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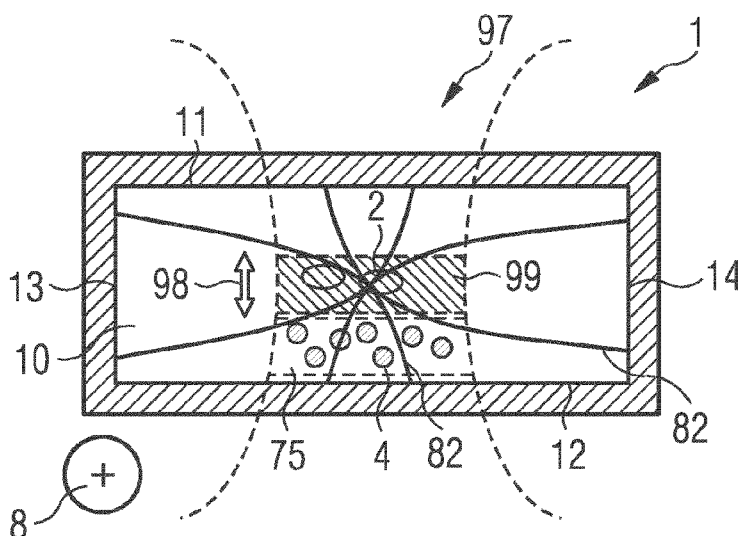
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(54) Title: A TECHNIQUE FOR SIMULTANEOUSLY SORTING AND ALIGNING A BIOLOGICAL ENTITY IN A FLOW CELL

FIG 11



(57) Abstract: A technique is presented for simultaneously sorting a first biological entity from a second biological entity and aligning the first biological entity, in a desired region within a flow chamber of a flow cell. The flow chamber has a rectangular cross-section. A bottom flow input module, a top flow input module and a sample input module provide a first fluid, a second fluid, and the sample, respectively, to the flow chamber. The sample laminarly flows sandwiched between the first and the second fluids. By controlling flow rate of the first and/or the second fluid the sample flow is aligned adjacent to but distinct from the desired region. An acoustic transducer generates a standing acoustic wave having pressure node linearly arranged along an axis passing through the desired region thus moving, i.e. sorting, and simultaneously orienting, the first biological entity from the sample flow into the desired region.

Description

A technique for simultaneously sorting and aligning a biological entity in a flow cell

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The present invention relates to techniques for sorting a first biological entity flowing in a sample having a mix of the first and a second biological entity, moving the first biological entity into a desired region of the flow cell and simultaneously orienting the first biological entity, while being moved to the desired region of the flow cell, that is to be inspected by an imaging device.

Medical technology in recent times has witnessed advent of numerous medical devices and microscopy techniques. A lot of these microscopy techniques are used for imaging microscopic specimens or samples for analyzing one or more characteristics of the sample, or more precisely for determining one or more characteristics of a component, for example leukocyte (WBC), red blood cell (RBC), etc. in the sample, for example blood sample. Examples of characteristics of the component, say WBC or RBC or other such biological entities, which may be determined, may include a volumetric measurement of the biological entity, a morphological study of the biological entity, and so on and so forth.

In general for any imaging dependant analysis, an 'in-focus' image or output from the imaging device is essential for carrying out specific and detailed analysis of the component i.e. the biological entity of the sample being studied. Furthermore, when the biological entity in the sample is non-spherical entity an orientation of the biological entity with respect to the imaging device, i.e. with respect to an imaging direction, is also essential, for example images of non spherical WBC or the RBC standing on their sides is an undesired orientation because in such orientation only sides of the WBC or the RBC are visible. However, with respect to the imaging direction, an image of the WBC or the RBC oriented

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such that a full face of the WBC or the RBC, for example one side of the disc shaped RBC, is visible is a desired orientation as in such orientation images will reveal lot more information which is essential for volumetric or morphological study of the WBC or the RBC or other such biological entities.

Furthermore, a sample may have more than one biological entity for example a first and a second biological entity and from this sample it may be desired that the first biological entity carried in the sample is studied or inspected by detecting and analyzing interference patterns formed in interferometric microscopy, for example digital holographic microscopy (DHM), representing the first biological entity. A desired result from the interferometric microscopy device may be obtained if the first biological entity is provided in a field of view (FOV) within a depth of field of the imaging device. Additionally, the first biological entity, if non-spherical is desired to be properly oriented when presented in the depth of field of the FOV of the imaging device. Moreover, a throughput of DHM device or any other imaging device, i.e. a rate of number of images or interference patterns, provided by the device, is increased if the rate of providing the first biological entity in the field of view within the depth of field of the imaging device is increased.

Providing the first biological entity in the sample as flowing in a flow cell, for example similar to the way sample is provided in flow cytometry, is an efficient way of providing the first biological entity to the imaging device to obtain a high throughput as compared to preparing the first biological entity on a slide or a solid substrate. It has several advantages for example it is easier to maintain a conformation of the first biological entity of the sample, for example the WBCs or the RBCs in the blood, in their native morphology in a fluid flow as compared to placing the entity on a slide. Furthermore, by providing the sample in a flow, the sample, and thus the entities in the sample, may be provided continu-

ously for a time period of imaging and thus a larger amount of sample, i.e. larger number of the first biological entities, may be imaged which means increased throughput and is also beneficial for statistical means as compared to scanning or imaging a smaller amount of the sample and thereby lesser numbers of the first biological entity.

However, providing the first biological entity in the sample as flowing in a flow cell has also certain disadvantages. One disadvantage that the first and the second biological entity in the sample are mixed and are presented in the depth of field of the FOV of the sample at the same time, thereby imaging results have representations from both the first and the second biological entity, whereas only the first biological entity was desired to be imaged. Simply put, in such samples, i.e. the samples having the first and the second biological entities, the first biological entity is not sorted from the second biological entity.

Another problem is that some of the first biological entities while flowing in the flow cell migrate to the walls of the flow cell and contact between the entities with the wall results into surface adhesion of the entities on the flow cell walls, or entities start disintegrating to form debris.

Yet another problem is focusing of the first biological entity in the flow cell. The first biological entity in the sample for example WBCs or RBCs in a diluted or whole blood sample flowing through the flow cell migrate to different sections of the flow cell and are not arranged in a desired region of the flow cell where the focus of the imaging device may be fixed. Since the first biological entities flow to different sections of the flow cell, some of the first biological entities of the sample in the flow cell may be either completely out of the FOV or may be in the FOV but out of focus. The first biological entities of the sample that are completely out of the FOV are not represented in the image of the interference pattern. The first biological entities of

the sample that are in the FOV but not in focus are imaged but parts or segments of the image or the interference pattern that represent such entities lack sharpness i.e. are out of focus or to say that the sharpness of segments of the interference pattern or the image representing such first biological entities are either low or not of acceptable quality or blurred.

Such first biological entities flowing as part of the sample in the flow cell or flow channel may be brought in focus by readjusting the focus of the interferometric microscopic device or the imaging device but the first biological entities of the flowing samples are dynamic so there is impractically little time to adjust the focus of the imaging device. It is a challenge to control the flow of sample in such flow cells, more particularly to control the first biological entities of the sample in the flow cell, so that the first biological entity, that is required to be imaged, are positioned or focused in a desired region of the flow cell. Furthermore, if the first biological entity is non-spherical biological entity then the first biological entity is also required to be simultaneously aligned in a desired orientation within the desired region.

Thus, there is a need to sort the first biological entity from a sample having the first and the second biological entities, to place the first biological entity within a desired region of the flow cell at which the imaging device may be focused, and to orient the first biological entity in the desired region, in short there is a need of simultaneously sorting and aligning the first biological entity, in the desired region, from the sample having mix of the first and the second biological entities.

Thus the object of the present disclosure is to provide a technique for simultaneously sorting a first biological entity from a second biological entity and aligning the first biological entity into a desired region in the flow cell.

The above object is achieved by a flow cell for simultaneously sorting a first biological entity from a second biological entity and aligning the first biological entity into a desired region in the flow cell according to claim 1, a method for simultaneously sorting the first biological entity from the second biological entity and aligning the first biological entity into the desired region in the flow cell according to claim 9, and a system for simultaneously sorting the first biological entity from the second biological entity and aligning the first biological entity into the desired region in the flow cell according to claim 16. Advantageous embodiments of the present technique are provided in dependent claims.

A first aspect of the present technique presents a flow cell for simultaneously sorting a first biological entity from a second biological entity and aligning the first biological entity into a desired region in the flow cell. The sorting of the first biological entity from the second biological entity is achieved by selectively moving at least few of the first biological entity to the desired region from a mix of the first biological entity from the second biological entity present outside the desired region. The aligning of the first biological entity in the desired region is achieved by focusing and orienting the first biological entity in the desired region. The first and the second biological entities are carried in a sample. The first biological entity is aligned in the desired region to be inspected by an imaging device. The flow cell includes a flow chamber, a bottom flow input module, a top flow input module, a sample input module and an acoustic transducer.

The flow chamber has a rectangular cross-section, a top wall, a bottom wall opposite to the top wall, a first side wall, a second side wall opposite to the first side wall and the desired region. The rectangular cross-section includes a square cross-section.

The bottom flow input module receives a first fluid and provides the first fluid to the flow chamber such that the first fluid laminarly flows in the flow chamber in form of a bottom
5 laminar flow along the bottom wall from one end of the flow chamber towards another end of the flow chamber. The bottom flow input module controls a rate of flow of the first fluid in the flow chamber. The top flow input module receives a second fluid and provides the second fluid to the flow chamber
10 such that the second fluid laminarly flows in the flow chamber in form of a top laminar flow along the top wall from one end of the flow chamber towards another end of the flow chamber. The top flow input module controls a rate of flow of the second fluid in the flow chamber.

15 The sample input module receives the sample and provides the sample to the flow chamber such that the sample laminarly flows in the flow chamber in form of a sample laminar flow from one end of the flow chamber towards another end of the
20 flow chamber. The sample laminar flow is sandwiched between the top laminar flow and the bottom laminar flow.

Further, the bottom and the top flow input modules are further configured to arrange the sample laminar flow such that
25 the sample laminar flow is aligned adjacent to and distinct from the desired region in the flow chamber.

The acoustic transducer generates a standing acoustic wave having a pressure node linearly arranged along an axis passing through the desired region. The standing acoustic wave
30 may be one dimensional or two dimensional. When the standing acoustic wave is two dimensional, the acoustic force acting along a first direction may be different, i.e. stronger or weaker, compared to the acoustic force acting along a second
35 direction.

Hereinafter, the 'rate of flow' has also been referred to as the flow rate. In the flow cell, by defining or by increasing

or by decreasing the flow rate of the first fluid, a height of the bottom laminar flow is controlled or varied. Similarly, by defining or by increasing or by decreasing the flow rate of the second fluid, a height of the top laminar flow is controlled or varied.

In the present technique, 'width' or 'height' have been interchangeably used for any laminar flow, not including the sample laminar flow, and mean an extension of that laminar flow along the rectangular cross-section of the flow chamber from a wall of the flow chamber along which the laminar flow is aligned towards the opposite wall, for example 'width' or 'height' of the bottom laminar flow means an extension of the bottom laminar flow along the rectangular cross-section of the flow chamber from the bottom wall of the flow chamber towards the top wall of the flow chamber. Similarly 'width' or 'height' of the top laminar flow means an extension of the top laminar flow along the rectangular cross-section of the flow chamber from the top wall of the flow chamber towards the bottom wall of the flow chamber.

For the sample laminar flow, width means an extension of the sample laminar flow along the rectangular cross-section of the flow chamber between the first and the second side walls. For the sample laminar flow, height means an extension of the sample laminar flow along the rectangular cross-section of the flow chamber between the top and the bottom walls. For the sample laminar flow, 'lateral position' means a location of a cross-section of the sample laminar flow along the rectangular cross-section of the flow chamber between the first and the second side walls, and 'longitudinal position' means a location of the cross-section of the sample laminar flow along the rectangular cross-section of the flow chamber between the top and the bottom walls.

In the flow cell, by controlling or varying the height of the bottom laminar flow and/or the top laminar flow, the width and/or the height and/or the longitudinal position of the

sample laminar flow is controlled or varied. By defining the width and/or the height and/or the longitudinal position of the sample laminar flow, the sample laminar flow is positioned adjacent to the desired region of the flow cell by
5 moving the sample laminar flow between the desired region and the top and/or the bottom walls. The imaging device is focused in the desired region. A depth of field of the imaging device may be confined within the desired region, or a depth of field of the imaging device with in the flow cell may define the desired region.
10

Since the sample having the first and the second biological entities flows adjacent to but physically removed from the desired region, neither the first nor the second biological
15 entities are in the desired region and thus not in focus of the imaging device. However, due to the pressure node of the standing wave being positioned in the desired region the first and the second biological entities have a tendency to move to the desired region, however owing to differences in
20 size i.e. including a mass and a volume, and conformation of the first and the second biological entities, the physical force on the first biological entity due to the standing wave is greater than that on the second biological entity, and thus the first biological entity moves to the desired region,
25 under the influence of the standing wave, faster compared to the second biological entity. Thus at least a few of the first biological entity are sorted, by moving to the desired region, from a mix of the first and the second biological entity.

30 In an alternative embodiment to the aforementioned embodiment, the first and the second biological entities may be one and the same biological entity, i.e. having similar size i.e. including a mass and a volume, and conformation. In this case
35 due to the pressure node of the standing wave being positioned in the desired region the entities that are physically closer to the desired will move to the desired region faster than the entities that are physically farther away from the

desired region, and thus the entities that reach the desired region, under the influence of the standing wave, faster are sorted, by moving to the desired region.

5 Since the focus of the imaging device is in the desired region, the first biological entity once in the desired region is in focus of the imaging device. Furthermore, a differential pressure is formed by the standing acoustic wave at different parts of the flow chamber and especially towards the
10 pressure node and this differential pressure acts on the non-spherical biological entity to orient the non-spherical biological entity carried in the sample into the desired region, and thus if the first biological entity has a non-spherical shape, then the first biological entity also gets aligned
15 simultaneously as it is sorted.

In an embodiment of the flow cell, the flow cell includes a first side flow input module. The first side flow input module receives a first side fluid and provides the first side
20 fluid to the flow chamber such that the first side fluid laminarly flows in the flow chamber in form of a first side laminar flow moving from the one end of the flow chamber towards the another end of the flow chamber. The first side laminar flow is sandwiched between the top laminar flow and
25 the bottom laminar flow and between the first side wall and the sample laminar flow. The first side flow input module controls a rate of flow of the first side fluid in the flow chamber.

30 The 'width' of the first side laminar flow means an extension of the first side laminar flow along the rectangular cross-section of the flow chamber from the first side wall of the flow chamber towards the second side wall of the flow chamber. In the flow cell, by controlling or varying the width of
35 the first side laminar flow, the width and/or the height and/or the lateral position of the sample laminar flow is controlled or varied in the flow chamber i.e. by moving the sample laminar flow between the desired region and the first

side wall. By defining the width and/or the height and/or the lateral position of the sample laminar flow, the sample laminar flow is placed adjacent to the desired region of the flow cell.

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In another embodiment of the flow cell, the flow cell includes a second side flow input module. The second side flow input module receives a second side fluid and provides the second side fluid to the flow chamber such that the second side fluid laminarly flows in the flow chamber in form of a second side laminar flow moving from the one end of the flow chamber towards the another end of the flow chamber. The second side laminar flow is sandwiched between the top laminar flow and the bottom laminar flow and between the second side wall and the sample laminar flow. The second side flow input module controls a rate of flow of the second side fluid in the flow chamber.

The 'width' of the second side laminar flow means an extension of the second side laminar flow along the rectangular cross-section of the flow chamber from the second side wall of the flow chamber towards the first side wall of the flow chamber. In the flow cell, by controlling or varying the width of the second side laminar flow, the width and/or the height and/or the lateral position of the sample laminar flow is controlled or varied in the flow chamber i.e. by moving the sample laminar flow between the desired region and the second side wall. By defining the width and/or the height and/or the lateral position of the sample laminar flow, the sample laminar flow is positioned adjacent to the desired region in the flow cell.

The first and the second side fluids may be provided either simultaneously or sequentially in any order.

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In another embodiment of the flow cell, the sample input module controls a rate of flow of the sample in the flow chamber. Thus amount of sample forming the sample laminar flow is

controlled, which in turn contributes to the width and/or the height of the sample laminar flow.

In another embodiment of the flow cell, the flow chamber is a
5 microfluidic channel. Thus the flow cell is compact.

In another embodiment of the flow cell, the acoustic transducer is an ultrasonic transducer and wherein the standing acoustic wave is a standing ultrasonic wave. This provides a
10 simple way of implementing the present technique.

In another embodiment of the flow cell, the ultrasonic transducer is a piezoelectric ceramic. The piezoelectric ceramic provides a simple way of implementing the present technique.
15 In a favorable embodiment the piezoelectric ceramic has a hole in the center area, especially when the flow cell is used for transmission microscopy. The piezoelectric ceramic is positioned onto the top or bottom of the microfluidic channel within the flow cell such that the probing radiation
20 from the imaging device, such as transmission microscope, passed through the hole in the piezoelectric ceramic. This at least partially reduces the exponential drop of acoustic force at the edge of the piezoelectric ceramic without the hole. The piezoelectric ceramic may comprise several holes to
25 perform transmission microscopy on multiple sites along the flow cell.

A second aspect of the present technique presents a method for simultaneously sorting a first biological entity from a
30 second biological entity and aligning the first biological entity into a desired region in the flow cell. The first and the second biological entities are carried in a sample. The first biological entity is to be aligned in the desired region to be inspected by an imaging device having a depth of
35 field in a field of view of the imaging device. The flow cell includes a flow chamber having a rectangular cross-section, a top wall, a bottom wall opposite to the top wall, a first

side wall, a second side wall opposite to the first side wall and the desired region.

In the method, a first fluid is provided to the flow chamber
5 such that the first fluid laminarly flows in the flow chamber
in form of a bottom laminar flow along the bottom wall from
one end of the flow chamber towards another end of the flow
chamber. Simultaneously along with or subsequent to the above
mentioned step, in the method, a second fluid is provided to
10 the flow chamber such that the second fluid laminarly flows
in the flow chamber in form of a top laminar flow along the
top wall from the one end of the flow chamber towards the an-
other end of the flow chamber.

15 Simultaneously along with or subsequent to the above men-
tioned step, in the method, the sample is provided to the
flow chamber such that the sample along with the one or more
non-spherical biological entity laminarly flows in the flow
chamber in form of a sample laminar flow from the one end of
20 the flow chamber towards the another end of the flow chamber
and wherein the sample laminar flow is sandwiched between the
top laminar flow and the bottom laminar flow.

In the method, a rate of flow of the first fluid and/or a
25 rate of flow of the second fluid in the flow chamber is con-
trolled in order to arrange the sample laminar flow such that
the sample laminar flow is aligned adjacent to and distinct
from the desired region in the flow chamber. The desired re-
gion is aligned with the depth of field in the field of view
30 of the imaging device. In the method, a standing acoustic
wave having a pressure node is generated such that the pres-
sure node of the standing acoustic wave is linearly arranged
along an axis that passes through the desired region. At
least a part of the standing acoustic wave passes through the
35 sample laminar flow to generate a relative movement of the
first biological entity towards the axis with respect to the
second biological entity. The differential pressure formed by
the standing acoustic wave at different parts of the flow

chamber and specially towards the pressure node acts on the first biological entity, if the first biological entity is non-spherical, to orient the first biological entity carried in the sample into the desired region.

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In the method, by defining or by increasing or by decreasing the flow rate of the first fluid, the height of the bottom laminar flow in the flow cell is controlled or varied. Similarly, by defining or by increasing or by decreasing the flow rate of the second fluid, the height of the top laminar flow in the flow cell is controlled or varied. By controlling or varying the height of the bottom and the top laminar flow, the width and/or the height and/or the longitudinal position of the sample laminar flow carrying the first and the second biological entity is controlled or varied. By defining the width and/or the height and/or the longitudinal position of the sample laminar flow, the sample laminar flow, and thus the first and the second biological entities are arranged adjacent to but distinct from the desired region. However, at least a few of the first biological entities under the influence of the standing acoustic wave move towards the pressure node of the standing acoustic wave and thus into the desired region. Once the first biological entities are in the desired region they are focused because the imaging device is arranged such that the focus of the imaging device is in the desired region of the flow cell. Furthermore, if the first biological entity is non-spherical in shape, the aligning of the first biological entity in the desired region is achieved under the influence of the standing acoustic wave in the desired region.

30

In an embodiment of the method, a first side fluid is provided to the flow chamber such that the first side fluid laminarly flows in the flow chamber in form of a first side laminar flow moving from the one end of the flow chamber towards the another end of the flow chamber. The first side laminar flow is sandwiched between the top laminar flow and the bottom laminar flow and between the first side wall and

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the sample laminar flow. Furthermore, a rate of flow of the first side fluid in the flow chamber is controlled. In the method, by controlling or varying the width of the first side laminar flow, the width and/or the height and/or the lateral position of the sample laminar flow is controlled or varied in the flow chamber i.e. by moving the sample laminar flow between the desired region and the first side wall. By defining the width and/or the height and/or the lateral position of the sample laminar flow, the sample laminar flow is moved into or positioned adjacent to the desired region of the flow cell.

In another embodiment of the method, a second side fluid is provided to the flow chamber such that the second side fluid laminarly flows in the flow chamber in form of a second side laminar flow moving from the one end of the flow chamber towards the another end of the flow chamber. The second side laminar flow is sandwiched between the top laminar flow and the bottom laminar flow and between the second side wall and the sample laminar flow. Furthermore, a rate of flow of the second side fluid in the flow chamber is controlled. In the method, by controlling or varying the width of the second side laminar flow, the width and/or the height and/or the lateral position of the sample laminar flow is controlled or varied in the flow chamber i.e. by moving the sample laminar flow between the desired region and the second side wall. By defining the width and/or the height and/or the lateral position of the sample laminar flow, the sample laminar flow is moved into or positioned adjacent to the desired region of the flow cell.

The first and the second side fluids may be provided either simultaneously or sequentially in any order.

In another embodiment of the method, the standing acoustic wave is a standing ultrasonic wave. This provides a simple way of implementing the present method.

In another embodiment of the method, the first biological entity is a leukocyte and the second biological entity is an erythrocyte. Thus the method is used to sort the leukocyte from the erythrocyte and to orient the sorted leucocytes in the desired region and hence the focus of the imaging device.

In another embodiment of the method, the first and the second biological entities are erythrocytes positioned at different distances with respect to the axis. Thus the method is used to sort the erythrocytes that are physically positioned closer to the axis i.e. the first biological entity from the erythrocyte that are physically positioned farther from the axis i.e. the second biological entity. Furthermore, aligning the first biological entity into the desired region includes orienting the first biological entity in the desired region, and thus the sorted erythrocytes are oriented in the desired region and hence in the focus of the imaging device.

A third aspect of the present technique presents a system for simultaneously sorting a first biological entity from a second biological entity and aligning the first biological entity into a desired region in the flow cell. The first and the second biological entities are carried in a sample. The system includes an imaging device and a flow cell. The imaging device has a field of view and the field of view includes a depth of field. The flow cell is same as the flow cell according to the first aspect of the present technique. The sample laminar flow is configured to be arranged such that the sample laminar flow is aligned adjacent to and distinct from the desired region in the flow chamber.

In an embodiment of the system, the imaging device is an interferometry microscopy device. Thus the sorting, focusing and/or aligning of the first biological entity in the depth of field of the interferometry microscopy device is achieved and this in turn leads to obtaining of high quality or focused images of the first biological entity in the desired orientation of the first biological entity which then may be

used for post imaging analysis for example volumetric measurements of components of the first biological entity, morphological studies of the contents of the first biological entity, and so and so forth.

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In another embodiment of the system, the interferometry microscopy device is a digital holographic microscopy device. This presents an advantageous example of interferometry microscopy device that may be used to image the first biological entity without requiring complex sample preparation.

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The present technique is further described hereinafter with reference to illustrated embodiments shown in the accompanying drawing, in which:

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FIG 1 schematically illustrates an exemplary embodiment of a system of the present technique;

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FIG 2 schematically illustrates an exemplary embodiment of a flow cell;

FIG 3 schematically illustrates the exemplary embodiment of the flow cell of FIG 2 with a sample flowing;

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FIG 4 schematically illustrates another exemplary embodiment of the flow cell of the present technique;

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FIG 5 schematically illustrates an exemplary embodiment of the flow cell depicting a bottom laminar flow and a top laminar flow;

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FIG 6 schematically illustrates the embodiment of the flow cell of FIG 5 depicting an exemplary scheme for working of the flow cell;

FIG 7 schematically illustrates an exemplary embodiment of the flow cell depicting a first side laminar flow and a second side laminar flow;

FIG 8 schematically illustrates the embodiment of the
 flow cell of FIG 7 depicting an exemplary scheme
 for working of the flow cell;

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FIG 9 schematically illustrates another exemplary embodi-
 ment of the flow cell of the present technique de-
 picting an exemplary standing acoustic wave;

10 FIG 10 schematically illustrates an exemplary embodiment
 of the flow cell of the present technique depicting
 a instance where a sample flow has been aligned ad-
 jacent to and distinct from a desired region;

15 FIG 11 schematically illustrates the exemplary embodiment
 of the flow of sample of FIG 9 depicting action of
 an exemplary standing acoustic wave;

20 FIG 12 schematically illustrates an exemplary view of
 working of the flow cell where the sample flow is
 aligned in the desired region;

25 FIG 13 schematically illustrates another exemplary view of
 working of the flow cell where the sample flow is
 being moved to be aligned adjacent to the desired
 region;

30 FIG 14 schematically illustrates another exemplary view of
 working of the flow cell where the sample flow has
 been moved to be aligned adjacent to and distinct
 from the desired region;

35 FIG 15 schematically illustrates a view of the first bio-
 logical entity in an undesired orientation with re-
 spect to the direction of imaging;

FIG 16 schematically illustrates a view of the first biological entity in the orientation depicted in FIG 15;

5 FIG 17 schematically illustrates a view of the first biological entity in a desired orientation with respect to the direction of imaging;

10 FIG 18 schematically illustrates a view of the first biological entity in the orientation depicted in FIG 17; and

15 FIG 19 schematically illustrates a hole in the piezoelectric ceramic in the flow cell; in accordance with aspects of the present technique.

Hereinafter, above-mentioned and other features of the present technique are described in details. Various embodiments are described with reference to the drawing, wherein like
20 reference numerals are used to refer to like elements throughout. In the following description, for purpose of explanation, numerous specific details are set forth in order to provide a thorough understanding of one or more embodiments. It may be noted that the illustrated embodiments are
25 intended to explain, and not to limit the invention. It may be evident that such embodiments may be practiced without these specific details.

It may be noted that in the present disclosure, the terms
30 "first", "second", etc. are used herein only to facilitate discussion, and carry no particular temporal or chronological significance unless otherwise indicated.

The basic idea of the present technique is to sort a first
35 biological entity from a second biological entity and to align the first biological entity in a desired region of a flow cell. The sorting and aligning of the first biological entity is done simultaneously. Aligning includes focusing the

first biological entity in the desired region in a flow chamber of the flow cell and orienting the first biological entity, especially when the first biological entity is non-spherical biological entity, in the desired region in the flow chamber of the flow cell.

In the technique the flow cell with the flow chamber having a rectangular cross-section is provided. In the flow chamber of such flow cell, the sample with its components, i.e. the first and the second biological entities such as a mix of WBC and RBC, a mix of different types of WBCs, a mix of several RBCs, is flowed in a laminar flow. The laminarly flowing sample is sandwiched at least between two laminar flows, for example a top and a bottom flow. By regulating a flow rate of one or both of these laminar flows, dimensions of these laminar flows may be influenced and since the sample laminar flow is sandwiched between these laminar flows, dimensions and position of the sample laminar flow is controlled within the flow chamber and thus the sample, and thereby the first and the second biological entities in the sample, is made to flow adjacent to and distinct from a desired region of the flow chamber.

Additionally, the laminarly flowing sample may also be sandwiched between two laminar flows, say side flows that are perpendicularly aligned to the top and the bottom flows. By regulating a flow rate of one or both of these side flows, dimensions of the side flows may be influenced and since the sample laminar flow is sandwiched between the side flows in addition to the top and the bottom flows, dimensions and position of the sample laminar flow are controlled within the flow chamber and thus the sample is further aligned adjacent to and distinct from the desired region in the flow chamber. The phrase 'adjacent to' means in vicinity of or arranged side by side or next to, either contiguously or non-contiguously, and the phrase 'distinct from' means substantially non-overlapping and includes no overlap.

Simultaneously while arranging the sample flow adjacent to and distinct from the desired region or subsequently a standing acoustic wave is generated using an acoustic transducer. The standing acoustic wave so created passes through the sample flow and has a pressure node in the desired region. The standing acoustic wave has a pressure node, i.e. a collection of pressure points, linearly arranged along an axis passing through the desired region. The different pressures within the flow chamber created by the standing acoustic wave act on the first biological entity differently than on the second biological entity and thus move the first biological entity the pressure node and/or maintain the first biological entity at the pressure node. Since the pressure node is in the desired region the first biological entity is thus sorted from the second biological entity by moving the first biological entity into the desired region. Furthermore, the differential pressure of the standing acoustic wave also orients the first biological entity, especially when the first biological entity is non-spherical, in a desired orientation i.e. in an orientation in which a largest or substantially larger side of the first biological entity is presented to the imaging light, for example when the first biological entity is RBC the desired orientation is when the RBC is oriented such that a disc face of the RBC is presented to the imaging light when the RBC is focused in the desired region and arranged along the axis. Thus sorting and aligning of the first biological entity is simultaneously achieved.

FIG 1 schematically presents a system 100 of the present technique. The system 100 includes an imaging device 90 for inspecting the sample (not shown in FIG 1) and a flow cell 1 with a flow chamber 10. The imaging device 90 may have, but not limited to, a first part 92 for example an illumination source 92, and a second part 94 for example a detector with or without an interferometric unit. The imaging device 90 has a field of view 97, hereinafter the FOV 97 which represents an observable range of the imaging device 90 i.e. an object (not shown) is imaged by the imaging device 90 only when the

object is positioned in the FOV 97. The imaging device 90 also has a focus within the FOV 97. The imaging device 90 has an axis 95 along which the imaging is performed by shining a probing radiation on the object for example a Laser or a lower-coherent light source, such as a superluminescent diode, from a direction 7 onto the object or specimen to be inspected by the imaging device 90.

The focus is extended according to a depth of field (not shown in FIG 1) of the imaging device 90. Thus when the object is positioned in the depth of field around the focus of the imaging device 90, an 'in-focus' image of the object is obtainable. The focus and the depth of field of the imaging device 90 in the system 1 are arranged such that the focus and the depth of field around the focus of the imaging device 90 lie or fall within the flow chamber 10. The region within the depth of field around the focus of the imaging device 90 is a region (not shown) in which the object should be ideally positioned or focused or concentrated within the flow chamber 10 for obtaining in-focus images or interference patterns of the object.

The flow cell 1 has an extended channel or cavity forming the flow chamber 10 through which a specimen, for example a sample having biological entities to be imaged or inspected by the imaging device 90 is passed or flowed in a direction 8, generally perpendicular to the direction 7. The specimen or the sample to be inspected flows in the flow chamber 10 from one end 17 to another end 19 of the flow chamber 10 and the FOV 97 of the imaging device 90 is arranged such that at least a part of the flow chamber 10 between the one end 17 and the another end 19 is positioned in the FOV 97 of the imaging device 90.

Additionally, the flow cell 1 includes an acoustic transducer 80 which generates a standing acoustic wave (not shown in FIG 1). The acoustic transducer and its functionality have been explained later in reference to FIGs 9 to 18.

Referring to FIG 2 in combination with FIG 1, the flow chamber 10 has been explained further. As depicted in FIG 2, the flow chamber 10 has a rectangular cross-section when viewed from a direction (not shown) opposite to the direction 8. The flow chamber 10 includes a top wall 11, a bottom wall 12 opposite to the top wall 11, a first side wall 13 and a second side wall 14 opposite to the first side wall 13. The flow chamber 10 has a desired region 99 within the flow chamber 10. If the biological entity (not shown in FIG 1 and 2) that is to be imaged or studied by the imaging device is positioned in the desired region 99 and if the FOV 97 and the depth of field around the focus of the imaging device 90 are arranged such that the depth of field around the focus of the imaging device 90 overlaps or aligns with the desired region 99, then in-focus images or interference patterns are obtainable for the biological entity in the desired region 99 of the flow chamber 10 when imaging or inspection is performed with the imaging device 90. It may be noted that the desired region 99, hereinafter the region 99, has been schematically depicted in FIG 2 to be positioned in a center location of the cross-section of the flow chamber 10, however, it is well within the scope of the present technique that the region 99 may be present in a non-central location of the cross-section of the flow chamber 10.

FIG 3, in contrast to FIG 2, schematically presents a sample 5 flowing through the flow chamber 10. The sample 5 has a first biological entity 2, for example WBCs and a second biological entity 4, for example corpuscles such as RBCs, and a fluidic carrier 6 for the biological entities 2, 4. For example, the fluidic carrier 6 may be diluted or undiluted blood plasma, a buffer, and so on and so forth. The first biological entity 2 and the second biological entity 4 have been hereinafter also referred to as the entity 2 and the entity 4. When the sample 5 flows through the flow chamber 10, as depicted in FIG 3, some of the entities 2, 4 are in the desired region 99 and some are outside the desired region 99.

If the FOV 97 and the depth of field around the focus of the imaging device 90 are arranged such that the depth of field around the focus of the imaging device 90 overlaps or aligns with the desired region 99, then some first entities 2 and
5 some second entities 4 are in the FOV 97, while some other first entities 2 and some other second entities 4 are outside the FOV 97. Furthermore, some of the entities 2, that are desired to be imaged, are in the FOV 97 but either completely or partially outside the region 99.

10

A complete working of the flow cell 1 may be understood in two parts - first with reference to FIGs 4 to 8 showing a scheme to align the sample 5, along with entities 2 and 4, to any part, as desired, within the flow cell 1 i.e. an exempl-
15 ary scheme of moving the sample 5 with the entities 2 and 4 within different positions or location within the flow cell 1 and second with reference to FIGs 9 to 18 showing a scheme to position the first biological entity 2 at the desired region 99 and simultaneously align the first biological entity 2
20 with help of action of the acoustic transducer 80.

Referring to FIG 4, 5 and 6 in combination of FIGs 1 and 2, the flow cell 1 of the present technique is explained herein-
after. As shown in FIG 4, the flow cell 1, besides having the
25 flow chamber 10 as explained in reference to FIG 2, also includes a bottom flow input module 20, a sample input module 30 and a top flow input module 40. In an exemplary embodiment of the flow cell 1, the flow chamber 10 is a microfluidic channel. Furthermore, the acoustic transducer 80 is present.
30 As mentioned hereinabove, the acoustic transducer and its functionality have been explained later in reference to FIG 9 to 18.

As shown in FIG 5 in combination with FIG 4, the bottom flow
35 input module 20 receives a first fluid (not shown) and provides the first fluid to the flow chamber 10. The bottom flow input module 20, hereinafter also referred to as the module 20, provides the first fluid, for example water, to the flow

chamber 10 in such a way that the first fluid laminarly flows along the bottom wall 12 in the flow chamber 10 from the one end 17 (shown in FIG 1) of the flow chamber 10 towards the another end 19 (shown in FIG 1) of the flow chamber 10. The laminarly flowing first fluid forms a bottom laminar flow 72. The bottom flow input module 20 controls a rate of flow of the first fluid in the flow chamber 10. The term 'control' as used herein includes defines or decides, restricts, sets up, increases and/or decreases the rate of flow of the first fluid in the flow chamber 10 forming the bottom laminar flow 72, hereinafter also referred to as the flow 72. Forming laminar flow of fluids in a flow chamber is a well known technique in the field of hydrodynamics or fluid dynamics and has not been described herein in details for sake of brevity. The module 20 may include, but not limited to, flow channels, valves, pumps, flow meters, etc. The flow 72 may be understood as a rectangular parallelepiped shaped flow extending along the direction 8 in the flow chamber 10 and contiguous with the bottom wall 12.

The top flow input module 40 receives a second fluid (not shown) and provides the second fluid to the flow chamber 10. The top flow input module 40, hereinafter also referred to as the module 40, provides the second fluid, for example water, to the flow chamber 10 in such a way that the second fluid laminarly flows along the top wall 11 in the flow chamber 10 from the one end 17 (shown in FIG 1) of the flow chamber 10 towards the another end 19 (shown in FIG 1) of the flow chamber 10. The laminarly flowing second fluid forms a top laminar flow 71. The top flow input module 20 controls a rate of flow of the second fluid in the flow chamber 10. The term 'control' as used herein includes defines or decides, restricts, sets up, increases and/or decreases the rate of flow of the second fluid in the flow chamber 10 forming the top laminar flow 71, hereinafter also referred to as the flow 71. The module 40 may include, but not limited to, flow channels, valves, pumps, flow meters, etc. The flow 71 may be understood as a rectangular parallelepiped shaped flow extending

along the direction 8 in the flow chamber 10 and contiguous with the top wall 11.

The sample input module 30 receives the sample 5 and provides
5 the sample 5, along with the first and the second biological
entities 2 and 4, to the flow chamber 10. The sample input
module 30, hereinafter also referred to as the module 30,
provides the sample 5 to the flow chamber 10 in such a way
10 that the sample 5 laminarly flows sandwiched between the flow
71 and the flow 72 from the one end 17 (shown in FIG 1) of
the flow chamber 10 towards the another end 19 (shown in FIG
1) of the flow chamber 10. The laminarly flowing sample 5
forms a sample laminar flow 75. The sample input module 30
controls a rate of flow of the sample 5 in the flow chamber
15 10. The term 'control' as used herein includes defines or de-
cides, restricts, sets up, increases and/or decreases the
rate of flow of the sample 5 in the flow chamber 10 forming
the sample laminar flow 75, hereinafter also referred to as
the flow 75. The module 30 may include, but not limited to,
20 flow channels, valves, pumps, flow meters, etc. The flow 75
may be understood as a rectangular parallelepiped shaped flow
extending along the direction 8 in the flow chamber 10 and
sandwiched between the flow 71 and the flow 72.

25 In the flow chamber 10, by defining or setting up or by in-
creasing or by decreasing the flow rate of the first fluid,
the height of the flow 72 is fixed or controlled or varied.
Similarly, by defining or setting up or by increasing or by
decreasing the flow rate of the second fluid, the height of
30 the flow 71 is fixed or controlled or varied.

In the flow cell 1, by controlling or varying the height of
the flow 71 and/or the flow 72, the width and/or the height
and/or the longitudinal position of the flow 75 is controlled
35 or varied. For example as schematically depicted in FIG 5,
the flow 75 is now restricted to or concentrated in at least
partly in the region 99. In an exemplary embodiment (not
shown) of the flow cell 1, the desired region 99 extends from

the first side wall 13 to the second side wall 14 and then the flow 75 is substantially positioned in the desired region 99.

5 As depicted in FIG 6, an exemplary working of the flow cell 1 has been schematically depicted. The relative heights of the flow 71 and the flow 72 may be adjusted such that the flow 75 is moved below or beneath the desired region 99, as shown in FIG 6 in comparison to FIG 5, for example by increasing the
10 flow rate of the second fluid via the module 40 and/or decreasing the flow rate of the first fluid via module 20 the relative heights of the flow 72 and the flow 71 are altered thereby moving the flow 75 at least partly or completely below or beneath the region 99. Similarly in another embodiment
15 (not shown), the flow 75 may be moved to a position above or top of the desired region 99 by increasing the flow rate of the first fluid via the module 20 and/or decreasing the flow rate of the second fluid via module 40. In short the height of the sample laminar flow 75 and/or the longitudinal position of the sample laminar flow 75 is decided or fixed or adjusted by altering the flow rates of the first and/or the second fluids via the modules 20 and/or 40.

Referring to FIG 4 in combination with FIGs 7 and 8, other
25 exemplary embodiments of the flow cell 1 have been explained hereinafter. In an embodiment of the flow cell 1 a first side flow input module 50, hereinafter the module 50, is included. The module 50 receives a first side fluid (not shown) and provides the first side fluid to the flow chamber 10. The
30 first side fluid, for example water, is provided by the module 50 in such a way that the first side fluid laminarly flows along the first side wall 13 in the flow chamber 10 from the one end 17 (shown in FIG 1) of the flow chamber 10 towards the another end 19 (shown in FIG 1) of the flow chamber 10. The laminarly flowing first side fluid forms a first
35 side laminar flow 73, hereinafter also referred to as the flow 73. The flow 73 is sandwiched between the flow 71 and

the flow 72 and between the first side wall 13 and the flow 75, as shown in FIG 7.

5 The module 50 controls a rate of flow of the first side fluid in the flow chamber 10. The term 'control' as used herein includes defines or decides, restricts, sets up, increases and/or decreases the rate of flow of the first side fluid in the flow chamber 10 forming the flow 73. The module 50 may include, but not limited to, flow channels, valves, pumps, 10 flow meters, etc. The flow 73 may be understood as a rectangular parallelepiped shaped flow extending along the direction 8 in the flow chamber 10 and contiguous with a part of the first side wall 13 on one face and the flow 75 on the opposite face, and also contiguous on another face with flow 71 15 and on a face opposite to the another face with the flow 72.

In another embodiment of the flow cell 1 a second side flow input module 60, hereinafter also referred to as the module 60, is included. The module 60 receives a second side fluid 20 (not shown) and provides the second side fluid to the flow chamber 10. The second side fluid, for example water, is provided by the module 60 in such a way that the second side fluid laminarly flows along the second side wall 14 in the flow chamber 10 from the one end 17 (shown in FIG 1) of the 25 flow chamber 10 towards the another end 19 (shown in FIG 1) of the flow chamber 10. The laminarly flowing second side fluid forms a second side laminar flow 74, hereinafter also referred to as the flow 74. The flow 74 is sandwiched between the flow 71 and the flow 72 and between the second side wall 30 14 and the flow 75, as shown in FIG 7.

The module 60 controls a rate of flow of the second side fluid in the flow chamber 10. The term 'control' as used herein includes defines or decides, restricts, sets up, increases 35 and/or decreases the rate of flow of the second side fluid in the flow chamber 10 forming the flow 74. The module 60 may include, but not limited to, flow channels, valves, pumps, flow meters, etc. The flow 74 may be understood as a rectan-

gular parallelepiped shaped flow extending along the direction 8 in the flow chamber 10 and contiguous with a part of the second side wall 14 on one face and the flow 75 on the opposite face, and also contiguous on another face with flow 71 and on a face opposite to the another face with the flow 72.

In the flow chamber 10, by defining or setting up or by increasing or by decreasing the flow rate of the first side fluid, the width of the flow 73 is fixed or controlled or varied. Similarly, by defining or setting up or by increasing or by decreasing the flow rate of the second side fluid, the width of the flow 74 is fixed or controlled or varied. In the flow cell 1, by controlling or varying the width of the flow 73 and/or the flow 74, the width and/or the height and/or the lateral position of the flow 75 is controlled or varied. For example as schematically depicted in FIG 7, the flow 75, is restricted to or concentrated in the region 99.

As depicted in FIG 8, an exemplary working of the flow cell 1 has been schematically depicted. The relative widths of the flow 73 and the flow 74 may be adjusted such that the flow 75 is at least partly or completely shifted from the desired region 99 towards the second side wall 14, as shown in FIG 8, for example by increasing the flow rate of the first side fluid via the module 50 and/or decreasing the flow rate of the second side fluid via module 60. Similarly in another embodiment (not shown), the flow 75 may at least partly or completely be shifted from the desired region 99 towards the first side wall 13 by decreasing the flow rate of the first side fluid via the module 50 and/or increasing the flow rate of the second side fluid via module 60. In short the width of the sample laminar flow 75 and/or the lateral position of the sample laminar flow 75 is decided or fixed or adjusted by altering the flow rates of the first side and/or the second side fluids via the modules 50 and/or 60.

As shown in FIG 4, in another embodiment of the flow cell 1, a flow exit 79 is present for allowing the flows 71, 72, 73, 74 and 75 to exit the flow chamber 10. In presence of the flows 71, 72, 73, 74 covering flow 75 on all sides, the biological entities 2, 4 are physically removed from the walls 11, 12, 13 and 14 and thus never in contact with the walls 11, 12, 13 and 14 and therefore none of the biological entities 2, 4 adhere to the walls 11, 12, 13 or 14 and thus disintegration of at least the first biological entity 2 to form debris is avoided.

FIG 9 shows the acoustic transducer 80 along with the flow cell 1. The transducer 80 may be positioned such that a part of the transducer 80 is in direct physical contact, for example by fixing such as by gluing, with one or more of the walls 11, 12, 13, 14 and outside the flow chamber 10. In another embodiment (not shown), the transducer 80 may even be positioned physically removed i.e. not touching any of the walls 11, 12, 13, 14. The transducer 80 generates a standing acoustic wave 82 having a pressure node 88 linearly arranged along an axis 9. The axis 9 passes through the desired region 99. The pressure node 88 of the standing acoustic wave 82 may include a collection of pressure points (not shown) that are substantially linearly arranged along the axis 9. The transducer 80 may be an ultrasonic transducer for example a piezoelectric ceramic, also called piezoceramic or piezoacoustic transducers (PZT). Such transducers 80 and their functionality are known in the field of wave propagation sciences and thus not described in details herein for sake of brevity. Although the standing wave 82 is shown to form just in one direction in FIG 9, it may be noted that standing wave 82 may also form in other directions (not shown in FIG 9), preferably in a direction perpendicular to the standing wave 82, i.e. the standing wave 82 may have two mutually perpendicular components as shown in FIG 11.

As shown in FIG 19, in the flow cell 1, the piezoelectric ceramic 80 may include at least one hole 96. The piezoelectric

ceramic 80 is positioned such that the axis 95 along which the imaging is performed passes through the hole 96. In one embodiment (not shown), the piezoelectric ceramic 80 may be positioned at the top wall 11. In another embodiment as depicted in FIG 19, the piezoelectric ceramic 80 is positioned at the bottom wall 12. The probing radiation from the imaging device (not shown in FIG 19) travels along the axis 95 and passes through the hole 96 either before or after passing through the flow chamber 10, as depicted in FIG 19.

FIGs 10 and 11 together represent an exemplary embodiment of working of the present technique to align the first biological entity 2 in the desired region. As shown in FIG 10, the flow 75 is aligned adjacent to and distinct from the desired region 99 for example the flow 75 is placed or positioned beneath the desired region 99. The positioning or aligning of the flow 75 is achieved, as explained in FIGs 4 to 8 by the flows 71 and 72. The flows 73 and 74 may also be used, as explained hereinabove. The first biological entity 2 and the second biological entity 4 are in the sample 5 i.e. the flow 75, and thus are present outside the desired region 99.

As shown in FIG 11, the standing acoustic wave 82, hereinafter the wave 82, is generated by the transducer 80. The wave 82 passes through the flow 75 and has a pressure node 88 linearly arranged along the axis 9 passing through the desired region 99. Due to the wave 82 having the pressure node 88 in the desired region 99 the first biological entity 2 and the second biological entity 4 have a tendency to move to the desired region 99. In this example of FIG 11, the first biological entity 2 is a WBC 2 and the second biological entity is a RBC 4. Owing to differences in size i.e. including a mass and a volume, and conformation of the WBC 2 and the RBC 4, the physical force on the WBC 2 due to the standing wave 82 is greater than that on the RBC 4, and thus the WBC 4, i.e. the first biological entity 2, moves to the desired region 99, as shown in FIG 11, under the influence of the wave 82, faster compared to the RBC 4. Thus at a given instance of

time, for example when the imaging is being performed by the imaging device 90, at least a few of the WBCs 2 i.e. the first biological entity 2, have moved to the desired region 99 and thus are sorted from the RBCs 4 in the flow 75.

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In an alternative embodiment (not shown), the first and the second biological entities 2, 4 may be one and the same biological entity type, i.e. having similar size i.e. including a mass and a volume, and conformation, for example the first and second biological entity 2, 4 may both be RBCs or WBCs. However, the entities 2, 4 while flowing in the flow 75 are at different distances from the pressure node 88 and thus experience different pressure due to the wave 82. For example the first entity 2 may be physically closer to the pressure node 88 than the second entity 4. In this case due to the pressure node 88 of the standing wave 82 being positioned in the desired region 99 the first entity 2 owing to its physical proximity with the pressure node 88 moves to the desired region 99 faster than the second entity 4. Thus at a given instance of time, for example when the imaging is being performed by the imaging device 90, at least one of the RBCs 2 i.e. the first biological entity 2, that is closer to the pressure node 88 has moved to the desired region 99 and thus is sorted from the other RBCs 4 in the flow 75.

25

Hereinafter with reference to FIGs 12, 13 and 14 another exemplary embodiment of the present technique has been explained. FIGs 12, 13 and 14 represent sequentially an exemplary embodiment of the working of the flow cell 1. As shown in FIG 12, the flow 75 including the entities 2, 4 are positioned in the desired region 99 and the wave 82 is also generated simultaneously. Subsequently, the flow 75 is moved from the desired region 99 for example towards a position beneath the desired region 99, as depicted in FIG 13. The moving of the flow 75 is achieved by the flows 71 and 72 and additionally but optionally by the flows 73 and 74, as explained hereinabove with reference to FIGs 4 to 8. FIG 13 represents an intermediate state between the FIG 12 and FIG

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14. As the flow 75 is being moved the wave 82 is still maintained. Finally, as shown in FIG 14, the flow 75 is moved such that the flow 75 is aligned adjacent to and distinct from the desired region 99 for example the flow 75 is placed or positioned beneath the desired region 99.

Due to the movement of the flow 75 to change a position of the flow 75 from the desired region 99 to a position outside the desired region 99, as sequentially depicted in FIGs 12 to 14, the first biological entity 2 and the second biological entity 4 have a tendency to move out of the desired region 99. In this example of FIGs 12 to 14, the first biological entity 2 is a WBC 2 and the second biological entity is a RBC 4. Owing to differences in size i.e. including a mass and a volume, and conformation of the WBC 2 and the RBC 4, the physical force on the WBC 2 due to the standing wave 82 is greater than that on the RBC 4, and thus the WBC 4, i.e. the first biological entity 2, is maintained in the desired region 99, as shown in FIGs 13 and 14, under the influence of the wave 82, whereas the RBC 4 moves out of the desired region 99 as a result of the movement of the flow 75 out of the desired region 99. Thus at a given instance of time, as depicted in FIG 14 for example when the imaging is being performed by the imaging device 90, at least a few of the WBCs 2 i.e. the first biological entity 2, are in the desired region 99 and thus are sorted from the RBCs 4 that have moved out of the desired region 99 along with the flow 75.

With FIGs 15 to 18 aligning of the first biological entity 2 has been explained, especially when the first biological entity 2 is non-spherical. The aligning of the first biological entity 2 is performed while the first biological entity 2 is being sorted from the second biological entity 4 as explained in reference to FIGs 4 to 14. The first biological entity 2 for purpose of explanation is a RBC 2. It may be noted that first biological entity may also be a WBC. As show in FIG 17, the desired region 99 is overlapping with a depth of field 98 in the FOV 97 of the imaging device 90 (shown in FIG 1).

FIG 15 represents a situation without the wave 82. Albeit the RBC 2 is in the desired region 99 and thus 'in-focus' of the imaging device 90, in absence of the wave 82 generated by the transducer 80, the RBC 2 though in the region 99 may be oriented to show up-ended side toward the axis 95 when viewed along the direction 7 as shown in FIGs 15 and 16. When the RBC 2 is in side showing orientation as depicted by FIGs 15 and 16 i.e. when the RBC 2 presents side to the axis 95 when viewed in the direction 7 the image or interference patterns obtained present less morphological features and are less useful for volumetric analysis as compared to a case when the RBC 2 is in an orientation when the RBC 2 presents disc face or flat side to the axis 95 when viewed in the direction 7.

As shown in FIG 17 when the transducer 80 generates the wave 82 acoustic forces resulting from the wave 82 orient the RBC 2 such that the RBC 2 rotates and is oriented to show flat face or disc face toward the axis 95 when viewed along the direction 7 as shown in FIGs 17 and 18. When the RBC 2 is in disc face showing orientation as depicted by FIGs 17 and 18 i.e. when the RBC 2 presents disc face or flat face to the axis 95 when viewed in the direction 7 the image or interference patterns obtained present more morphological features and are more useful for volumetric analysis as compared to a case when the RBC 2 is in the orientation shown in FIGs 15 and 16. The flow chamber 10 serves as a conduit for the flows 71, 72, 73, 74 and 75 and at the same time acts as an acoustic resonator defined by the flow chamber 10 dimension. The RBC 2 exposed to an acoustic force resulting from the standing acoustic wave 82 in the flow chamber 10 will be oriented such that a net acoustic force acting on the RBC 2 is minimized, for example the RBC 2 will be oriented substantially parallel to the top wall 11 and the bottom wall 12, for the embodiment depicted in FIG 17.

It may be noted that although FIGs 1, 4 and 9 depict only one transducer 80, it is well within the scope of the present

technique to have more than one transducer 80. Each of the multiple transducers 80 may be actuated by individual electric signals which may be same or different. It may be noted that the imaging device 90 (shown in FIG 1) may perform
5 counting and imaging of the first biological entity 2, and when performing both imaging and counting, only few of the first biological entities 2 may be desired to be oriented and not all as representative specimens of the first biological entity 2 in the sample 5.

10 It may also be noted that when the standing wave 82 forms in two directions or to say in two dimensions, as shown in FIGs 11 to 14, the first biological entity 2 rotates and aligns itself in an orientation in which the first biological entity
15 2 presents its smallest dimension parallel with the strongest acoustic force out of the two acoustic forces from the two directions. Thus besides the pressure node 88, another pressure node (not shown) is formed. The frequencies for generating the standing waves 82 from two different sides may be
20 different frequencies, depending on the orientation of the first biological entity 2 that is desired to be achieved.

As shown in FIG 1, the system 100 includes the imaging device 90. In one embodiment of the system 100, the second part 94
25 of the imaging device 90 includes an interferometry unit (not shown) and a detector (not shown). The interferometry unit may be a common path interferometry unit or different path interferometry unit. In common path interferometry unit, a light beam is shone or impinged on the first biological enti-
30 ty 2 from the first part 92 of the imaging device 90 and then the light beam emerging after interacting with the first biological entity 2 is split into a reference beam (not shown) and an object beam (not shown). Subsequently, object information is filtered out or deleted from the reference beam and
35 then the filtered reference beam is superimposed with the object beam to detect the interference pattern at the detector. In different path interferometry unit, a light beam to be incident on the first biological entity 2 is first split into

an object beam (not shown) and a reference beam (not shown)
i.e. the light beam is split into the reference beam and the
object beam before interacting with the first biological en-
tity 2. The object beam is then shone or impinged upon the
5 first biological entity 2 but the reference beam is directed
to another optical path (not shown) within the different path
interferometric unit and is not shone or impinged upon the
first biological entity 2. Subsequently, the object beam car-
rying object information is superimposed with the reference
10 beam to obtain interference pattern at the detector. The in-
terference pattern obtained as an output of the common path
or different path interferometry is analyzed. The interfer-
ence pattern also referred to as image of the first biologi-
cal entity 2 represents characteristics of the first biologi-
15 cal entity 2 such as physical structures in the first biolog-
ical entity 2, morphology of the first biological entity 2,
and so on and so forth. Designs, setups and principle of
working of the common path interferometry and the different
path interferometry are known in the field of interferometric
20 microscopy and not described herein in details for sake of
brevity.

The present technique also encompasses a method for simulta-
neously sorting the first biological entity 2 from the second
25 biological entity 4 and aligning the first biological entity
2 into the desired region 99 in the flow cell 1. The entities
2, 4 are carried in the sample 5. The first biological entity
2 is to be aligned in the desired region 99 to be inspected
by the imaging device 90 having the depth of field 98 as
30 shown in FIG 17 in the field of view 97 of the imaging device
90. The flow cell 1 is same as the flow cell 1 described in
reference to FIGs 1 to 10 and presented in accordance with
the first aspect of the present technique.

35 In the method, the first and the second fluids are provided
to the flow chamber 1 to form the flows 72 and 71 along with
the flow 75 sandwiched between the flows 72 and 71, as ex-
plained in reference to FIGs 4 to 8. The providing of the

first fluid, the sample 5 and the second fluid to form the flow 72, the flow 75 and the flow 71, respectively, is either performed simultaneously or sequentially.

5 In the method, the rate of flow of the first fluid and/or the rate of flow of the second fluid in the flow chamber 10 is controlled in order to arrange the flow 75 such that the flow 75 is aligned adjacent to and distinct from the desired re-
10 gion 99 in the flow chamber 10, as explained in reference to FIGs 11, 14 and 17. As shown in FIGs 15 and 17, the desired region 99 is aligned with the depth of field 98 in the field of view 97 of the imaging device 90. In the method, the wave 82 having the pressure node 88 is generated, as explained hereinabove with reference to FIG 9, FIGs 11 to 14 and FIG
15 17. At least a part of the wave 82 passes through the flow 75 to generate a relative movement of the first biological entity 2 with respect to the second biological entity 4 towards the axis 9. The differential pressure formed by the wave 82 at different parts of the flow chamber 10 and specially to-
20 wards the pressure node 88 acts on the first biological entity 2 to sort and align the first biological entity 2 as explained in reference to FIGs 10 to 17. As explained hereinabove, the first biological entity 2 and the second biological entity 4 may be different types of biological entity for
25 example the first biological entity 2 may be a WBC and the second biological entity 4 may be a RBC, or the first biological entity 2 and the second biological entity 4 may be same type of biological entity for example the first and the second biological entities 2, 4 may be either a WBC or a RBC.

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In an embodiment of the method, the first side fluid is provided to the flow chamber 10 forming the flow 73, and additionally the second side fluid is provided to the flow chamber 10 forming the flow 74, as explained in reference to FIGs
35 7 and 8. The first and the second side fluids may be provided either simultaneously or sequentially in any order.

While the present technique has been described in detail with reference to certain embodiments, it should be appreciated that the present technique is not limited to those precise embodiments. Rather, in view of the present disclosure which
5 describes exemplary modes for practicing the invention, many modifications and variations would present themselves, to those skilled in the art without departing from the scope and spirit of this invention. The scope of the invention is, therefore, indicated by the following claims rather than by
10 the foregoing description. All changes, modifications, and variations coming within the meaning and range of equivalency of the claims are to be considered within their scope.

Patent claims

1. A flow cell (1) for simultaneously sorting a first biological entity (2) from a second biological entity (4) and
5 aligning the first biological entity (2) into a desired region (99) in the flow cell (1), the first and the second biological entities (2,4) carried in a sample (5), the first biological entity (2) to be aligned in the desired region (99) for inspection by an imaging device (90), the flow cell (1)
10 comprising:
- a flow chamber (10) having a rectangular cross-section, a top wall (11), a bottom wall (12) opposite to the top wall (11), a first side wall (13), a second side wall (14) opposite to the first side wall (13) and the desired region (99);
15 - a bottom flow input module (20) configured to receive a first fluid and to provide the first fluid to the flow chamber (10) such that the first fluid laminarly flows in the flow chamber (10) in form of a bottom laminar flow (72) along the bottom wall (12) from one end (17) of the flow chamber (10) towards another end (19) of the flow chamber (10),
20 wherein the bottom flow input module (20) is further configured to control a rate of flow of the first fluid in the flow chamber (10);
 - a top flow input module (40) configured to receive a second
25 fluid and to provide the second fluid to the flow chamber (10) such that the second fluid laminarly flows in the flow chamber (10) in form of a top laminar flow (71) along the top wall (11) from the one end (17) of the flow chamber (10) towards the another end (19) of the flow chamber (10), wherein
30 the top flow input module (40) is further configured to control a rate of flow of the second fluid in the flow chamber (10);
 - a sample input module (30) configured to receive the sample (5) and to provide the sample (5) to the flow chamber (10)
35 such that the sample (5) laminarly flows in the flow chamber (10) in form of a sample laminar flow (75) from the one end (17) of the flow chamber (10) towards the another end (19) of the flow chamber (10) and the sample laminar flow (75) is

sandwiched between the top laminar flow (71) and the bottom laminar flow (72);

wherein the bottom and the top flow input modules (20, 30) are further configured to arrange the sample laminar flow

5 (75) such that the sample laminar flow (75) is aligned adjacent to and distinct from the desired region (99) in the flow chamber (10); and

- an acoustic transducer (80) configured to generate a standing acoustic wave (82) having a pressure node (88) linearly
10 arranged along an axis (9) passing through the desired region (99).

2. The flow cell (1) according to claim 1, comprising:

- a first side flow input module (50) configured to receive a
15 first side fluid and to provide the first side fluid to the flow chamber (10) such that the first side fluid laminarly flows in the flow chamber (10) in form of a first side laminar flow (73) sandwiched between the top laminar flow (71) and the bottom laminar flow (72) and between the first side
20 wall (13) and the sample laminar flow (75), wherein the first side flow input module (50) is further configured to control a rate of flow of the first side fluid in the flow chamber (10) and wherein the first side laminar flow (73) moves from the one end (17) of the flow chamber (10) towards the another
25 end (19) of the flow chamber (10).

3. The flow cell (1) according to claim 1 or 2, further comprising:

- a second side flow input module (60) configured to receive
30 a second side fluid and to provide the second side fluid to the flow chamber (10) such that the second side fluid laminarly flows in the flow chamber (10) in form of a second side laminar flow (74) sandwiched between the top laminar flow (71) and the bottom laminar flow (72) and between the
35 second side wall (14) and the sample laminar flow (75), wherein the second side flow input module (60) is further configured to control a rate of flow of the second side fluid in the flow chamber (10) and wherein the second side laminar

flow (74) moves from the one end (17) of the flow chamber (10) towards the another end (19) of the flow chamber (10).

4. The flow cell (1) according to any of claims 1 to 3,
5 wherein the sample input module (30) is configured to control a rate of flow of the sample (5) in the flow chamber (10).

5. The flow cell (1) according to any of claims 1 to 4,
wherein the flow chamber (10) is a microfluidic channel.

10 6. The flow cell (1) according to any of claims 1 to 5, wherein the acoustic transducer (80) is an ultrasonic transducer and wherein the standing acoustic wave (82) is an standing ultrasonic wave.

15 7. The flow cell (1) according to claim 6, wherein the ultrasonic transducer (80) is a piezoelectric ceramic.

20 8. The flow cell (1) according to claim 7, wherein the piezoelectric ceramic comprises at least one hole (96) and is positioned such that an axis (95) along which the imaging is performed passes through the hole (96).

25 9. A method for simultaneously sorting a first biological entity (2) from a second biological entity (4) and aligning the first biological entity (2) into a desired region (99) in the flow cell (1), the first and the second biological entities (2, 4) carried in a sample (5), the first biological entity (2) to be aligned in the desired region (99) for inspection
30 by an imaging device (90) having a depth of field (98) in a field of view (97) of the imaging device (90), the flow cell (1) comprising a flow chamber (10) having a rectangular cross-section, a top wall (11), a bottom wall (12) opposite to the top wall (11), a first side wall (13), a second side wall (14) opposite to the first side wall (13) and the de-
35 sired region (99); the method comprising:

- providing a first fluid to the flow chamber (10) such that the first fluid laminarly flows in the flow chamber (10) in

form of a bottom laminar flow (72) along the bottom wall (12) from one end (17) of the flow chamber (10) towards another end (19) of the flow chamber (10);

- providing a second fluid to the flow chamber (10) such that the second fluid laminarly flows in the flow chamber (10) in form of a top laminar flow (71) along the top wall (11) from the one end (17) of the flow chamber (10) towards the another end (19) of the flow chamber (10);

- providing the sample (5) to the flow chamber (10) such that the sample (5) laminarly flows in the flow chamber (10) in form of a sample laminar flow (75) from the one end (17) of the flow chamber (10) towards the another end (19) of the flow chamber (10) and the sample laminar flow (75) is sandwiched between the top laminar flow (71) and the bottom laminar flow (72);

- controlling a rate of flow of the first fluid and/or a rate of flow of the second fluid in the flow chamber (10) to arrange the sample laminar flow (75) such that the sample laminar flow (75) is aligned adjacent to and distinct from the desired region (99) in the flow chamber (10);

- aligning the desired region (99) with the depth of field (98) in the field of view (97) of the imaging device (90); and

- generating an standing acoustic wave (82) having a pressure node (88) linearly arranged along an axis (9) passing through the desired region (99) and wherein at least a part of the standing acoustic wave (82) passes through the sample laminar flow (75) to generate a relative movement of the first biological entity (2) towards the axis (9) with respect to the second biological entity (4).

10. The method according to claim 9, comprising:

- providing a first side fluid to the flow chamber (10) such that the first side fluid laminarly flows in the flow chamber (10) in form of a first side laminar flow (73) sandwiched between the top laminar flow (71) and the bottom laminar flow (72) and between the first side wall (13) and the sample lam-

inar flow (75) from the one end (17) of the flow chamber (10) towards the another end (19) of the flow chamber (10); and
- controlling a rate of flow of the first side fluid in the flow chamber (10).

5

11. The method according to claim 9 or 10, comprising:

- providing a second side fluid to the flow chamber (10) such that the second side fluid laminarly flows in the flow chamber (10) in form of a second side laminar flow (74) sandwiched between the top laminar flow (71) and the bottom laminar flow (72) and between the second side wall (14) and the sample laminar flow (75) from the one end (17) of the flow chamber (10) towards the another end (19) of the flow chamber (10); and
- controlling a rate of flow of the second side fluid in the flow chamber (10).

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12. The method according to any of claims 9 to 11, wherein the standing acoustic wave (82) is a standing ultrasonic wave.

13. The method according to any of claims 9 to 12, wherein the first biological entity (2) is a leukocyte and the second biological entity (4) is an erythrocyte.

25

14. The method according to any of claims 9 to 12, wherein the first and the second biological entities (2,4) are erythrocytes positioned at different distances with respect to the axis (9).

30

15. The method according to claim 14, wherein the aligning the first biological entity (2) into the desired region (99) includes orienting the first biological entity (2) in the desired region (99).

35

16. A system (100) for simultaneously sorting a first biological entity (2) from a second biological entity (4) and aligning the first biological entity (2) into a desired re-

gion (99) in the flow cell (1), the first and the second biological entities (2,4) carried in a sample (5), the system (100) comprising:

- an imaging device (90) having a field of view (97), wherein
5 the field of view (97) includes a depth of field (98); and
- a flow cell (1) according to any of claims 1 to 8, and wherein the sample laminar flow (75) is configured to be arranged such that the sample laminar flow (75) is aligned adjacent to and distinct from the desired region (99) in the
10 flow chamber (10).

17. The system (100) according to claim 16, wherein the imaging device (90) is an interferometry microscopy device.

- 15 18. The system (100) according to claim 17, wherein the interferometry microscopy device is a digital holographic microscopy device.

FIG 2

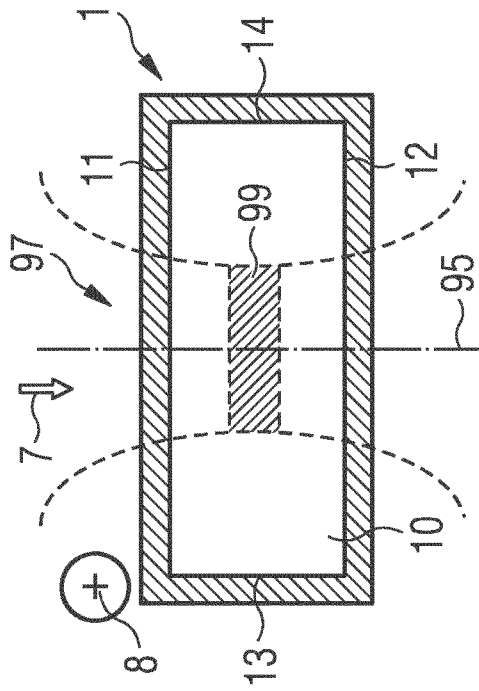


FIG 3

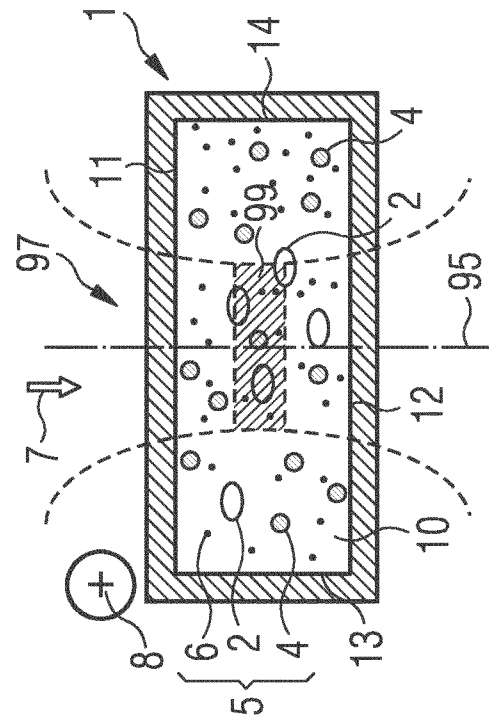


FIG 1

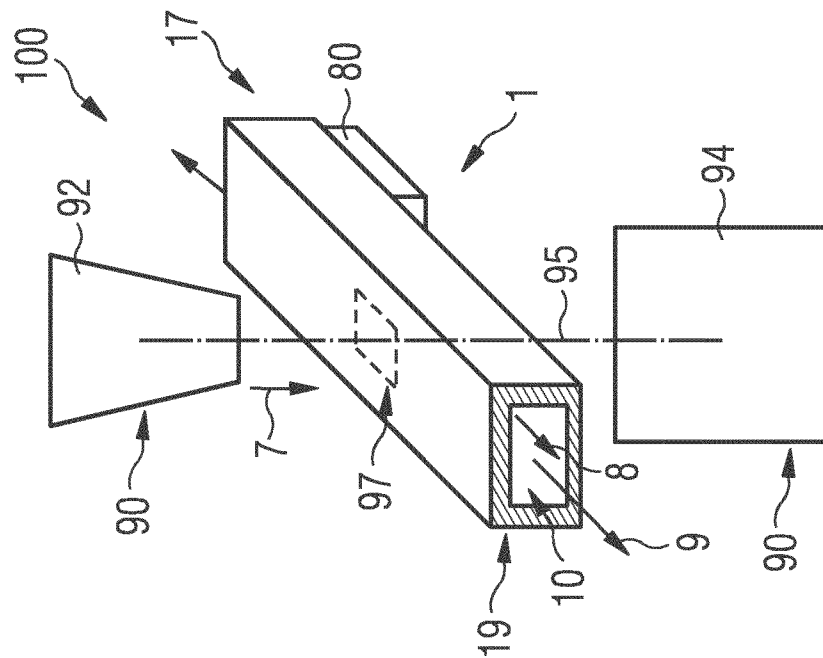
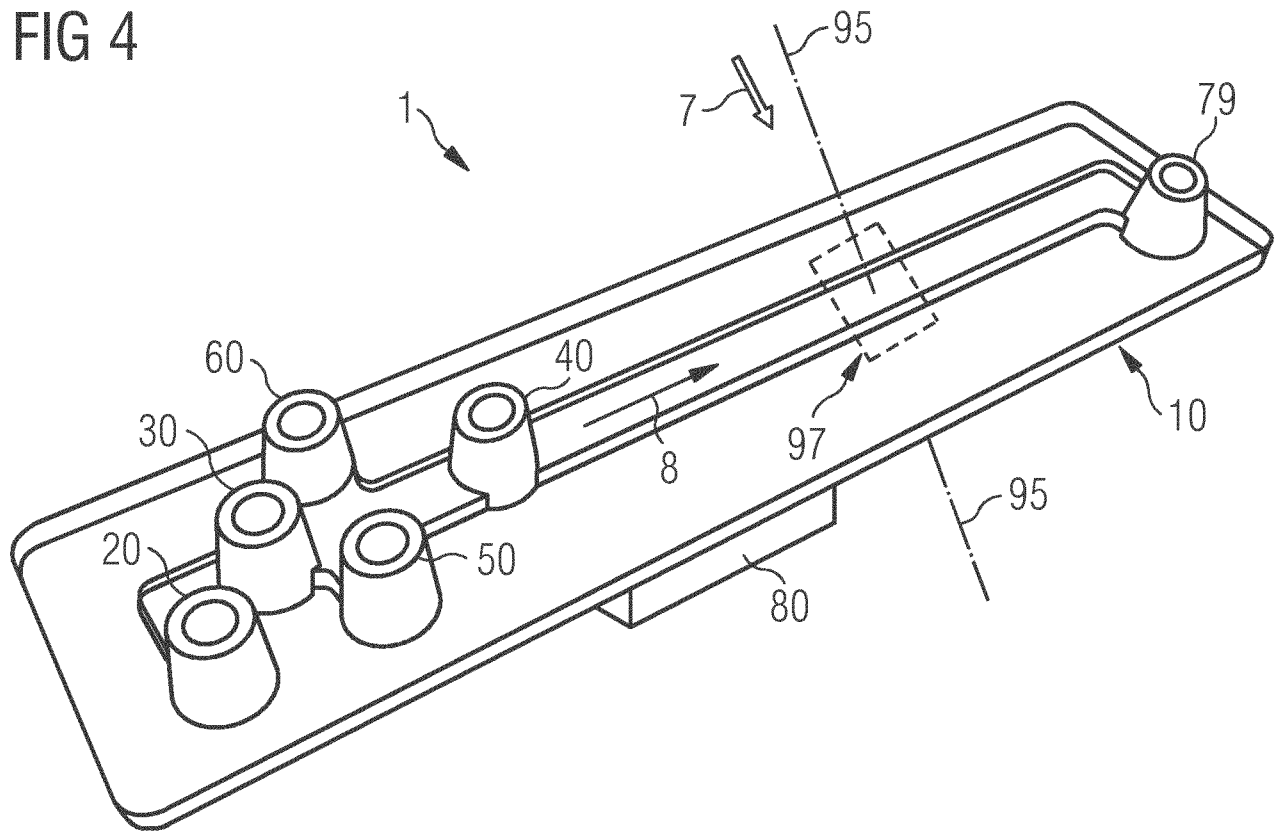
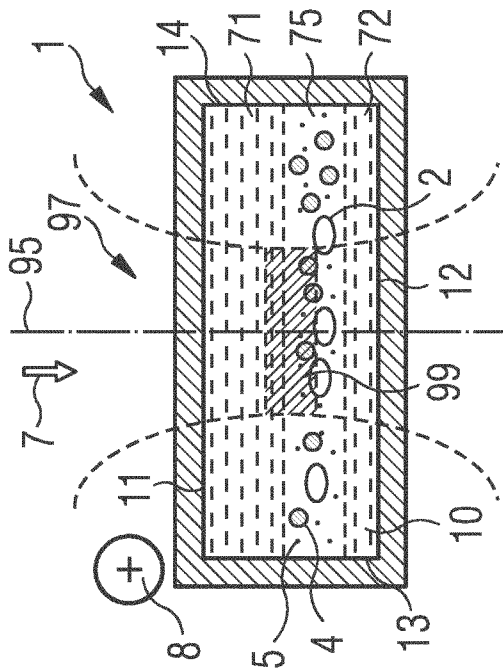
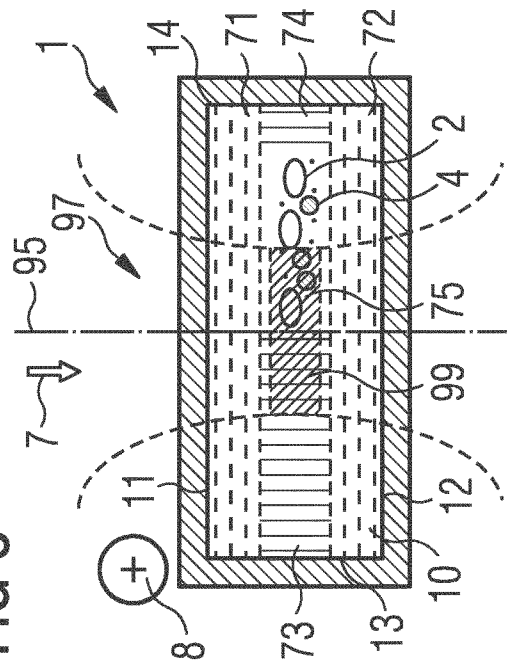


FIG 4



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G
F

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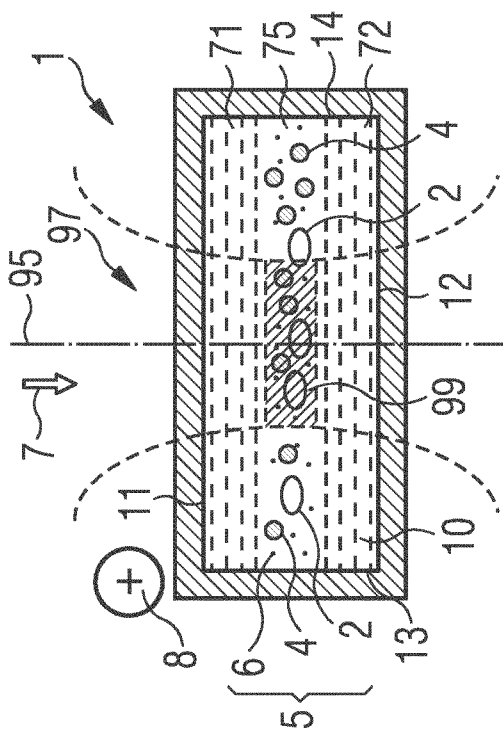
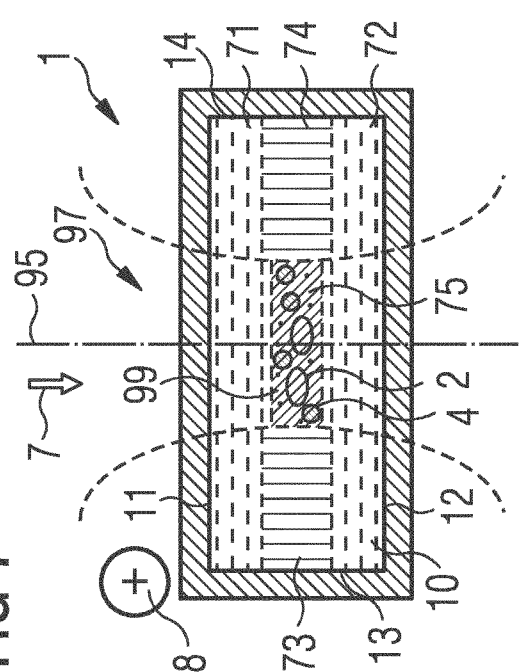


FIG 7



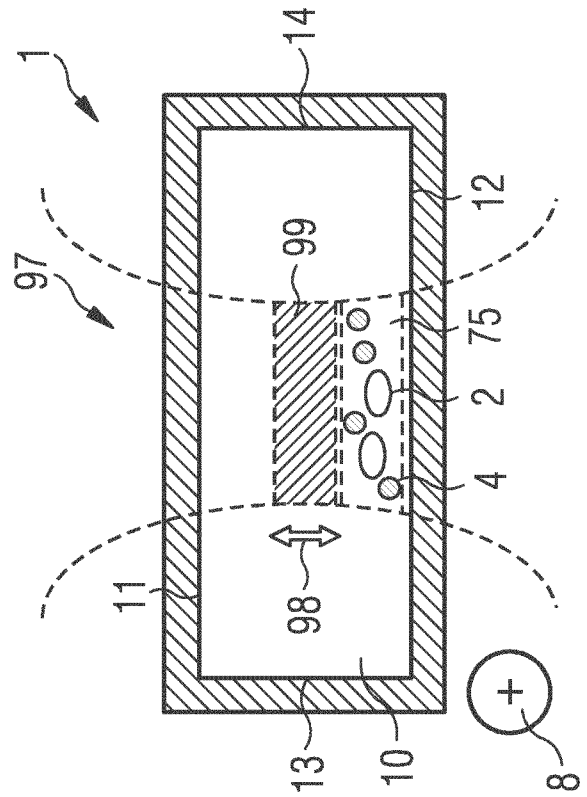
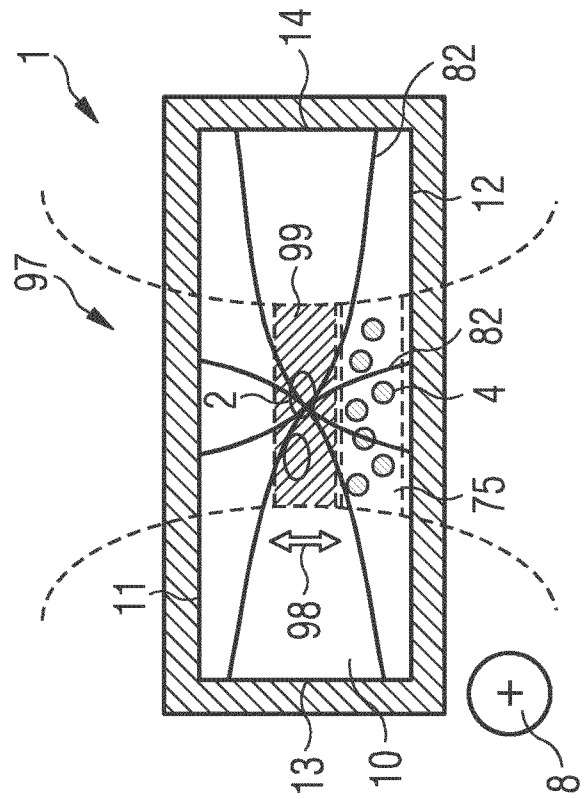
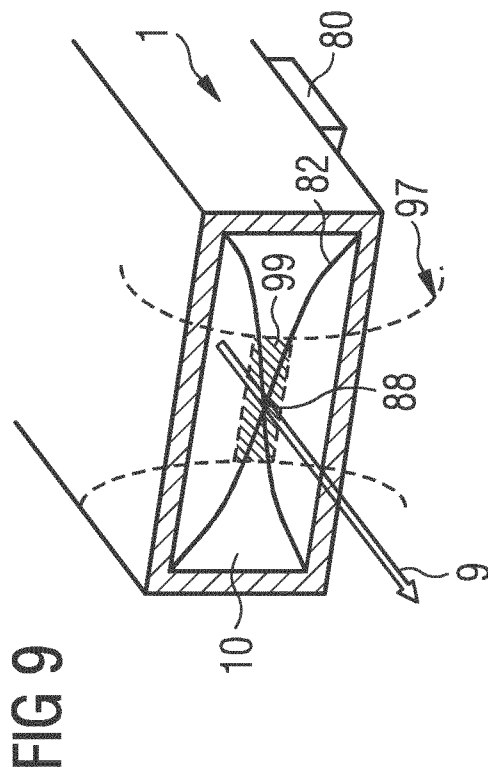


FIG 12

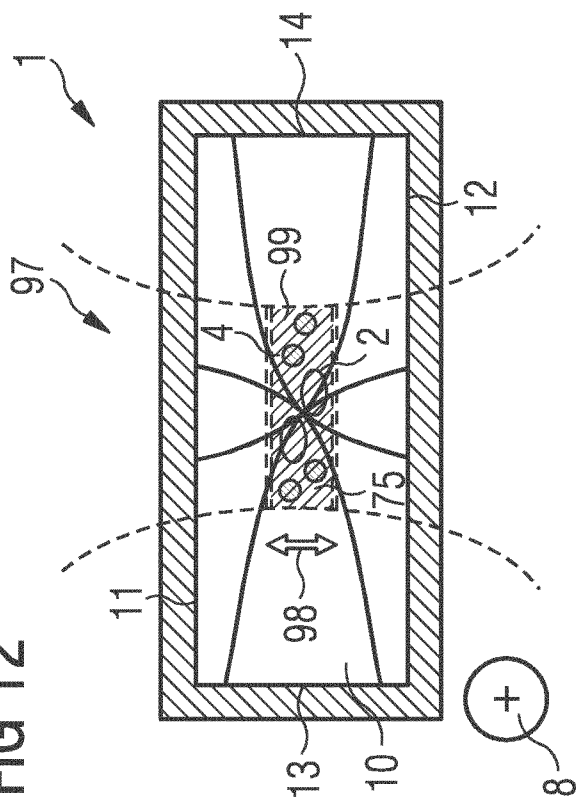


FIG 13

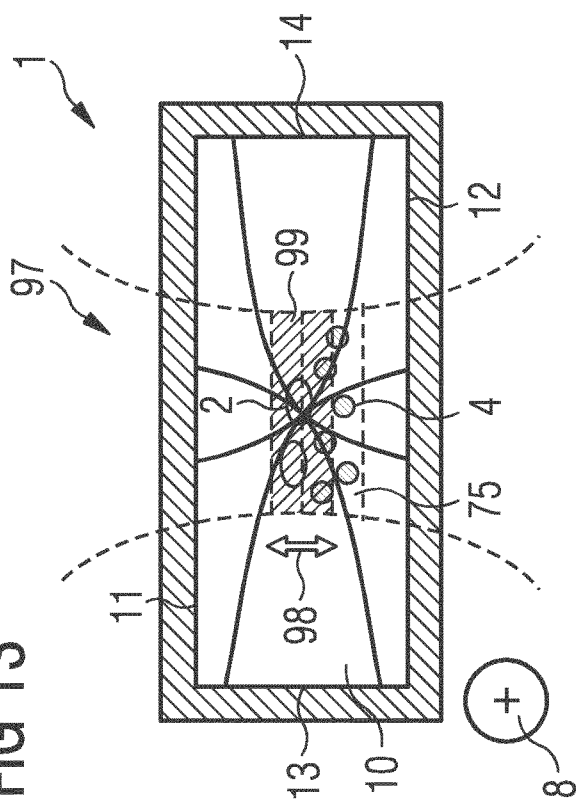


FIG 14

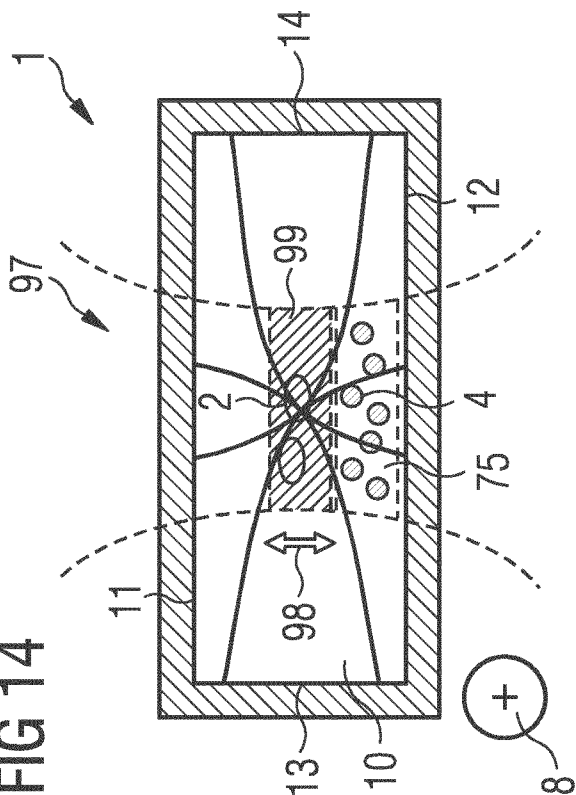
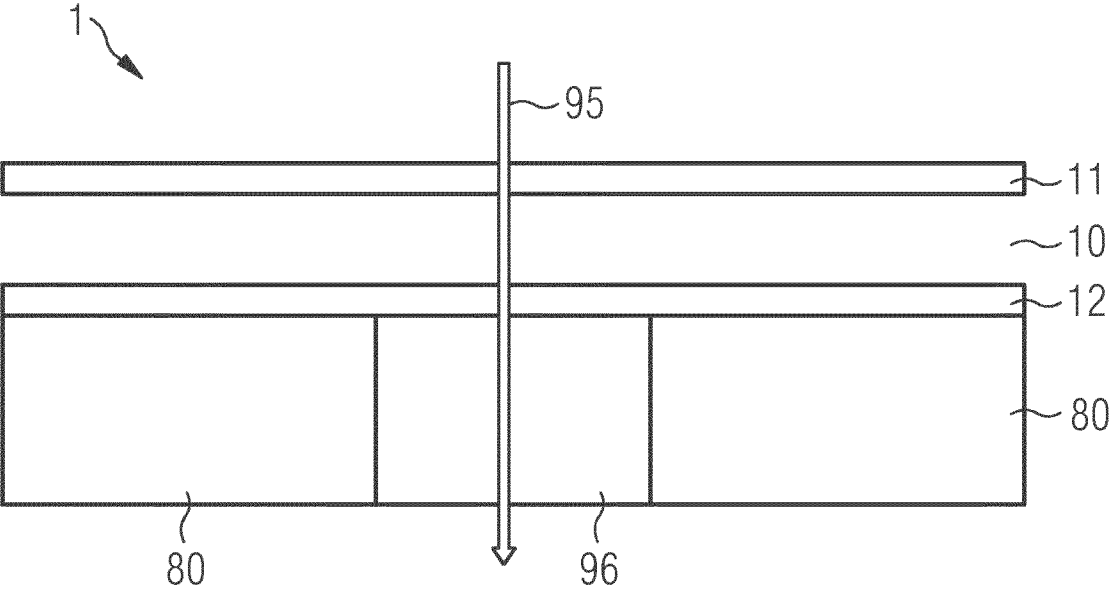


FIG 19



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/055794

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N15/14

ADD. B01L3/00 G01N15/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	X. DING ET AL: "Cell separation using tilted-angle standing surface acoustic waves", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 111, no. 36, 25 August 2014 (2014-08-25), pages 12992-12997, XP055283160, US ISSN: 0027-8424, DOI: 10.1073/pnas.1413325111	1,9,16
Y	Section: Results, Design and Characterization; page 12993; figures 1,5 ----- -/-	2-7, 10-15, 17,18



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

30 November 2016

Date of mailing of the international search report

08/12/2016

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/055794

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	C. PERFETTI ET AL: "Quantitative analysis of 3D hydrodynamic focusing of microparticles by digital holographic microscopy", EXPERIMENTS IN FLUIDS., vol. 55, no. 2, 1 February 2014 (2014-02-01), XP055324483, DE ISSN: 0723-4864, DOI: 10.1007/s00348-014-1667-y Overview of focusing techniques; page 2, column 1, paragraph 3 - column 2, paragraph 1 -----	2-7, 10-15, 17,18
A	J. H. NIEUWENHUIS ET AL: "Integrated flow-cells for novel adjustable sheath flows", LAB ON A CHIP: MINIATURISATION FOR CHEMISTRY, PHYSICS, BIOLOGY, MATERIALS SCIENCE AND BIOENGINEERING, vol. 3, no. 2, 1 January 2003 (2003-01-01), page 56, XP055324572, GB ISSN: 1473-0197, DOI: 10.1039/b210724d figure 3 -----	1-18
A	US 2012/160746 A1 (THORSLUND SARA [SE] ET AL) 28 June 2012 (2012-06-28) paragraph [0038]; figure 3 -----	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/055794

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		JP 2012501437 A	19-01-2012
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