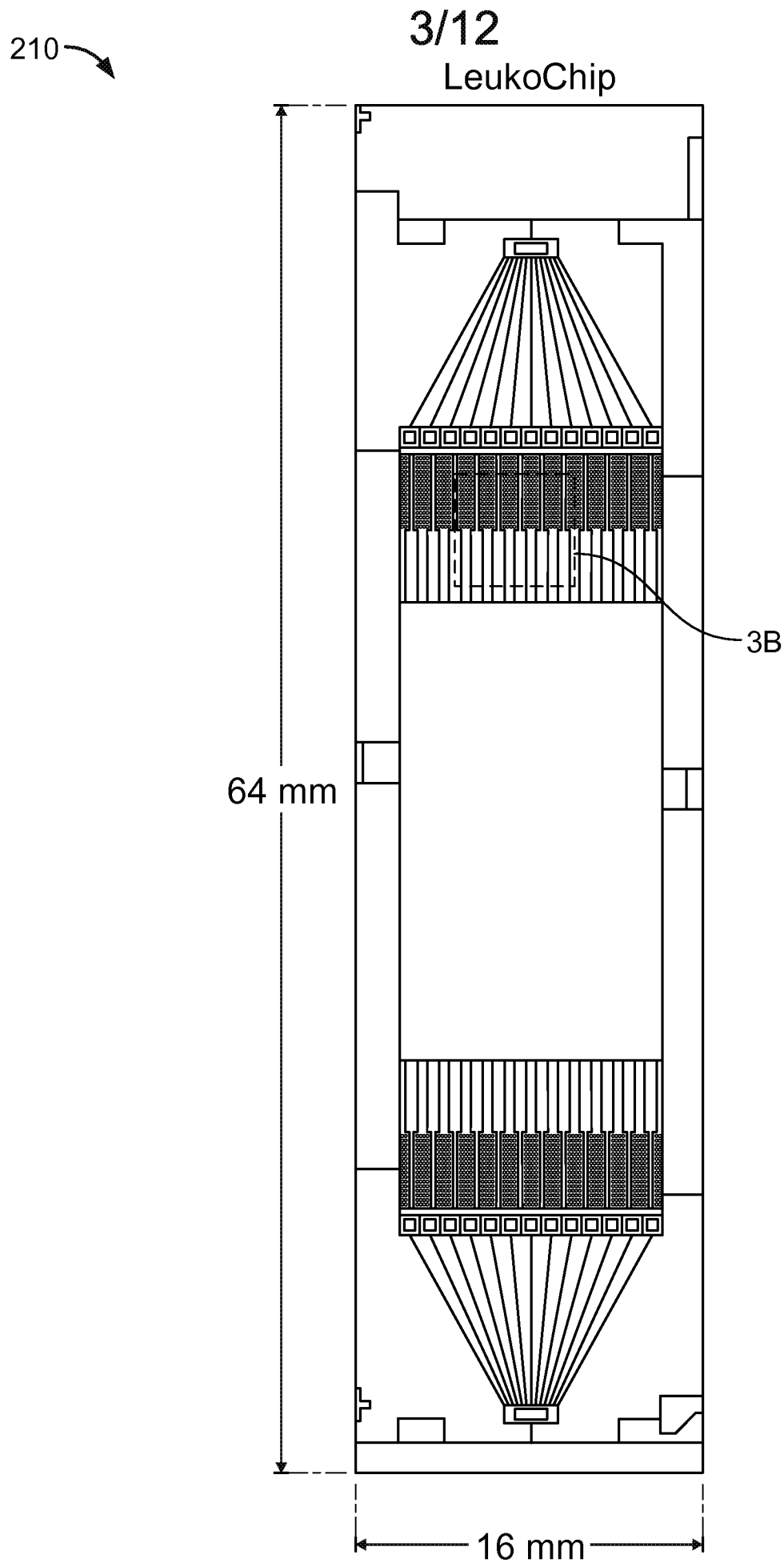


FIG. 3A

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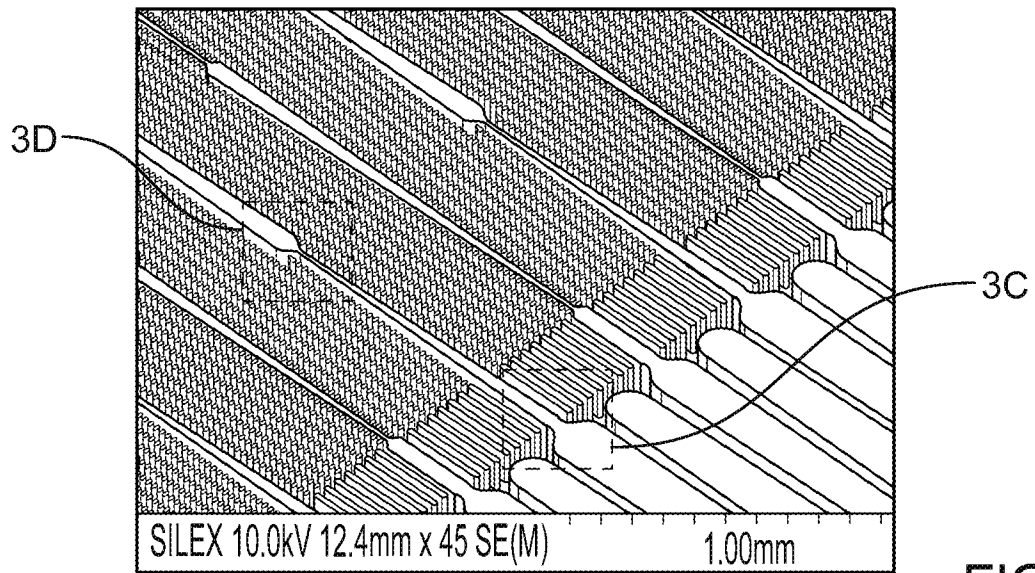


FIG. 3B

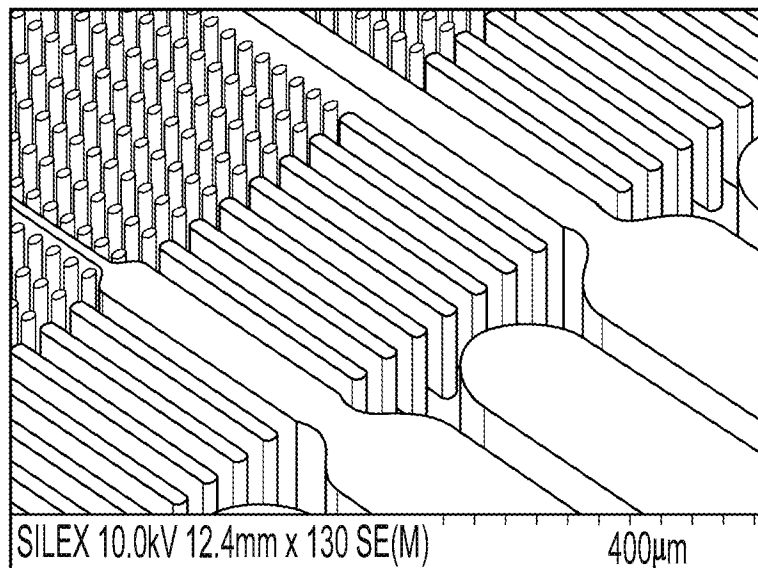


FIG. 3C

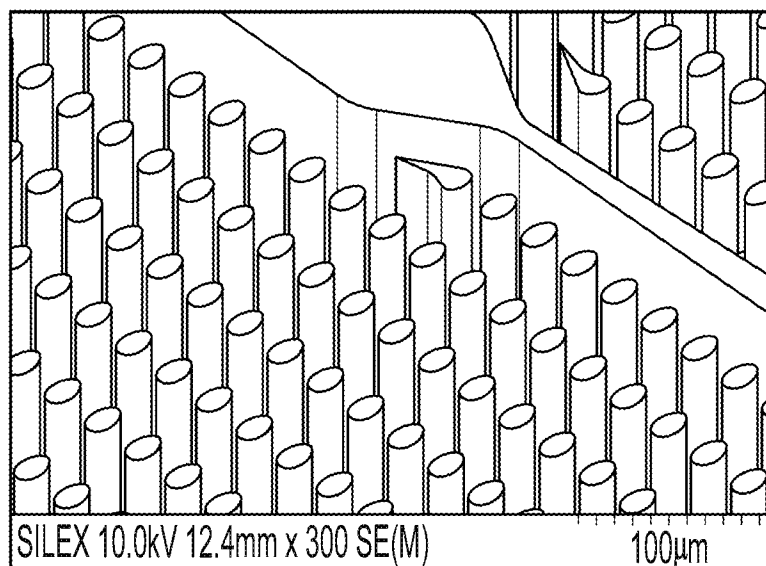


FIG. 3D

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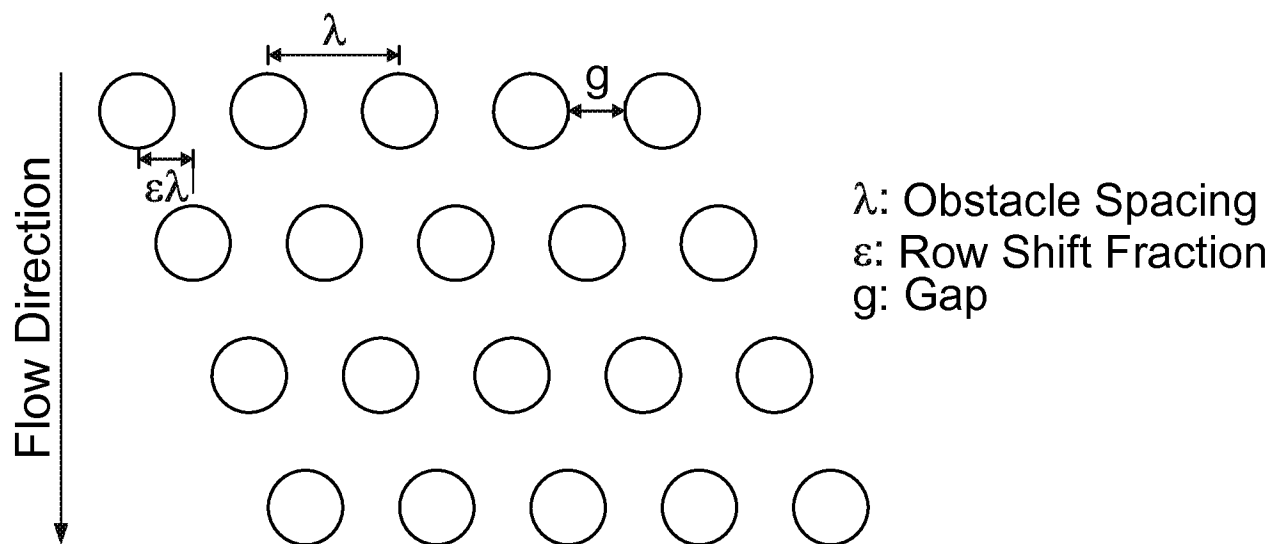


FIG. 3E

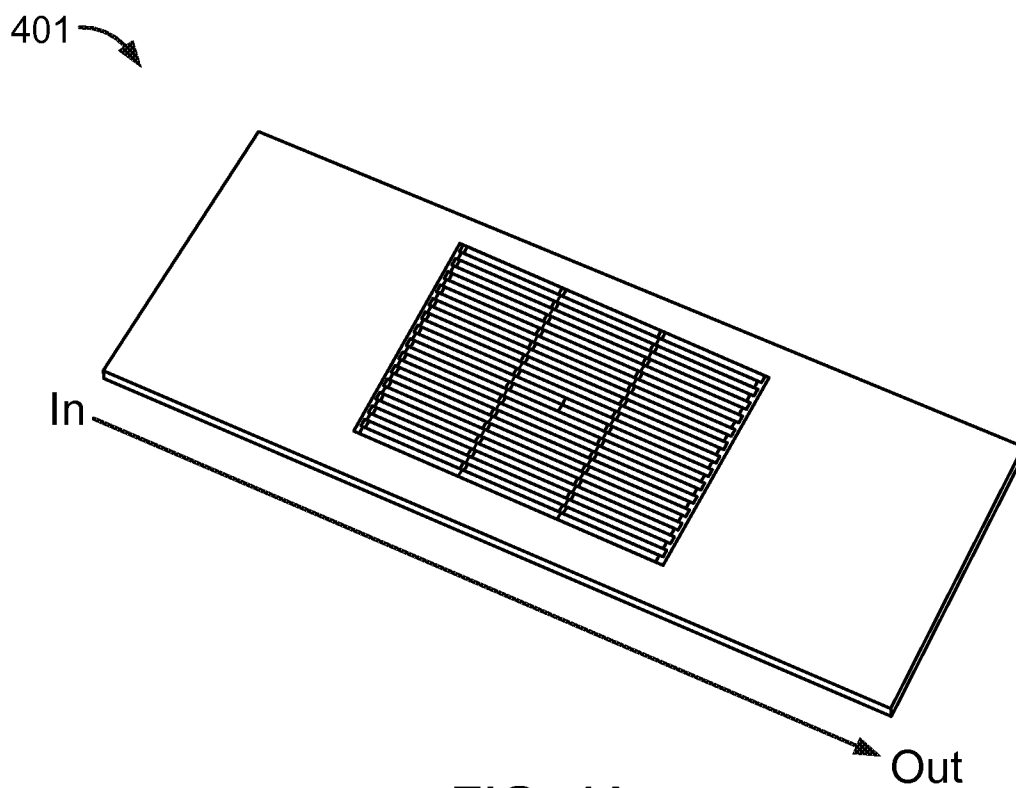


FIG. 4A

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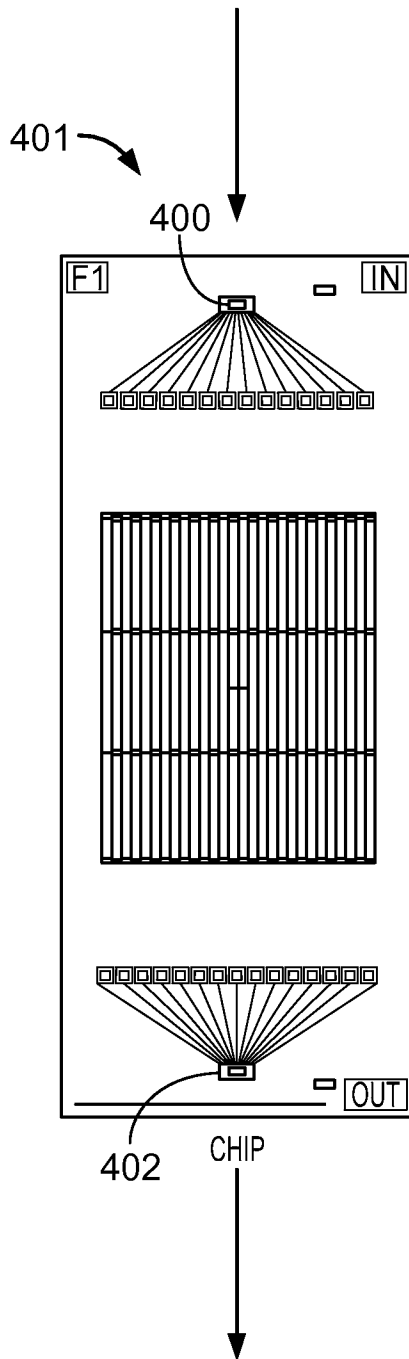


FIG. 4B

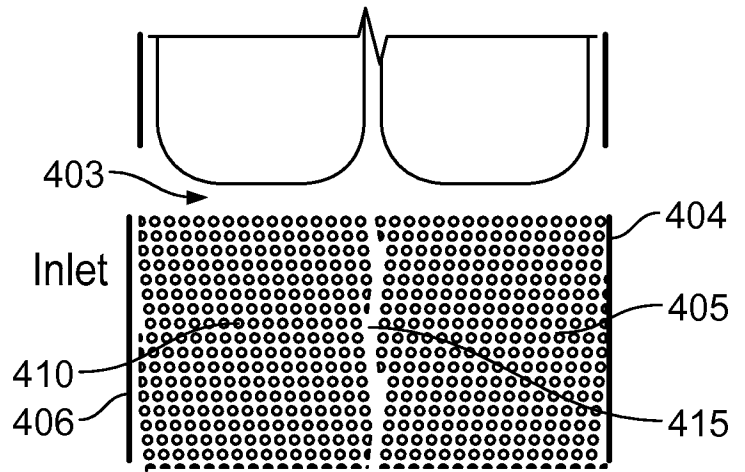


FIG. 4C

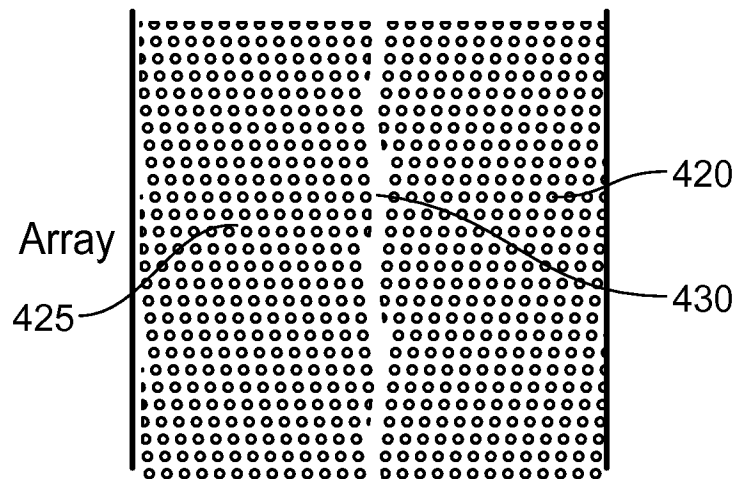


FIG. 4D

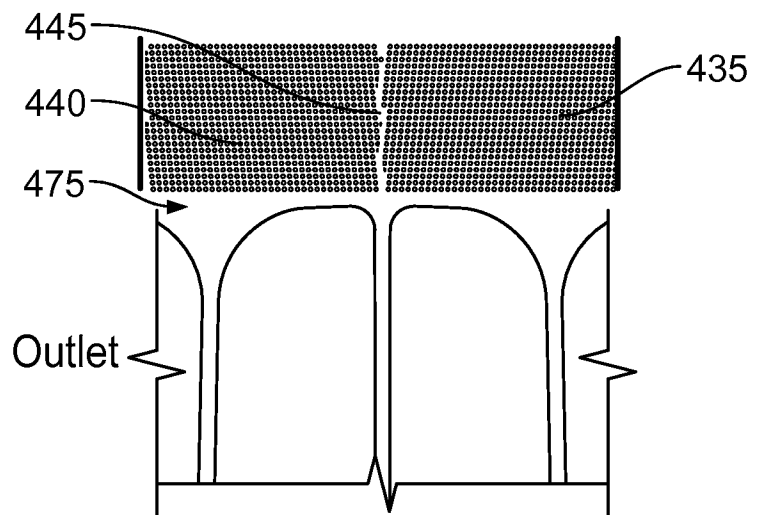


FIG. 4E

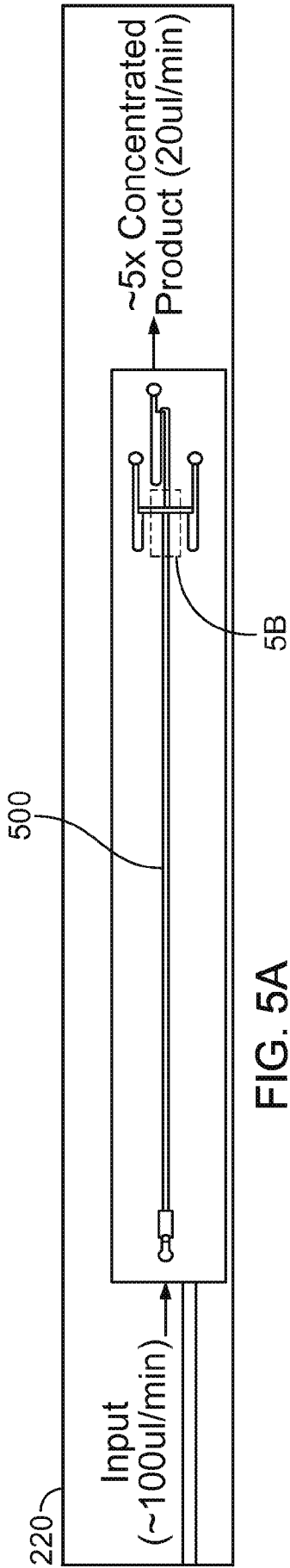


FIG. 5A

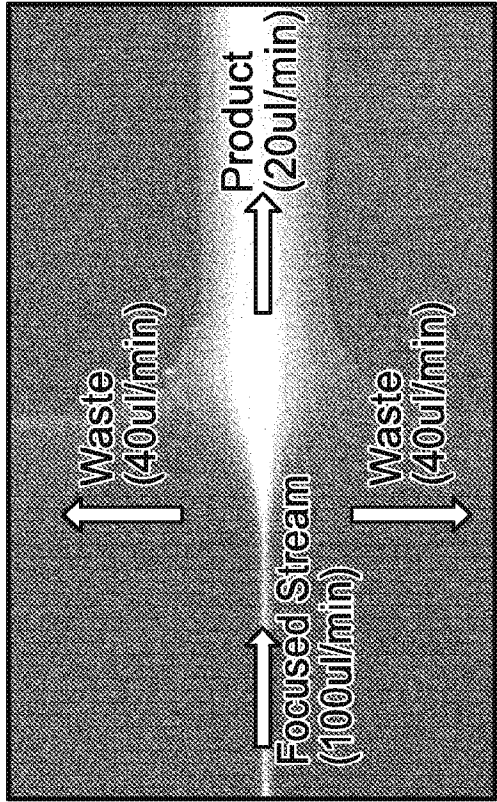


FIG. 5B

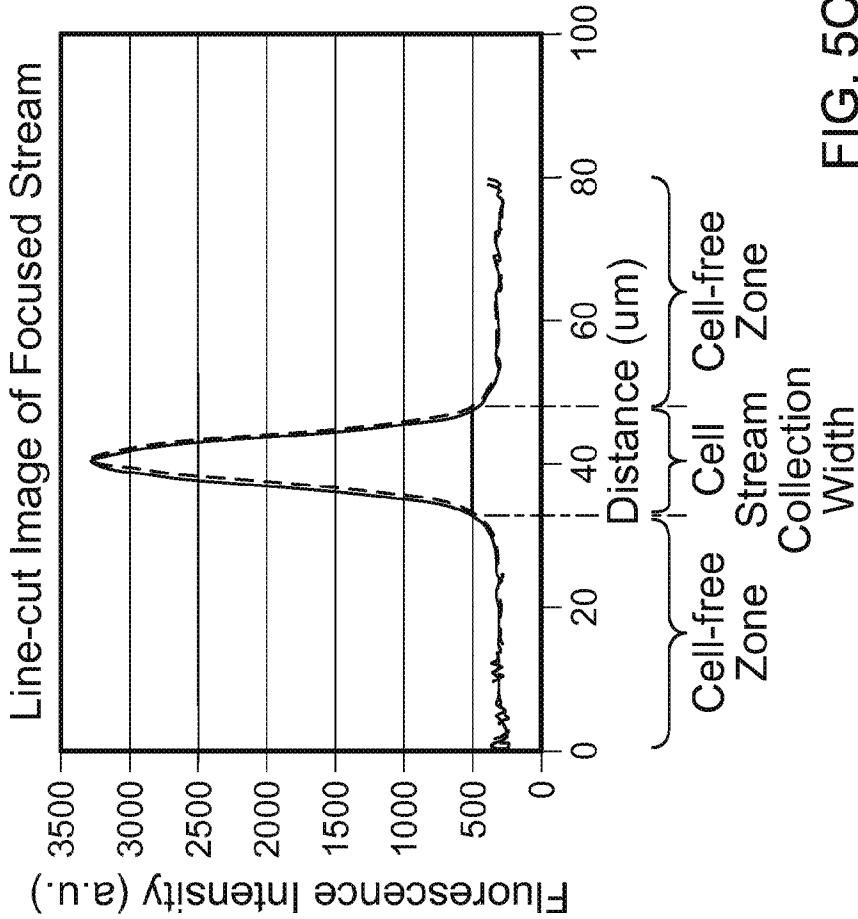


FIG. 5C

600

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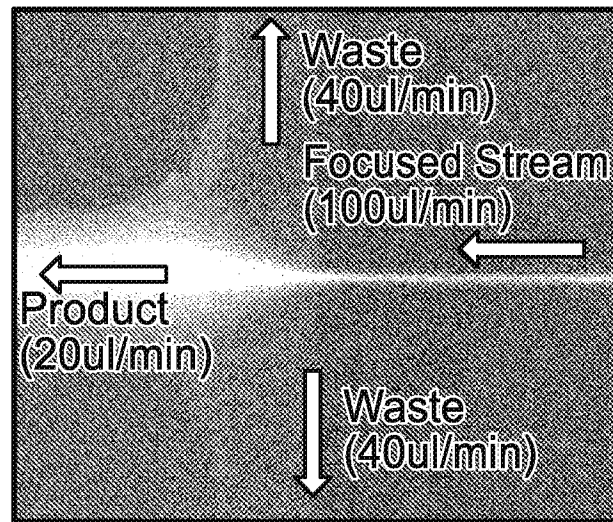


FIG. 6A

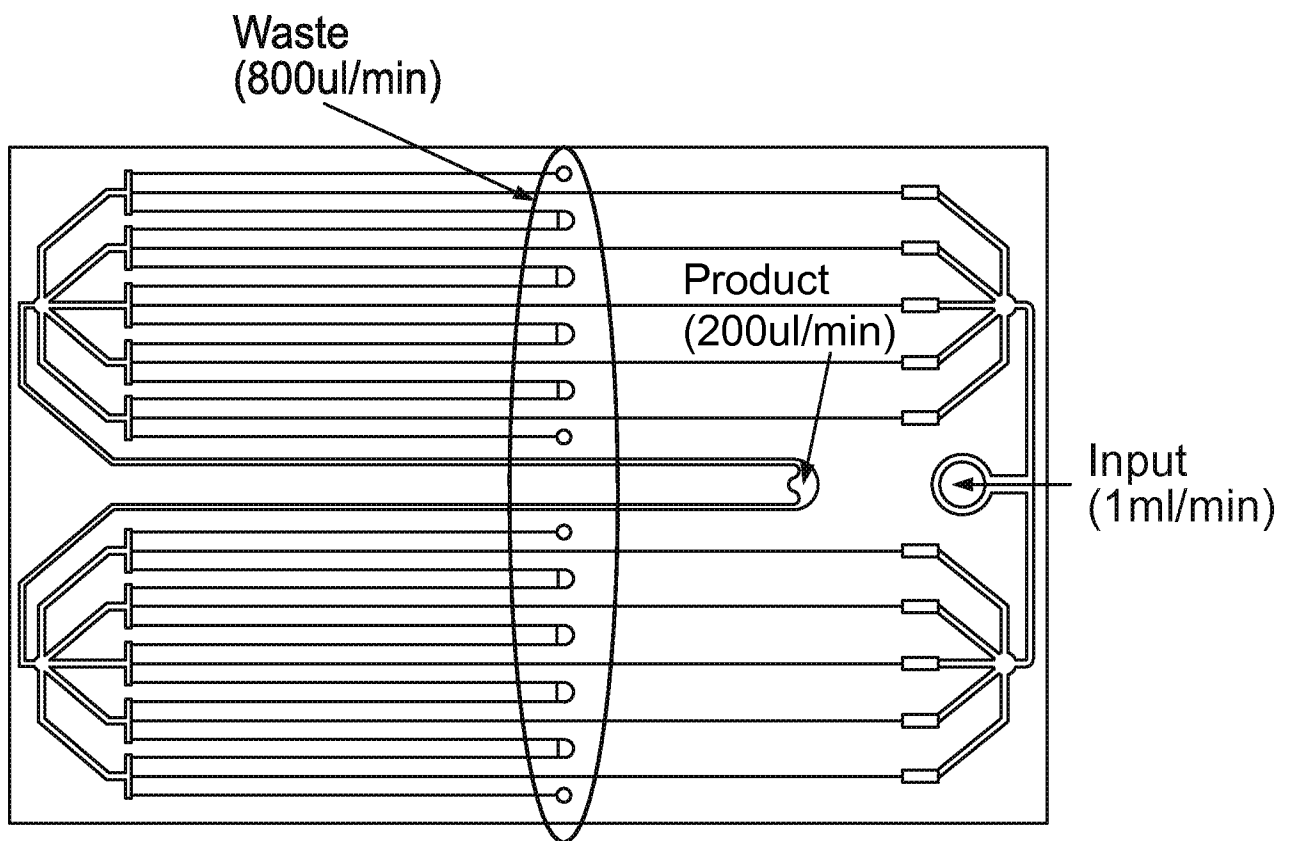


FIG. 6B

700 ↗

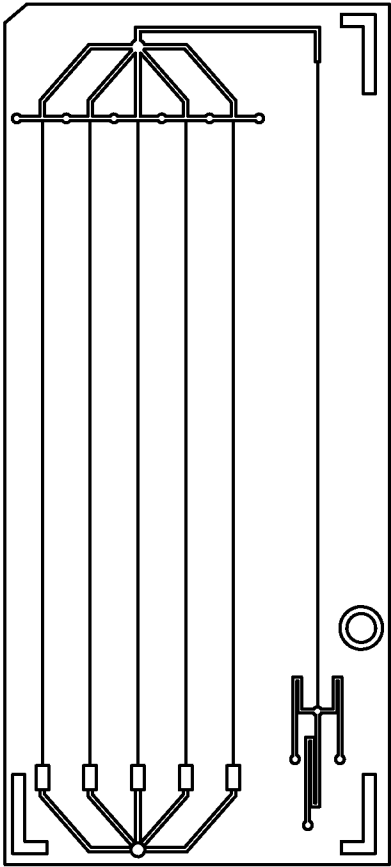


FIG. 7A

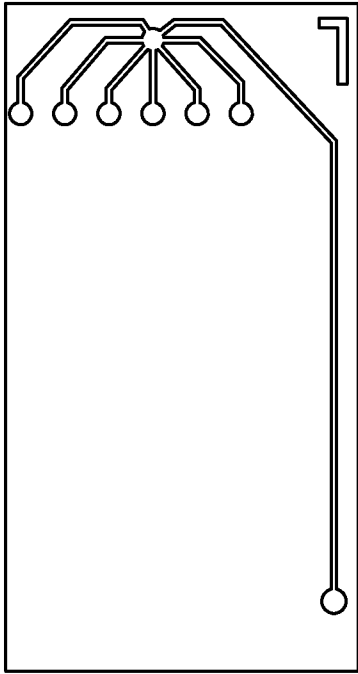
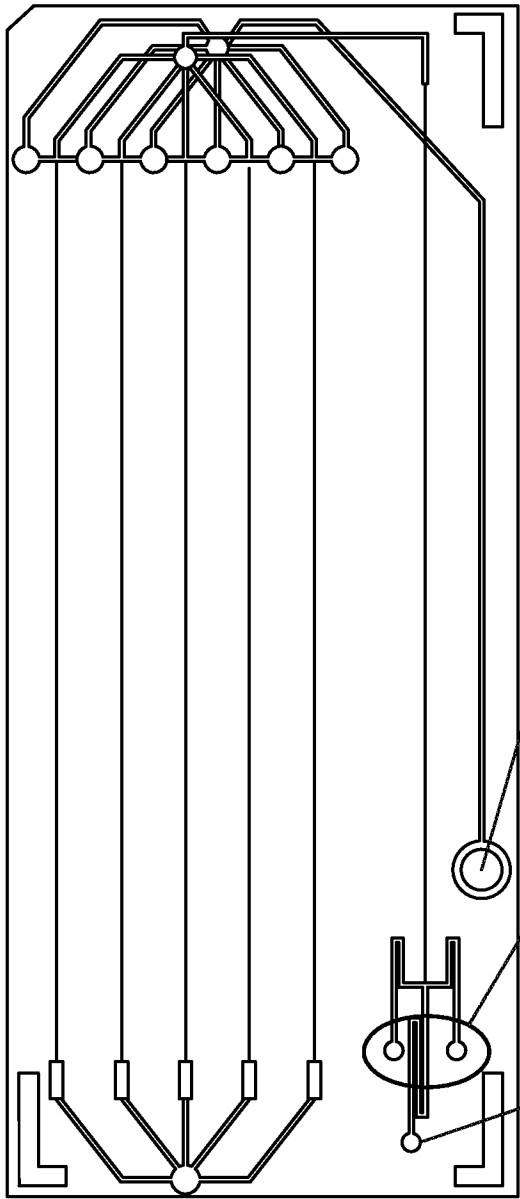


FIG. 7B



Product Waste2 Waste1

FIG. 7C

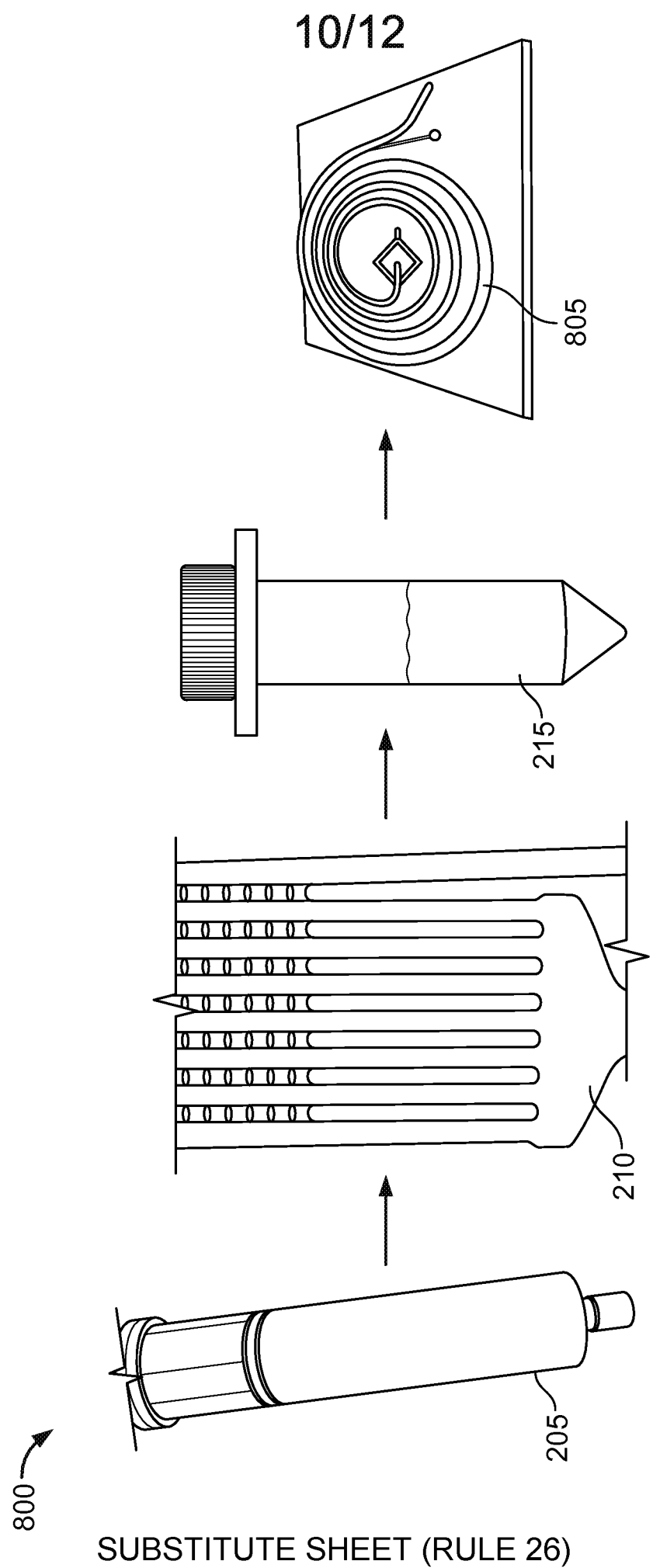
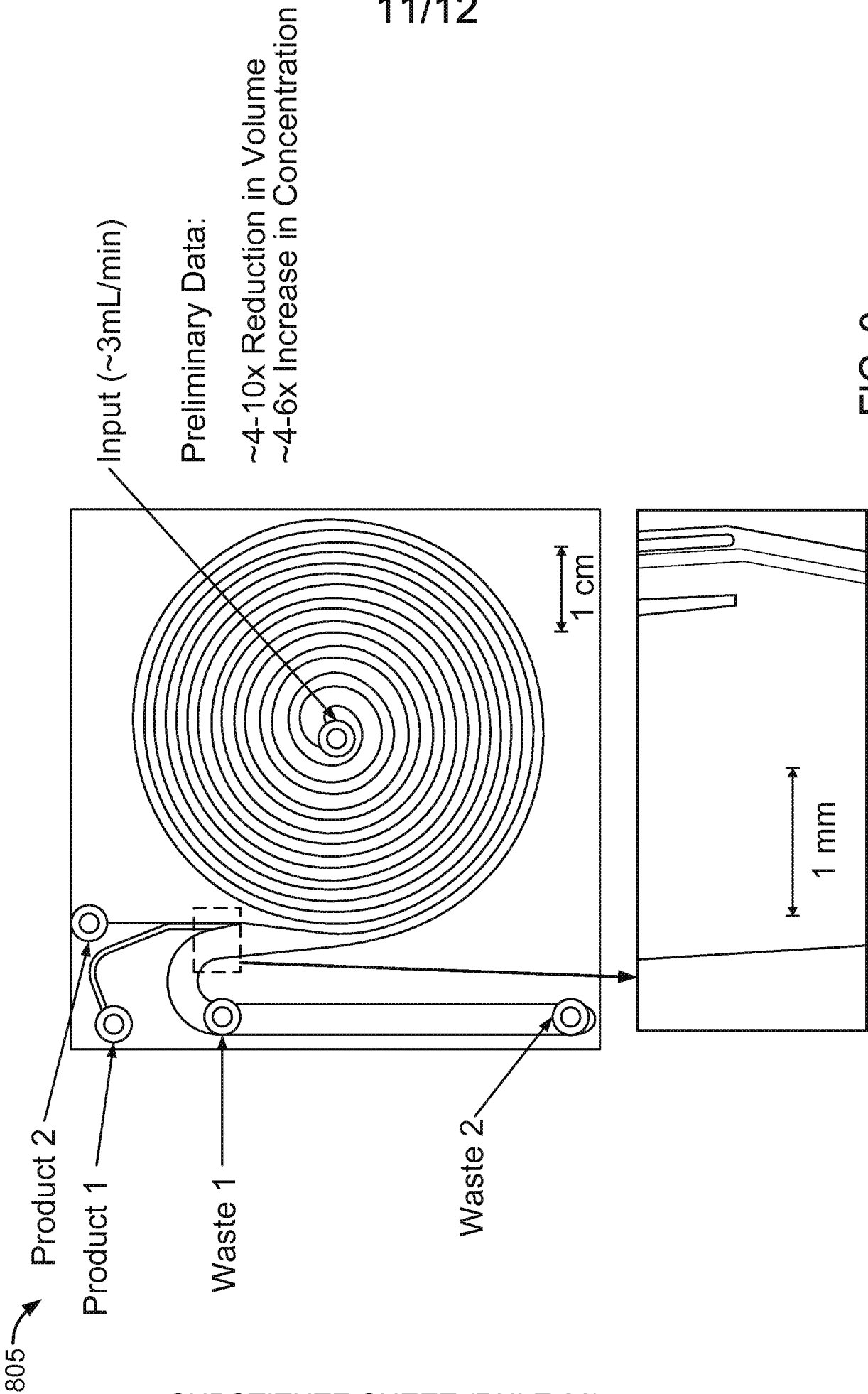


FIG. 8



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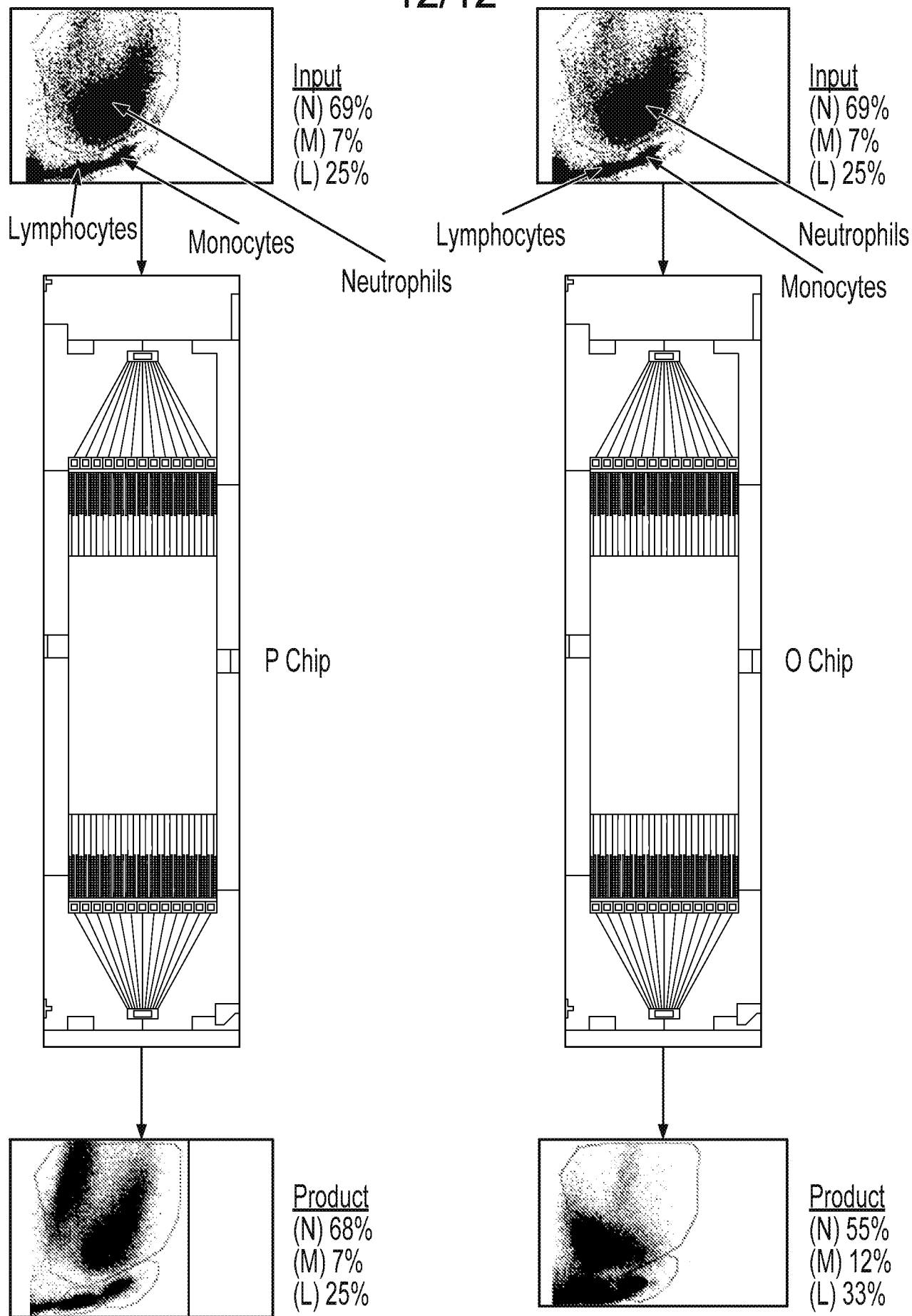


FIG. 10A

FIG. 10B