(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 2 790 020 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Drawings
Drawing(s) replaced or added

(48) Corrigendum issued on:
18.05.2016 Bulletin 2016/20

(45) Date of publication and mention of the grant of the patent:
09.03.2016 Bulletin 2016/10

(21) Application number: **14159725.2**

(22) Date of filing: **14.03.2014**

(51) Int Cl.:
G01N 33/574 *(2006.01)* ***B01L 3/00*** *(2006.01)*
G01N 15/02 *(2006.01)* ***C12Q 1/24*** *(2006.01)*
G01N 33/49 *(2006.01)* ***C12N 5/09*** *(2010.01)*

(54) Methods for segregating particles using an apparatus with a size-discriminating separation element having an elongate leading edge

Verfahren zur Trennung von Partikeln mittels einer Vorrichtung mit größenunterscheidendem Trennelement mit einer länglichen Vorderkante

Procédés de ségrégation de particules au moyen d'un appareil équipé d'un élément de séparation par discrimination de taille présentant un bord d'attaque allongé

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

(30) Priority: **15.03.2013 US 201361794468 P**
12.11.2013 US 201314077811

(43) Date of publication of application:
15.10.2014 Bulletin 2014/42

(60) Divisional application:
16150499.8

(73) Proprietor: **ANGLE North America, Inc.**
Philadelphia, PA 19104-5504 (US)

(72) Inventor: **Hvichia, Georgi**
Philadelphia, PA Pennsylvania 19116 (US)

(74) Representative: **Irvine, Jonquil Claire**
HGF Limited
140 London Wall
London EC2Y 5DN (GB)

(56) References cited:
WO-A1-2005/036139
WO-A1-2012/139209
WO-A2-2009/131645
WO-A2-2010/011934
WO-A2-2010/129441
WO-A2-2011/066497
US-B2- 7 993 908

- **SEONYOUNG KIM ET AL: "Circulating Tumor Cell Microseparator Based on Lateral Magnetophoresis and Immunomagnetic Nanobeads", ANALYTICAL CHEMISTRY, vol. 85, no. 5, 5 March 2013 (2013-03-05), pages 2779-2786, XP055152908, ISSN: 0003-2700, DOI: 10.1021/ac303284u**

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- VANKRUNKELSVEN S ET AL: "A NOVEL MICROSTEP DEVICE FOR THE SIZE SEPARATION OF CELLS", ELECTROPHORESIS, WILEY INTERSCIENCE, DE, vol. 25, 2 June 2004 (2004-06-02), pages 1714-1722, XP002320623, ISSN: 0173-0835, DOI: 10.1002/ELPS.200405900

Description

Back ground of the disclosure

[0001] Mechanical devices intended for manipulation of biological cells and other small particles and having structural elements with dimensions ranging from tens of micrometers (the dimensions of biological cells) to nanometers (the dimensions of some biological macromolecules) have been described. For example, U.S. Patent number 5,928,880, U.S. Patent number 5,866,345, U.S. Patent number 5,744,366, U.S. Patent number 5,486,335, and U.S. Patent number 5,427,946 describe devices for handling cells and biological molecules

[0002] U.S. Patent 7,993,908 and published PCT applications no. WO 03/008931, WO 2009/131645 and WO 2011/066497, all of which are in common ownership with the now claimed invention, describes microscale devices for separating cells and other particles based on their size. The form of device described in those specifications includes a stepped separation element interposed between two regions of a void formed by a cover and body, and separation of particles within the apparatus is governed by the ability of particles initially present in one region to traverse the stepped separation element to arrive at the other region. The subject matter disclosed herein is considered an improvement upon previously disclosed such devices.

[0003] Vankrunkelsven et al., " A novel microstep device for the size separation of cells", Electrophoresis, Wiley Interscience, Vol. 25, June 2004, pages 1714-1722 and published PCT Application WO 2005/036139 also describe devices for size separation of particles such as cells in a fluid.

[0004] The subject matter disclosed herein can be used to segregate and manipulate biological cells, organelles, cell conglomerates, and other particles from mixed populations of particles or cells.

Summary of the disclosure

[0005] The present invention is as set out in the claims.

[0006] In one aspect there is thus provided a device for segregating smaller and larger particles, e.g separating circulating tumor cells from blood cells in a whole blood sample, the apparatus comprising:

a body (2) and
a cover (4) that define a void (50) therebetween, the void (50) containing
a separation element (1) that segregates an inlet region (52) and an outlet region (58) of the void (50), the separation element (1) defining, together with a surface of the void (50),
a channel that fluidly connects the inlet and outlet regions by way of a separating portion, the channel having

an overall width at the separating portion and
a height defined by the distance between the separation element (1) and the surface of the void (50),

at least one of the body (2), the cover (4), and the separation element (1) bearing a segregating step disposed within and having a leading edge extending completely across the separating portion of the channel, whereby the channel is divided into an upstream portion on the inlet side of the leading edge and a lamellar downstream portion on the outlet side of the leading edge,

the height of the upstream portion being sufficient to facilitate passage therethrough of both larger and smaller particles,
the height of the downstream portion being sufficiently large to facilitate passage therethrough of the smaller particles and sufficiently small to inhibit passage therethrough of the larger particles, and
the length of the leading edge being at least 20 times greater than the overall width of the channel at the separation region,
wherein the upstream portion of the channel is lamellar in a region between the inlet region (52) and the separation element (1) and
wherein the leading edge has an undulating shape,

whereby the particles can be segregated by passing them through the channel, and recovering particles based on their ability to traverse the segregating step.

[0007] More generally, the present disclosure thus relates to an apparatus for segregating smaller and larger particles. The apparatus includes a body and a cover that define a void between them. The void contains a separation element that segregates an inlet region and an outlet region of the void. Together with one or more surfaces of the void, the

separation element defines a channel that fluidly connects the inlet and outlet regions by way of a separating portion. The channel has an overall width at the separating portion and a height defined by the distance between the separation element and the surface of the void. At least one of the body, the cover, and the separation element bears a segregating step disposed within and having a leading edge extending substantially completely across the separating portion of the channel. The channel is divided into an upstream portion on the inlet side of the leading edge and a substantially lamellar downstream portion on the outlet side of the leading edge. The height of the upstream portion is sufficient to facilitate passage therethrough of both larger and smaller particles. The height of the downstream portion is sufficient large to facilitate passage therethrough of the smaller particles and sufficiently small to inhibit passage therethrough of the larger particles. The breadth of the leading edge is substantially greater than the overall width of the channel at the separation region (which is normally the same width as that of the segregating step, meaning that leading edge of the segregating step is normally longer than the width of that step). The particles can be segregated by passing them through the channel and recovering particles based on their ability to traverse the segregating step.

[0008] In one embodiment, the upstream portion of the channel is substantially lamellar, meaning that it is defined by two broad surfaces that are substantially parallel to one another.

[0009] The breadth of the leading edge can be substantially (e.g., at least 100 times) greater than a characteristic dimension of the larger particles, so that many such particles can be trapped at the leading edge without substantially preventing bulk fluid flow past the leading edge. The breadth of the leading edge in a device of the invention will also be substantially (i.e. at least 20, e.g. 50, 100, 500, 1000, 10000, or 100000 times) greater than the overall width of the channel at the separation region (or the width of the segregating step within the channel). By way of example, the height of the downstream portion (i.e., the portion of the channel of the leading edge of the segregating step) can be selected so that it is sufficiently small to inhibit passage therethrough of a selected cell type (e.g., a circulating tumor cell or human fetal stem-like cells), sufficiently large that it does not inhibit passage therethrough of a selected cell type (e.g., human red blood cells), or a combination of these.

[0010] The leading edge of any such segregating step in a device of the invention will have an undulating shape. This may be an invaginated or irregular shape. The segregating step can have, on its inlet side, an upstream face that is substantially perpendicular to the portion of the step that defines the downstream portion of the channel.

[0011] The separation element can be integral with at least one of the body and the cover. It can also be a separate item interposed between the body and the cover. The device can have one or more supports for maintaining the height of the channel. Such supports can be disposed within the channel and extend between the separation element and the surface of the void, for example.

[0012] The disclosure also relates to methods of segregating larger and smaller particles. These methods include providing a fluid suspension of larger and smaller particles at the inlet of the apparatus described herein. Fluid is urged through the channel and one can collect at least one of i) smaller particles (e.g., red blood cells) at the outlet region, and ii) larger particles (e.g., circulating tumor cells) upstream of the leading edge of the segregating step.

[0013] The disclosure also relates to methods of diagnosing occurrence of a tumor in a vertebrate subject. These methods include steps of i) providing a blood sample obtained from the subject to the inlet region of the apparatus described herein (the height of the lamellar portion of the downstream portion of the channel is smaller than the size of a CTC), passing the sample through the channel of the apparatus, and thereafter examining the portion of the apparatus upstream of the leading edge of the segregating step for the presence of a cell. Presence of at least one cell is an indication that a tumor occurs in the subject. One or more diagnostic tests can thereafter be used to assess a characteristic of a tumor cell for at least one cell that was present upstream of the leading edge of the segregating step after passing the sample through the channel. Examples of such tests include binding the cell or an extract thereof with a tissue-specific or tumor-specific antibody, analyzing nucleic acids obtained from such a cell that was present upstream of the leading edge, or assessing the proliferative capacity of the cell.

[0014] The disclosure further relates to methods of assessing the efficacy of a tumor treatment for a subject afflicted with a tumor. These methods include isolating CTCs from blood samples obtained from the subject before and after the treatment using the methods described herein. At least one characteristic of the CTCs isolated from the samples is compared among the samples. A difference in the characteristics of CTCs (e.g., CTC concentration or number) isolated from the blood samples is an indication of the efficacy of the treatment.

[0015] The disclosure also relates to methods of reducing CTC load in a vertebrate subject. Such methods include steps of i) providing blood obtained from the subject at the inlet of the apparatus described herein (wherein the height of the lamellar portion of the downstream portion of the channel is smaller than the size of a CTC), ii) urging the blood through the channel to deplete CTCs from the blood, iii) collecting CTC-depleted blood at the outlet region, and iv) returning the CTC-depleted blood to the subject.

Brief description of the several views of the drawings

[0016] The foregoing summary, as well as the following detailed description of the invention, will be better understood

when read in conjunction with the appended drawings. These drawings are included for the purpose of illustrating the disclosure. The disclosure is not limited to the precise arrangements and instrumentalities shown.

[0017] Figure 1 consists of Figures 1A, 1B, 1C, and 1D, and illustrates a prior art separation element **1** having two integral, rectangular slab-shaped steps, including a focusing step **10** and a segregating step **11**. Devices having a separation element of this sort are disclosed in U.S. Patent 7,993,908, for example. Figure 1A is an elevated view of the separation element **1** in which the rectangular shape of the face **20** of the focusing step **10** can be seen adjacent the broad face **40** thereof and the rectangular shape of the face **21** of the segregating step **11** can be seen adjacent the broad face **41** thereof. Figure 1B is a side view of the separation element **1** shown in Figure 1A, showing the height difference between the focusing and segregating steps (**10** and **11**, respectively). Figure 1C is an orthogonal view of the separation element **1** shown in Figures 1A and 1B. Figure 1D is a cross-sectional view of the separation element **1** disposed in a fluid channel defined by a gap between a cover **4** and the body **2** of an apparatus described herein. In Figure 1D, the height (h_1) of a downstream portion of the fluid channel, the height (h_0) of an upstream portion of the fluid channel, and the height (h_c) of the fluid channel itself are shown. The height (h_1) of the downstream portion is defined by the distance between the segregating step **11** and the cover **4**, and the height (h_0) of the upstream portion is defined by the distance between the focusing step **10** and the cover **4**.

[0018] Figure 2 consists of Figures 2A, 2B, and 2C and illustrates a separation element **1** having a rectangular slab-shaped focusing step **10** and a segregating step **11** having a slab shape but having an undulating face **21** and leading edge **31**. Figure 2A is an elevated view of the separation element **1** in which the undulating shape of the face **21** of the segregating step **11** can be seen adjacent the broad face **41** thereof. Figure 2B is a side view of the separation element **1** shown in Figure 2A, showing the height difference between the focusing and segregating steps (**10** and **11**, respectively). Figure 2C is an orthogonal view of the separation element **1** shown in Figures 2A and 2B.

[0019] Figure 3 consists of Figures 3A, 3B, and 3C and illustrates a separation element **1** having a rectangular slab-shaped focusing step **10** and a segregating step **11** having a slab shape but having an irregular face and leading edge. Figure 3A is an elevated view of the separation element **1** in which the rectangular shape of the face of the focusing step **10** can be seen adjacent the broad face **40** thereof and the irregular shape of the face of the segregating step **11** can be seen adjacent the broad face **41** thereof. Figure 3B is a side view of the separation element **1** shown in Figure 3A, showing the height difference between the focusing and segregating steps (**10** and **11**, respectively). Figure 3C is an orthogonal view of the separation element **1** shown in Figures 3A and 3B.

[0020] Figure 4 consists of Figures 4A, 4B, and 4C, and illustrates a separation element **1** having a rectangular focusing step **10** and three steps atop it and downstream (relative to BFF) from its leading edge. Each of the first segregating step **11**, second segregating step **12**, and third segregating step **13** has a chevron-shaped leading edge (leading edges **31**, **32**, and **33**, respectively. Bulk fluid flow **BFF** direction is indicated. Figure 4A is an elevated view of the separation element **1**. Figure 4B is a side view of the separation element **1** shown in Figure 4A, showing the height differences among the steps. A recessed portion of the separation element **1** downstream of steps **11-13** forms part of an outlet passageway by way of which material that has traversed all of steps **10-13** can be carried away from the separation element **1**. Figure 4C is a cross-sectional view of the separation element 1 disposed in a fluid channel defined by a gap between a cover **4** and the body **2** of an apparatus described herein. In Figure 4C, the heights (h_3, h_2, and h_1, respectively) of serial downstream portions of the fluid channel, the height (h_0) of an upstream portion of the fluid channel, and the height (h_c) of the fluid channel itself are shown. The height (h_3) of a third downstream portion is defined by the distance between the third segregating step **13** and the cover **4**. The height (h_2) of a second downstream portion is defined by the distance between the second segregating step **12** and the cover **4**. The height (h_1) of a first downstream portion is defined by the distance between the first segregating step **11** and the cover **4**. The height (h_0) of the upstream portion is defined by the distance between the focusing step **10** and the cover **4**.

[0021] Figure 5 consists of Figures 5A and 5B and illustrates a separation element **1** having a focusing step **10** having a curved transitional face **20** that extends completely across the separation element **1** and three segregating steps atop it and downstream (relative to BFF) from the focusing step **10**. Each of the first segregating step **11**, second segregating step **12**, and third segregating step **13** has a curved leading edge, meaning that the breadth of the leading edge of each of segregating steps **11-13** is greater than its width (unlike the length of the leading edge **30** of focusing step **10**, which is equal to its width). Bulk fluid flow **BFF** direction is indicated. Figure 5A is an elevated view of the separation element **1**. Figure 5B is a side view of the separation element **1** shown in Figure 5A, showing the height differences among the steps. A recessed portion of the separation element **1** downstream of steps **10-13** forms part of an outlet passageway by way of which material that has traversed all of steps **10-13** can be carried away from the separation element **1**.

[0022] Figure 6 consists of Figures 6A and 6B and illustrates a separation element **1** having a rectangular focusing step **10** and three segregating steps atop it and downstream (relative to BFF) from its leading edge. Each of the first segregating step **11**, second segregating step **12**, and third segregating step **13** has a serpentine leading edge. Bulk fluid flow **BFF** direction is indicated. Figure 6A is an elevated view of the separation element **1**. Figure 6B is a side view of the separation element **1** shown in Figure 6A, showing the height differences among the steps.

[0023] Figure 7 consists of Figures 7A, 7B, 7C, and 7D (which are drawn approximately to scale relative to one another)

and illustrates four step configurations having equal breadth (B, equal to 5 times the width of the channel) in a fluid channel indicated by heavy lines. The direction of bulk fluid flow (BFF) is indicated, and step height increases from the upstream to the downstream side of the step, which is indicated by a line extending across the fluid channel in the figures. In Figure 7A, step height rises across half the fluid channel at a relatively upstream position and across the other half of the fluid channel at a relatively downstream position, with the step face extending between those two positions. The length (L) of the extended step face is equal to four times the width (W) of the fluid channel in figure 7A, yielding a total B of the step equal to 5W. In Figure 7B, the step has two portions extending between an upstream position and a downstream position. Although the length of the step face extension in the direction of BFF is only 2W, there are two such extensions. As a result the total breadth of the face in Figure 7B is 2 x 2W + W, or 5W. Similarly, the step shown in Figure 7C, which has three portions extending between upstream and downstream positions (i.e., four step face extensions of length W) exhibits a B of 4 x W + W, or 5W. The step shown in Figure 7D, which has five portions extending between upstream and downstream positions (i.e., eight step face extensions of length W/2) exhibits a B of 8 x W/2 + W, or 5W. Of note, L of steps having equal B decreases with increasing invagination of the steps. This illustrates that miniaturization of particle separation functionality of a step can be achieved by increasing the complexity (B/L) of the step face.

[0024] Figure 8 is an embodiment of a particle segregation device as now claimed constructed to have a size approximately equal to a common microscope slide. Inlet and outlet regions **52** and **58** are shown, as is the separation portion **55** of the channel that extends between inlet and outlet regions **52** and **58**.

[0025] Figure 9 is a magnified image of PC3 prostate cancer cells captured using a segregation apparatus described herein. In the image, cells can be seen on or upstream (bulk fluid flow is from left to right in the Figure) from the first segregation step **11** and the second segregation step **12**, while few or no cells are present on focusing steps **10**.

[0026] Figure 10 consists of Figures 10A and 10B. Each of these is a magnified image of PC3 prostate cancer cells captured using a segregation apparatus described herein. In each image, cells can be seen on or upstream (bulk fluid flow is from left to right in the Figure) from the first segregation step **11**, the second segregation step **12**, and the third segregation step **13**, while few or no cells are present on focusing steps **10**.

Detailed description

[0027] The disclosure relates to an apparatus for segregating particles on the basis of their ability to traverse a passageway. Particles (e.g., particles suspended in a liquid or gaseous fluid or particles in a vacuum) are moved through a stepped passageway **55** defined by a separation element **1** in the apparatus. The stepped passageway **55** connects portions of a void **50** defined by a body **2** and a cover **4**, and the separation element **1** is present within the void **50** and separates inlet and outlet regions (**52** and **58**, respectively) regions of the void **50**. The separation element **1** may be a discrete element, or it may be attached to or integral with one of body **2** and cover **4**.

[0028] The stepped passageway **55** fluidly connects the inlet region **52** and the outlet region **58** of the void **50**, and contains at least one segregating passageway **101** that has a narrow dimension defined by the distance between the face **41** of a (first) segregating step **11** and another portion of the (first) segregating passageway **101**, such as the face of the body **2** or the cover **4**. Only some particles in the fluid are able to move into the segregating passageway **101**. The net result is that some particles can move through the entire stepped passageway **55**, while other particles are retained within the apparatus, such as upstream of the segregating passageway **101**. Segregation of particles is thus achieved. Movement of particles can be motivated by fluid flow, gravity, vibration, or any combination of these, for example.

[0029] In contrast to analogous devices disclosed, for example, in U.S. Patent 7,993,908 (illustrated in Figure 1), the leading edge **31** and transitional face **41** of at least one of the segregating steps of a device of the invention has a breadth substantially greater (by a factor of at least 20, e.g. 25) than the width of the segregating step (i.e., greater than the width of the stepped passageway **55** in which the segregating step **11** is disposed. Because separation of particles in bulk fluid flowing past a segregating step **11** tends to occur mostly at the leading edge and face **21** of the step, increasing the breadth of these, relative to the width of the segregating step **11** and passageway can have several beneficial effects.

[0030] Particles flowing past a segregating step **11** in a bulk fluid will necessarily have a size, in at least one dimension, not greater than the height of the segregating passageway **101** above that segregating step **11** (i.e., the narrow dimension of the segregating passageway; otherwise the particles would be unable to pass therethrough with the bulk fluid). Likewise, particles having dimensions greater than the height of the segregating passageway **101** above a segregating step **11** will cease to flow with bulk fluid at or near the leading edge **31** or the transitional face **21** of the segregating step **11** and will tend to accumulate there. Increasing the breadth of the transitional face **21** and leading edge **31** beyond the overall width of the passageway in which the segregating step **11** is disposed permits multiple particles to be accommodated at the leading edge **31** or elsewhere along the transitional face **21** (e.g., if the face is sloped). Thus, the apparatus in which the leading edge has a breadth greater than the overall width of the segregating passageway **101** can be used to capture one or more size-segregated particles at or near the leading edge **31** of the segregating step **11**. As the breadth of the leading edge **31** of the segregating step **11** increases, a greater number of size-segregated particles can

be captured at the transitional face **21** thereof without clogging the device. As hereinbefore indicated, it is provided by a device of the invention that the ratio of the breadth of a segregating step is substantially greater (at least 20, e.g. 50, 100, 500, or 1000 fold greater) than the width of the passageway that bounds the ends of the leading edge of the segregating step.

[0031] In order to accommodate a leading edge **21** having a breadth greater than the width of the segregating passageway **101**, the leading edge **21** and the transitional face **31** of a segregating step **11** must not extend straight across the narrowest width of the segregating passageway **101**. The leading edge can be straight (e.g., extending obliquely across the passageway **101** in a direction other than the narrowest dimension thereof) or composed of multiple straight segments (see Figures 4 and 7). The leading edge **21** can also be curved (See Figure 5), invaginated (See Figures 2, 3, and 6), or meandering (See Figure 3) in shape, thereby increasing its breadth (and that of its corresponding segregating step **11** and transitional face **21**) relative to the width of the segregating passageway **101**. As a result of the leading edge undulating shapes now taught for a segregating step of a device of the invention, the capture capacity can be increased, relative to prior art devices in which segregating steps **11** extended directly across the width of the segregating passageway **101**.

[0032] In one embodiment, the leading edge of the step is highly curved (e.g., has many invaginations, such as the invagination shown in segregating steps **11-13** in Figure 6), so that its breadth is significantly greater than the overall width of the passageway in which the step is contained. By way of example, Figures 4, 5, and 6 illustrate a four-step separation element **1** that can be accommodated within a passageway having a substantially rectangular cross-section In Figures 4, 5, and 6, the separation element **1** has an overall width equal to the width (i.e., in the direction perpendicular to bulk fluid flow **BFF**) of the segregating passageway **101**. The separation element **1** in each of these figures includes a focusing step **10** that extends directly across the segregating passageway **101** (like steps in prior art devices) and thus has a breadth equal to the overall width of the passageway.

[0033] In Figure 4, the leading edge of each of segregating steps **11-13** has a breadth greater than the overall width of the segregating passageway **101** -- if the vertex of the chevron-shaped leading edge of each step is a right angle, then the length of the leading edge of each step is (by application of the Pythagorean equation) equal to twice the square root of (the square of the width of the passageway divided by two) (i.e., if the width of the segregating passageway **101** is one unit, then the breadth of each step is about 1.4 units).

[0034] In Figure 5, the breadth of the leading edge of each of segregating steps **11-13** is greater than the overall width of the segregating passageway **101**, on account of the curvature of the leading edge of each step.

[0035] In Figure 6, the breadth of the leading edge of each of segregating steps **11-13** is longer still, owing to the curvature and invagination of each step.

[0036] The geometries shown in Figures 4-6 are for illustrative purposes. Step leading edges can have innumerable geometric shapes. The shapes shown in those figures simply illustrate the concept that increasing the complexity (especially 'folding' or invagination) of the leading edge can cause the breadth of the leading edge of any step to greatly exceed the overall width of the passageway within which the step occurs. In another embodiment of the separation element shown in Figures 4-6, the separation element lacks focusing step **10** and the segregating steps **11-13** are integral with three adjacent walls of the substantially rectangular segregating passageways **101-103** in which the separation element **1** is disposed.

[0037] Particles unable to traverse a segregating step can be urged in the direction of bulk fluid flow along the leading edge of the segregating step. Thus, for example, particles that are able to traverse the focusing step, but are unable to traverse the segregating steps of the device shown in Figure 6 will tend to be urged by bulk fluid flow toward the central invagination in the segregating steps and toward the peripheral edges of those steps. Although not shown, the shapes of the leading edges of the segregating steps illustrated in Figures 4 and 5 can be inverted relative to the direction of BFF shown in those figures (i.e., so that the apices of the chevron-shaped and curved steps lie downstream from the edges of the steps). Steps can thus be shaped to facilitate or promote accumulation of particles at selected locations along their leading edges.

[0038] Particles captured at the leading edge **31** or along the transitional face **21** of a segregating step **11** (i.e., a step past which some, but not all, particles in a bulk fluid can move with the bulk fluid flowing past the step) will tend to occlude fluid flow past the step at or on which they are captured (i.e., at the position at which their movement with the bulk fluid stops or is substantially inhibited). If captured particles occlude fluid flow past a substantial portion (e.g., >0.01 %, > 0.1 %, > 1%, > 10%, > 50%, > 90%, or > 99%) of the stepped passageway, this will decrease the throughput of the segregating passageway **101** (i.e., the volume of fluid that can be passed through the narrow passageway in a unit of time at a selected fluid pressure drop across the step) can be significantly diminished.

[0039] By increasing the breadth of the leading edge **31** of at least one segregating step **11** (i.e., relative to the overall width of the space within which the segregating passageway **101** is contained), captured particles will individually occupy a smaller percentage of the flow area of the segregating passageway **101**, reducing flow occlusion and increasing the ability of the apparatus to maintain a near-constant throughput. Constant throughput can reduce the need for complicated or expensive fluid flow control equipment, since the pressure drop across the apparatus should remain substantially

constant so long as throughput remains substantially constant. A very broad step leading edge **31** can therefore significantly reduce the tendency of the apparatus to experience decreased throughput for samples having significant numbers of captured particles. Such apparatus can also capture a greater number of size-segregated particles without exhibiting significantly decreased throughput.

[0040] The invention claimed herein is complementary to the subject matter disclosed in international patent applications nos. PCT/US2002/022689, PCT/US2009/002421, PCT/US2010/046350, and PCT/US2010/058172 published as WO 03/008931, WO 2009/131645, WO 20111/028483 and WO 2011/066497 respectively.

Definitions

[0041] As used herein, each of the following terms has the meaning associated with it in this section.

[0042] For fluid flowing through a passageway in which a separation element **1** as described herein is disposed, the "height" of the passageway is the minimum distance between the surfaces of the passageway between which the separation element **1** is interposed. For example, in each of Figures 1D and 4C, a separation element **1** is interposed between a body **2** and a cover **4**. The minimum distance between the parallel faces of the body **2** and cover **4** defines the height (h_c) of the passageway. Also visible in these figures are the height (h0) of the passageways above the focusing steps **10** of the separation elements **1** and the heights (h_1, h_2, and h_3) of the segregating passageways **101**, **102**, and **103** above segregating steps **11**, **12**, and **13**, respectively. It is not critical that the 'height' dimension be oriented vertically relative to gravity during operation of the devices described herein.

[0043] A "focusing" step is merely a step which is disposed in (and preferably extends most of the way or completely across) the channel on the inlet side of a segregating step. A focusing step essentially directs fluid flow through the channel toward the portion of the narrow passageway defined by the segregating step, reducing potential areas of "dead volume" in which little or no local fluid flow occurs. The channel should have a greater height on the inlet side of the focusing step than on its outlet side, such as with an inclined focusing step, or the focusing step can have a more staircase-like conformation, including multiple steps. Devices described herein need not include a focusing step, but inclusion of a separating step can be important in embodiments in which minimization of dead volume (and retention therein of particles intended to pass beyond the segregating step(s)) is desired.

[0044] The "width" of a passageway in which a separation element **1** as described herein is disposed is the minimum distance, in the direction perpendicular to the direction of bulk fluid flow through the passageway (i.e., the overall general direction of such flow, ignoring localized flow redirection induced by step geometries) and perpendicular to the height of the passageway, between opposite faces of the passageway. For example, the width of a passageway is indicated as "W" in Figure 7 for each of four passageways containing steps of various geometries. Further by way of example, the width dimension of the stepped passageways **55** depicted in figures 1D and 4C extend perpendicularly out of the figure. The "width" of a step is assessed in the same direction as the width of a passageway in which the step is disposed; thus, the width of a step that extends completely across the width a passageway is equal to the width of the passageway (even though the breadth of the leading edge of the step may be significantly greater than the width of the step owing, for example, to curvature or invagination of the leading edge).

[0045] The "breadth" of the leading edge **31** of a segregating step **11** is the length of the leading edge **31**, measured following the curvature of the step. If the leading edge **31** is envisioned as an inflexible cord, the breadth of the leading edge is the length of the cord when it is pulled taut. Thus, the breadth of the leading edge of a curved or invaginated step can be significantly greater than the width of the step. This is illustrated in Figures 7A-7D, in which four leading edges **31** having a length 5W are configured in a variety of conformations, each leading edge substantially exceeding the width (W) of the stepped passageway **55** in which it is disposed.

[0046] The "broad face" of a step is the portion of a step that exists at a topographically altitude higher than a reference surface with respect to which the step exists. The broad face of a step described herein will generally, but need not, be planar.

[0047] The "transitional face" of a step is the portion of the step that bridges its broad face and the reference surface. The transitional face preferably has a smooth or flat contour, and can be a surface perpendicular to both the reference surface and the broad face **41**, as shown for transitional face **21** in Figure 1. Transitional faces can also be inclined planar surfaces (see transitional face **20** in Figure 6) or curved (see transitional face **20** in Figure 5).

[0048] The "leading edge" **31** of a step is the portion of the step at which its broad face **41** meets its transitional face **21**, for example as shown in Figure 1.

[0049] The "flow area" of a passageway is a cross-section of the passageway taken in a plane perpendicular to the direction of bulk fluid flow in the passageway.

Detailed Description

[0050] The disclosure relates to an apparatus for segregating particles on the basis of their ability to flow through a

segregating passageway **101** having a minimum dimension (height) defined by the separation between a segregating step **11** of a separation element **1** and a surface of a void **50** in which the separation element is disposed. The apparatus includes a separation element **1** disposed in a void **50** formed by a body **2** and cover **4**. Within the void **50**, the separation element **1** separates an inlet region **52** of the void from an outlet region **58** of the void. The inlet and outlet regions are in fluid communication by way of a stepped passageway **55** defined by the separation element **1** and one or both of the body **2** and cover **4**. One or more segregating steps **11** formed in the separation element **1** define one or more segregation passageways **101**. Fluid that flows between the inlet and outlet regions passes through the stepped passageway **55**, including through at least a first segregation passageway **101**.

[0051] In operation, particles in an inlet region **52** of the void **50** pass into the stepped passageway **55** and, if they are able, into the segregating passageway **101**. Particles in the segregating passageway **101** can pass to the outlet region **58** of the void **50**. Cells that are not able to pass into or along the segregating passageway **101** do not reach the outlet region **58**. In this way, particles able to reach the outlet region **58** are segregated from particles that are not able to reach the outlet region **58**. The two populations of particles can be separately recovered from the apparatus. For example, particles at the outlet region **58** can be recovered in a stream of liquid withdrawn from the outlet region **58** (e.g., by way of an outlet port or by way of a catheter inserted into the outlet region **58**). Particles unable to pass through the segregating passageway **101** to the outlet region **58** can be recovered by flushing them, in the reverse direction, through the stepped passageway **55** and into the inlet region **52**. Such particles can be withdrawn from the inlet region **52**. Alternatively, particles unable to pass through the segregating passageway **101** to the outlet region **58** can be left in the apparatus or recovered by disassembling the apparatus.

[0052] The apparatus described herein can be used in a wide variety of applications. In addition to segregating particles from a mixed population of particles, the device can be used in applications in which one or more of the segregated particle populations are identified or further manipulated, for example. The construction and operation of the apparatus resist clogging by the particles being segregated, relative to devices previously used for particle separation.

[0053] By way of example, the apparatus can be used to isolate tumor cells from a mixed suspension of cells, such as to isolate circulating tumor cells (CTCs) present in the blood of a human or other vertebrate subject. The apparatus can also be used to isolate fetal cells from the blood of a woman carrying (or who previously carried) a fetus. The apparatus can furthermore be used to isolate from a mixed suspension of cells substantially any cell(s) that can be differentiated from others in the suspension on the basis of their size, their compressibility, or a combination of these.

[0054] Parts and portions of the apparatus are now discussed separately in greater detail.

The Body and Cover

[0055] The apparatus has a body 2 and a cover 4 defining a void 50 therebetween. A portion of the void 50, defined in part by the separation element 1, is a stepped passageway 55. The stepped passageway 55 is also defined by a surface of the body 2, a surface of the cover 4, or by a combination of these, that is opposed to one or more stepped surfaces (e.g., 31 and 32) of the separation element 1. In order to simplify construction of the apparatus, most or all of the stepped passageway-defining surfaces can be formed or machined into a separation element 1 that is an integral part formed in a recess of the cover 4 or the body 2, the recessed portion being surrounded by a flat surface, so that the opposed surface of the body 2 or the cover 4 need only be another flat surface in order to form the void 50 and enclose the separation element 1 therein upon contact between the flat surfaces of the body 2 and cover 4.

[0056] The general format of the body 2 and cover 4 having an interposed separation element 1 is discussed generally in prior art documents previously noted above and substantially any arrangement described therein can be used for the apparatus described here. Described herein are elements of the separation element 1 that are not disclosed in those documents.

[0057] The body 2, the cover 4, or both can define an inlet port through which fluid can be introduced into or withdrawn from the void 50. For example, the body 2 can define an inlet port that fluidly communicates with the inlet region 52. Fluid introduced into the inlet port can flow into the inlet region 52, displacing fluid already there (because the void is sealed) into the stepped passageway, and thence sequentially into the first passageway **51**, the second passageway **52**, and the outlet region 58. Particles suspended in fluid in one of these regions and passageways can be carried into a downstream region or passageway if the particle can flow through the present and intervening passageways and regions. Similarly, withdrawal of fluid from the outlet region 58 by way of an outlet port formed in the body 2 can induce fluid flow from passageways in fluid communication with the outlet region 58 and from passageways and regions in fluid communication therewith.

[0058] Ports can be simple holes which extend through the cover or body, or they can have fixtures (burrs, rings, hubs, or other fittings) associated with them for facilitating connection of a fluid flow device to the port. The body 2, cover 4, or both can define an inlet port in the inlet region 52 of the void 50, an outlet port in the outlet region 58 of the void 50, or both an inlet port and an outlet port. Fluid can be introduced into the inlet region 52 through the inlet port. Fluid can be withdrawn from the outlet region 58 through the outlet port. Continuous introduction of fluid into the inlet region 52

and simultaneous withdrawal or emission of fluid from the outlet region 58 can create a continuous flow of fluid through the apparatus. Similarly, continuous withdrawal of fluid from the outlet region 58 and simultaneous influx or introduction of fluid into the inlet region 52 can create continuous flow.

The Void

[0059] The body 2 and the cover 4 form a void 50 when they are assembled. The void 50 has an inlet region 52, an outlet region 58, and a separation region interposed between the inlet region 52 and the outlet region 58. A separation element 1 is disposed within the separation region and, together with the body 2, the cover 4, or both, defines a stepped passageway 55. The stepped passageway 55 includes at least a first segregating passageway **101** that is defined by at least a first segregating step **11** in the separation element 1. The stepped passageway 55 can include any number of additional segregating steps, each of which can define an additional segregating passageway in the void. Preferably, the only fluid path connecting the inlet and outlet regions **52** and **58** is the stepped passageway 55, although that stepped passageway can be separated into multiple stepped passageways, arranged in series, in parallel, or in some combination of these. Likewise, multiple devices as described herein can be operated in series (e.g., to selectively capture particles in selected size ranges) or in parallel (e.g., to enhance cell capture capacity).

[0060] During operation of the device, at least the inlet region 52, the outlet region 58, and the stepped passageway of the void 50 are filled with a fluid. Preferably, the entire void 50 is filled with fluid during operation. In one embodiment, the only fluid path that connects the inlet region 52 and the outlet region 58 is the stepped passageway. Particles present in the inlet region 52 can enter the stepped passageway 55. The void (i.e., as defined by one or more of the body, cover, and separation element) can be formed so as to taper in the direction of (or opposite) bulk fluid flow from the inlet region toward the stepped passageway. Such void shapes can focus particles flow toward the stepped passageway, maintain fluid linear flow velocity through the shaped region within a desired range (e.g., substantially constant), facilitate viewing of particles passing therethrough, or have other beneficial consequences.

[0061] Particles present in the stepped passageway **55** can enter and pass through the first segregating passageway **101** unless they are excluded by the height (i.e., the narrow dimension) of the first segregating passageway **101**, or unless their movement through the first segregating passageway **101** is inhibited by particles which block that passageway (e.g., cells immobilized at or upstream from the leading edge **31** of the first segregating passageway **101**. Particles which pass through the first segregating passageway **101** can enter the outlet region 58 and thence be recovered. Movement of particles within the apparatus can be induced by fluid flow through the apparatus, by intrinsic motility of the cells, or a combination of the two. Over time, particles unable to enter the first passageway **51** will be segregated in the inlet region 52; particles able to traverse the first segregating passageway **101** will be segregated in or upstream from the stepped passageway **55**; particles able to enter the first segregating passageway **101** but unable to freely move therethrough will be segregated in the first segregating passageway **101**; and particles able to move through first segregating passageway **101** will be segregated in the outlet region 58 (or in fluid withdrawn or emitted from the outlet region 58).

[0062] Particles segregated in this manner can be recovered (using any of a variety of known methods, including some described herein) from their respective locations. By way of example, a catheter can be inserted into a region or passageway (e.g., the inlet region 52 or first segregating passageway **101**) of the apparatus, and particles present therein can be withdrawn by inducing suction in lumen of the catheter. Further by way of example, backflushing (i.e., fluid flow from the outlet region 58 in the direction of the inlet region 52) can be used to collect particles present in one or more of the inlet region 52 or the first segregating passageway **101**.

The Separation Element

[0063] The separation element 1 of the devices claimed herein is distinguished from those described previously in U.S. Patent number 7,993,908, in PCT publication WO 2011/066497, or elsewhere. The separation element 1 of the devices of the invention includes at least one segregating step 11 that has an undulating leading edge 31 with a breadth significantly greater than (at least 20x greater than) the overall width of a passageway within which the segregating step occurs. Put another way, the shape of the leading edge of at least one step of the separation element 1 is such that the breadth of that leading edge is substantially greater than the overall width of the step. Put yet another way, the breadth of the leading edge of the step, assessed along its contour, is greater than the shortest linear distance between the two endpoints of the step edge (i.e., regardless of whether the step edge follows that shortest line). By way of example, the leading edge can be invaginated (see e.g. Fig. 6) e.g. serpentine (see Fig.8). The upper limit of the ratio of step breadth to passageway width is bounded substantially only by the tolerance of the manufacturing methods used to form the step and the size of the particles that pass the step.

[0064] The stepped passageway 55 is the orifice through which particles move, fluid flows, or both, from the inlet region 52 to the outlet region 58 during operation of the apparatus. The separation element 1 has a stepped structure, which defines the stepped shape of at least one side of the stepped passageway 55. The separation element 1 has at

least one segregating step **11**, and it can have multiple segregating steps (e.g., **11-13** in Figures 6-8). Fluid must flow through the segregating passageway **101** defined in part by the corresponding segregating step **11** in order to traverse the stepped passageway 55 from the inlet region 52 to the outlet region 58 when the apparatus is assembled.

[0065] During operation of the apparatus described herein, a mixture of particles having different sizes can be caused to flow through the stepped passageway 55, including at least one segregating passageway 101. Passage of particles having a characteristic size in excess of the narrow dimension (i.e., the height) of the segregating passageway 101 is impeded at or near the leading edge **31** of the segregating step **11** that bounds the segregating passageway, and such particles will tend to accumulate at or near the leading edge **31** rather than passing through the segregating passageway 101. So long as the segregating passageway 101 is not completely occluded by impeded particles across the entire breadth of the segregating step **11**, flow of fluid and particles around or past the impeded cells can continue. Development of the subject matter described herein arose, at least in part, as a result of attempts to design apparatus less susceptible to fouling and clogging by impeded particles than prior art apparatuses. Preferably, the breadth of separating step **11** leading edges **31** are selected so that, for an anticipated mixture of particles, that the portion(s) of the segregating passageway 101 at which passage of particles are impeded has a sufficient flow area that fluid flux through such portion(s) is not significantly (i.e., not more than 50%, 20%, 10%, 5%, 1%, 0.33%, or 0.1% or less) impeded when a desirable or foreseeable number of particles are lodged at the portion(s).

[0066] The separation element 1 can include a focusing step 10 (as illustrated in Figures 1-6), which serves to deflect fluid flow within the stepped passageway 55 toward the first segregating passageway 101, to fill 'dead spaces' upstream of the first segregating step 11, to provide a structurally sound foundation for carrying segregating steps on the separation element, or some combination of these. The separation need not include a focusing step.

[0067] The steps of the separation element 1 can have any of a variety of shapes. In one embodiment (e.g., in the apparatus depicted in Figure 1), both the focusing step **10** and the first segregating step **11** have a common 'staircase-type' step structure, i.e., two planar surfaces that intersect at a right angle. That is, the transitional face 20 of the focusing step 10 and the broad face 40 of the focusing step **10** meet at a right angle, as do the transitional face **21** of the first segregating step **11** and the broad face 41 thereof. Alternatively, the transitional and broad faces of steps can meet at an angle between 90 and 180 degrees, for example. The transitional and broad faces of the steps can also meet at an angle between 0 and 90 degrees, forming an overhang. For a device of the invention however, at least one segregating step 11 has an undulating leading edge consistent with a length at least 20 x greater than the overall width of the channel at the separation region, e.g. an invaginated leading edge 31 and transitional face 21, so that the breadth of the step is significantly greater than the width of the step.

[0068] Steps having transitional and broad faces that meet at an angle between 90 and 180 degrees can occlude passage of particles having a variety of sizes (i.e., those having sizes intermediate between the narrow dimension of the passageway defined by the broad face of the step and the narrow dimension of the space upstream from the step. By halting passage of particles having slightly different sizes at different positions on the transitional face of the step, a step having transitional and broad faces that meet at an angle between 90 and 180 degrees can prevent clogging of the passageway defined by the broad face of the step to a greater degree than a step having transitional and broad faces that meet at an angle of 90 degrees or less.

[0069] Clogging of fluid flow past a step by particles that occlude the passageway defined by the broad face of the step can also be reduced or avoided by increasing the width of the step, as was recognized in the art. Because each particle occludes fluid flow only for the flow area obscured by the particle, a wider step will necessarily be clogged by a greater number of occluding particles. However, increasing the width of a step is not always practical, especially when significant widening is required to accommodate numerous particles or when miniaturization is desired.

[0070] A significant aspect of the subject matter disclosed herein is recognition by the present inventors that the capacity of a segregating step 11 to accommodate impeded particles can be significantly increased without increasing the width of the step. Rather than (or in addition to) increasing the width of a segregating step 11, its particle-retention capacity can be increased by increasing the breadth of the leading edge 31 of the step (i.e., where particles impedance occurs), for example by decreasing the straightness of the step.

[0071] By way of example, in a fluid channel having a rectangular cross-section, a step that extends directly across (i.e., at right angles to the sides) of the channel has a leading edge with a breadth simply equal to the width of the channel (see, e.g., Figure 1). If the shape of the step is a hemicircle, with the arc of the hemicircle extending such that the center of the hemicircle lies downstream from the upstream-most edge of the hemicircle, then the breadth of the leading edge of the step is equal to the perimeter of the hemicircle, which is the number pi multiplied by the width of the channel and divided by two (i.e., roughly 1.57 x the width of the channel). Similarly, steps having leading edges shaped like an arc of a circle or ellipse, like chevrons (i.e., like the letter V), like zig-zags, like serpentine lines, or like irregular lines (See Figures 2-6) will all have breadth values greater than the breadth of a step that simply extends perpendicularly across a fluid channel having a rectangular cross-section

[0072] As indicated above, an essential feature of a device of the invention is provision of a leading edge 31 of a segregating step 11 shaped such that the breadth of the leading edge 31 is substantially greater (at least 20 times

greater) than the overall width of the segregation passageway 101 defined by the step. This can be achieved, for example, by forming the step such that its leading edge has an undulating or highly irregular edge shape, as illustrated in Figures 2 and 3, which are representations of steps having undulating and irregular edges, respectively. In Figure 2, the segregation step **11** is a flat slab having finger-shaped projections at its transition face **21**. The breadth of the leading edge 31 of the step formed by the perimeter of the finger-shaped projections is substantially greater than the width of the step, as can be seen clearly in Fig. 2A. Likewise, the undulations and irregularities in the leading edge of the segregation step **11** illustrated in Figure 3 cause the breadth of the leading edge to be substantially greater than the overall width of the step, as can be seen clearly in Fig. 3A.

[0073] Multiple steps can have similarly or differently-shaped leading edges. Figures 4-6, for example, illustrate separation elements **1** in which a focusing step **10** (which does not necessarily impede passage of any particles is shaped differently from each of segregating steps **11-13**. In these illustration segregating steps **11-13** have the same or similar shapes, but they need not. Regardless of the shape of the leading edge 31 of a segregating step 11, what is important to passage of cells or other particles through the segregating passageway 101 bounded by the steps is the narrow dimension (height; e.g., h_1 in Figure 1D) defined by each segregating step 11. Particles unable to pass through the narrow dimension defined by a segregating step 11 will not traverse the step (unless it is able to deform and the pressure drop across the step is sufficient to induce such deformation).

[0074] A series of segregating steps having progressively narrowing passageways defined thereby, a segregating step having an inclined broad face (i.e., so that the narrow passageway defined thereby narrows in the direction of bulk fluid flow therethrough), or a combination of these can be used to capture deformable cells (i.e., cells which can deform to fit within, but not pass through, the passageway defined by a segregating step) and to segregate them from cells that are either sufficiently small or sufficiently deformable to pass the segregating step(s).

[0075] The breadth of each segregating step **11** can be selected based on the anticipated accumulation of particles on the step, in view of the particle composition of sample anticipated to be processed using the apparatus and the narrow dimension of each corresponding segregating passageway 101. The breadth of a segregating step 11 can be selected to be significantly (e.g., 10, 1,000, or 100,000 times) greater than the narrow dimension of the corresponding segregating passageway 101. By way of example, for segregation of fetal-like cells from maternal blood, a breadth approximately at least 1,000 (one thousand), and preferably 10,000 (ten thousand), times the narrow dimension of the corresponding passageway is considered desirable. Segregating steps 11 having relatively large breadth permit accumulation of particles within a segregating passageway 101 while limiting clogging of the segregating passageway 101.

[0076] Although the apparatus has been described herein with reference to a single segregating step **11** (Figures 1-3 and 7) and with reference to three segregating steps **11** (Figures 4-6), substantially any number of segregating steps 11 (e.g., two, four, ten, or one hundred steps) can be included in the apparatus, each segregating step 11 defining a corresponding segregating passageway 101 within the stepped passageway 55 and having a characteristic narrow dimension.

Materials and Methods of Construction

[0077] The materials and methods used to make the devices described herein can be substantially the same as those described previously in U.S. Patent number 7,993,908 and in PCT publication WO 2011/066497, or elsewhere, so long as the leading edge 31 of at least one segregating step 11 of the apparatus can be constructed as described herein - e.g., having a breadth significantly greater than its width, such as a leading edge 31 having an undulating shape. That is, the methods must be able to make a device having at least one segregating step 11 having a leading edge 31 breadth greater than the overall width of the step (e.g., greater than the width of a passageway within the device in which the step occurs).

Segregable Particles

[0078] The devices described herein can be used to segregate substantially the same kinds of particles as those described previously in U.S. Patent number 7,993,908 and in PCT publication WO 2011/066497. Attributes of the particles that affect their ability to traverse the segregation passageway(s) **101** of the apparatus described herein include the size, shape, surface properties, and deformability of the particles.

[0079] In an important embodiment, the apparatus is used to segregate tumor cells (which tend to be significantly larger than corresponding non-tumor cells of the same cell type) from non-tumor cells. It is known that tumor cells circulate in the bloodstream of many individual humans (as well as other vertebrate animals), even for tumors that are considered solid, unitary tumors, such as ovarian, prostate, and breast cancers. Detection and/or enumeration of circulating tumor cells (CTCs) can be an important indicator of the presence, nature (e.g., stage or grade), malignancy, and response to treatment of a tumor. Furthermore, isolation of CTCs permits identification of the type of tumor that is present. These characteristics can be significantly important for diagnosis, treatment, and prevention of metastasis of tumors.

[0080] In one embodiment, blood obtained from an individual (e.g., human) subject is processed using an apparatus described herein to segregate CTCs from the blood. Segregated CTCs can be recovered and analyzed by any known method to obtain important diagnostic, therapeutic, and preventative information specific to the individual subject. Because CTCs are believed to be present at early stages of tumor formation, detection and characterization of CTCs can enable early, effective intervention to prevent tumor development and spread.

[0081] Substantially any diagnostic procedure amenable to use of isolated cells can be performed using cells that are obtained from the device described herein. Examples of such methods include assessing the affinity of an antibody preparation with such cells or an extract prepared from them, assessing nucleic acids contained within such cells, or assessing the ability of the cells to grow in the presence of a selected medium or to interact with other cells. Cells obtained using the devices described herein can thus be used to assess gene expression, genetic changes, biomarker display, or other morphological or biochemical features of the cells (or changes to such features).

[0082] In another embodiment, the apparatus described herein is used to segregate circulating endothelial cells (CECs) from a sample including such cells, such as a blood sample taken from a patient. CECs having an enlarged size (relative to normal CECs) can also be segregated by selecting appropriate narrow passageway dimensions in the apparatus. By way of example, an apparatus can be used which has narrow passageway dimensions selected to segregate enlarged CECs from normal CECs. Further by way of example, an apparatus can be used which has narrow passageway dimensions selected to segregate all CEC (or only enlarged CECs) from the cells normally present in blood. CECs are known to be indicative of the presence or occurrence of trauma in an individual, and the presence of enlarged CECs can be particularly indicative of certain conditions, such as acute or impending myocardial infarction (see, e.g., Damani et al., 2012, Sci. Transl. Med. 4:126ra33). CECs isolated using the apparatus described herein can also be recovered as described herein and/or analyzed by conventional methods (e.g., by detection of immunological cell-surface markers) to identify their tissue of origin and thereby further indicating the type and/or body location of the trauma that induced their circulation. By way of example, isolation of enlarged CECs of cardiac origin is indicative that the patient has recently undergone, is currently undergoing, or is imminently at risk for occurrence of a myocardial infarction.

Fluid Displacement Devices

[0083] The apparatus described herein can be operated using substantially the same types of fluid displacement devices as those described previously in U.S. Patent number 7,993,908 and in PCT publication WO 2011/066497, or in the literature pertaining to other microfluidic devices.

Using the Apparatus

[0084] Use and operation of the apparatus described herein are substantially the same as described previously in documents discussed herein. The apparatus described herein have the significant advantage of exhibiting less susceptibility to clogging, flow/throughput impairment, and other undesirable phenomena attributable to capture of cells on a segregating step 11 thereof.

Examples

[0085] The subject matter of this disclosure is now described with reference to the following Examples.

[0086] A device as above is constructed with a stepped passageway having an overall width of 2.5 centimeters and including a second step **62** having an undulating leading edge having a breadth of 8.0 centimeters. The narrow dimension of the second passageway **52** between the second step **62** and the opposed cover 4 is 10 micrometers.

[0087] When a suspension of cells (e.g., 10 milliliters of human blood having a selected number of tumor cells included therein) is passed through the stepped passageway, followed by a rinsing solution that does not lyse the tumor cells, substantially all blood cells pass through the apparatus and most or all of the tumor cells are retained within it.

Table 1. Parts List

1	Separation Element
2	Body
4	Cover
10	Focusing Step
11	(First) Segregating Step
12	Second Segregating Step
32	Leading Edge of Second Segregating Step
13	Third Segregating Step

(continued)

20	Transitional Face of Focusing Step
21	Transitional Face of (First) Segregating Step
22	Transitional Face of Second Segregating Step
23	Transitional Face of Third Segregating Step
30	Leading Edge of Focusing Step
31	Leading Edge of (First) Segregating Step
32	Leading Edge of Second Segregating Step
33	Leading Edge of Third Segregating Step
40	Broad Face of Focusing Step
41	Broad Face of (First) Segregating Step
42	Broad Face of Second Segregating Step
43	Broad Face of Third Segregating Step
50	Void defined by body and cover
52	Inlet Region of Void
53	Upstream Portion of channel
54	Channel connecting inlet and outlet regions of void
55	Separating Portion of channel
56	Downstream Portion of channel
58	Outlet Region of Void
60	Part of Separating Portion bounded by Focusing step
61	Part of Separating Portion bounded by (First) Segregating Step
62	Part of Separating Portion bounded by Second Segregating Step
63	Part of Separating Portion bounded by Third Segregating Step
101	(First) Segregating Passageway
102	Second Segregating Passageway
103	Third Segregating Passageway

Table 2. Abbreviations List

BFF	Bulk Fluid Flow
hc	Height of Channel
h0	Height of Channel in portion bounded by Focusing Step
h1	Height of Channel in portion bounded by (First) Segregating Step
h2	Height of Channel in portion bounded by Second Segregating Step
h3	Height of Channel in portion bounded by Third Segregating Step
W	Overall Width of Channel in the Separating Portion
L	Length of Separating Portion
B	Breadth of Leading Edge of a Segregating Step
D	ratio B/L
W	Width of a Segregating Step

[0088] **Figure 8** illustrates a device according to the claims wherein the leading edge of the segregating step is serpentine in form and as indicated above has a size approximately equal to a common microscope slide.

Claims

1. A device for segregating smaller and larger particles, the device comprising:

a body (2) and
a cover (4) that define a void (50) therebetween, the void (50) containing

a separation element (1) that segregates an inlet region (52) and an outlet region (58) of the void (50), the separation element (1) defining, together with a surface of the void (50),
a channel that fluidly connects the inlet and outlet regions by way of a separating portion, the channel having

an overall width at the separating portion and
a height defined by the distance between the separation element (1) and the surface of the void (50),

at least one of the body (2), the cover (4), and the separation element (1) bearing a segregating step disposed within and having a leading edge extending completely across the separating portion of the channel, whereby the channel is divided into an upstream portion on the inlet side of the leading edge and a lamellar downstream portion on the outlet side of the leading edge,

the height of the upstream portion being sufficient to facilitate passage therethrough of both larger and smaller particles,
the height of the downstream portion being sufficiently large to facilitate passage therethrough of the smaller particles and sufficiently small to inhibit passage therethrough of the larger particles, and
the length of the leading edge being at least 20 times greater than the overall width of the channel at the separation region,
wherein the upstream portion of the channel is lamellar in a region between the inlet region (52) and the separation element (1), and
wherein the leading edge has an undulating shape,

whereby the particles can be segregated by passing them through the channel, and recovering particles based on their ability to traverse the segregating step.

2. The device of claim 1, wherein the height of the downstream portion inhibits passage therethrough of circulating tumor cells and facilitates passage therethrough of human red blood cells.

3. The device of claim 2, wherein the circulating tumor cells are derived from a solid tumor such as an ovarian, prostate or breast cancer tumor.

4. The device of any one of claims 1 to 3, wherein the height of the downstream portion inhibits passage therethrough of at least one of: human fetal cells; human fetal stem-like cells; circulating endothelial cells; and enlarged circulating endothelial cells of cardiac origin, and facilitates passage therethrough of human red blood cells.

5. The device of any one of claims 1 to 4, wherein at least one of the body (2), the cover (4), and the separation element (1) bears a focusing step disposed in and extending completely across the channel on the inlet side of the segregating step, the channel having a greater height on the inlet side of the focusing step than on its outlet side; optionally, wherein the focusing step extends perpendicularly across the channel.

6. The device of any one of claims 1 to 5, wherein at least one of the body (2), the cover (4), and the separation element (1) bears a plurality of segregating steps disposed serially within the separation portion, each segregating step:

a) having a leading edge that extends across the separating portion and has a length greater than the overall width of the channel at the separating portion; and
b) dividing the channel into an upstream portion and a lamellar downstream portion relative to the leading edge of the segregating step, the height of the channel at the downstream portion immediately following the segregating step being smaller than the height of the channel at the upstream portion immediately preceding the segregating step.

7. The device of any of claims 1 to 6, wherein the leading edge has a serpentine shape.

8. The device of any one of claims 1 to 7, wherein the segregating step has an upstream face on its inlet side that is perpendicular to the portion of the step that defines the downstream portion.

9. The device of any one of claims 1 to 8, wherein the separation element (1) is integral with at least one of the body and the cover.

10. The device of any one of claims 1 to 9, further comprising a support for maintaining the height of the channel, the support being disposed within the channel and extending between the separation element and the surface of the void.

11. The device of any one of claims 1 to 10, wherein the device has a size approximately equal to a common microscope slide.

12. A method of segregating larger and smaller particles, the method comprising providing a fluid suspension of larger and smaller particles at the inlet of the device of any one of claims 1 to 11, urging the fluid through the channel and segregating particles based on their ability to traverse the segregating step, and optionally recovering the larger particles thus segregated that are unable to pass to the outlet region (58).

13. The method of claim 12, wherein the method further comprises back-flushing fluid from the outlet region in the direction of the inlet of the device to recover the larger particles.

14. The method of claim 12 or claim 13, wherein the fluid is a blood sample.

15. The method of any of claims 12 to 14, wherein the larger particles are circulating tumor cells (CTCs) and the smaller particles are blood cells.

16. The method of any of claims 12 to 14, wherein the larger particles are fetal cells and the smaller particles are blood cells.

17. The method of any of claims 12 to 14 wherein the larger particles are circulating endothelial cells and the smaller particles are blood cells.

18. The method of claim 17, wherein the circulating endothelial cells are enlarged circulating endothelial cells of cardiac origin.

19. The method of any of claims 15 to 17, wherein the blood cells are red blood cells.

20. A method of assessing the efficacy of a tumor treatment for a subject afflicted with a tumor, the method comprising isolating CTCs from blood samples obtained from the subject before and after the treatment using the method of claim 15 or claim 19 when dependent on claim 15 and comparing at least one characteristic of the CTCs isolated from the samples, whereby a difference in the characteristics of CTCs isolated from the blood samples is an indication of the efficacy of the treatment; optionally,
wherein the characteristic is CTC concentration in the sample.

21. A method of diagnosing occurrence of a tumor in a vertebrate subject, the method comprising segregating circulating tumor cells (CTCs) from a blood sample obtained from the subject using the method of claim 15 or claim 19 when dependent on claim 15,
wherein the height of the downstream portion inhibits passage therethrough of a CTC,
whereby presence of at least one segregated circulating tumor cell is an indication that a tumor occurs in the subject.

22. The method as claimed in claim 21, further comprising examining the portion of the apparatus upstream of the leading edge of the segregating step for the presence of at least one segregated CTC.

23. The method of claim 21 or claim 22, further comprising thereafter performing a diagnostic test that assesses a characteristic of a tumor cell on the at least one segregated circulating tumor cell that was present.

24. The method as claimed in claim 23, wherein the diagnostic test is binding with a tissue-specific antibody; AND/OR,
wherein the diagnostic test is binding with a tumor-specific antibody; AND/OR,
wherein the diagnostic test comprises analyzing nucleic acids obtained from the at least one segregated circulating tumor cell that was present; AND/OR,
wherein the diagnostic test is assessing the proliferative capacity of the segregated circulating tumor cell; AND/OR,
wherein the diagnostic test is microscopic observation of the morphology of the segregated circulating tumor cell.

25. The method as claimed in any of claims 21 to 24, wherein the at least one circulating tumor cell is derived from a solid tumor such as an ovarian, prostate or breast cancer tumor.

26. A method of determining an indication of the presence or occurrence of trauma in an individual based on the presence of circulating endothelial cells in a blood sample, the method comprising segregating circulating endothelial cells from the blood sample obtained from the individual using the method of claim 17 or claim 19 when dependent on claim 17.

27. A method of determining an indication that a patient has recently undergone, is currently undergoing, or is imminently at risk for occurrence of myocardial infarction based on the presence of enlarged circulating endothelial cells of cardiac origin in a blood sample, the method comprising segregating enlarged circulating endothelial cells from the blood sample obtained from the patient using the method of claim 18.

Patentansprüche

1. Vorrichtung zum Trennen kleinerer und größerer Teilchen, wobei die Vorrichtung folgendes umfasst:

einen Körper (2), und
eine Abdeckung (4), die dazwischen einen Zwischenraum (50) definieren, wobei der Zwischenraum (50) folgendes enthält:
ein Trennelement (1), das einen Einlassbereich (52) und einen Auslassbereich (58) des Zwischenraums (50) trennt, wobei das Trennelement (1) gemeinsam mit einer Oberfläche des Zwischenraums (50) folgendes definiert:

einen Kanal, der fluidfähig die Einlass- und Auslassbereiche über einen Trennungsabschnitt verbindet, wobei der Kanal folgendes aufweist:

eine Gesamtbreite an dem Trennungsabschnitt, und
eine durch den Abstand zwischen dem Trennelement (1) und der Oberfläche des Hohlraums (50) definierte Höhe;

wobei wenigstens ein Element des Körpers (2), der Abdeckung (4) und des Trennelements (1) eine Trennstufe aufweist, die sich in dem Trennungsabschnitt befindet und eine Vorderkante aufweist, die sich vollständig über diesen erstreckt, wodurch der Kanal in einen Stromaufwärtsabschnitt auf der Einlassseite der Vorderkante und einen lamellaren Stromabwärtsabschnitt auf der Auslassseite der Vorderkante unterteilt ist;
wobei die Höhe des Stromaufwärtsabschnitts ausreicht, um es zu ermöglichen, dass sowohl größere als auch kleinere Teilchen dort hindurch verlaufen;
wobei die Höhe des Stromabwärtsabschnitts ausreichend große ist, um es zu ermöglichen, dass die kleineren Teilchen dort hindurch verlaufen, und wobei sie ausreichend klein ist, um es zu verhindern, dass die größeren Teilchen dort hindurch verlaufen; und
wobei die Länge der Vorderkante mindestens 20 Mal größer ist als die Gesamtbreite des Kanals an dem Trennungsbereich;
wobei der Stromaufwärtsabschnitt des Kanals in einem Bereich zwischen dem Einlassbereich (52) und dem Trennelement (1) lamellar ist; und
wobei die Vorderkante eine gewellte Form aufweist,
wobei die Teilchen getrennt werden können, indem sie durch den Kanal geleitet werden, und wobei die Teilchen wiedergewonnen werden auf der Basis ihrer Fähigkeit, die Trennstufe zu passieren.

2. Vorrichtung nach Anspruch 1, wobei die Höhe des Stromabwärtsabschnitts es verhindert, dass zirkulierende Tumorzellen dort hindurch verlaufen, und wobei sie es ermöglicht, dass rote Blutkörperchen dort hindurch verlaufen.

3. Vorrichtung nach Anspruch 2, wobei die zirkulierenden Tumorzellen von einem festen Tumor stammen, wie etwa einem Eierstockkrebstumor, einem Prostatakrebstumor oder einem Brustkrebstumor.

4. Vorrichtung nach einem der Ansprüche 1 bis 3, wobei die Höhe des Stromabwärtsabschnitts das Hindurchtreten wenigstens eines der folgenden verhindert:

menschlicher fötaler Zellen, menschlicher fötaler Stammzellen, zirkulierender Endothelzellen und vergrößerter zirkulierender Endothelzellen kardialer Herkunft, und wobei die Höhe es ermöglicht, dass menschliche rote Blutkörperchen dort hindurchtreten.

5. Vorrichtung nach einem der Ansprüche 1 bis 4, wobei wenigstens ein Element des Körpers (2), der Abdeckung (4) und des Trennelements (1) eine Fokussierungsstufe aufweist, die sich in dem Kanal auf der Einlassseite der Trennstufe befindet und vollständig über diesen erstreckt, wobei der Kanal eine größere Höhe auf der Einlassseite der Fokussierungsstufe aufweist als auf dessen Auslassseite; wobei sich optional die Fokussierungsstufe senkrecht über den Kanal erstreckt.

6. Vorrichtung nach einem der Ansprüche 1 bis 5, wobei wenigstens ein Element des Körpers (2), der Abdeckung (4) und des Trennelements (1) eine Mehrzahl von Trennstufen aufweist, die seriell in dem Trennungsabschnitt angeordnet sind, wobei jede Trennstufe folgendes aufweist:

a) eine Vorderkante, die sich über den Trennungsabschnitt erstreckt und eine Länge aufweist, die größer ist als die Gesamtbreite des Kanals an dem Trennungsabschnitt; und
b) den Kanal in einem Stromaufwärtsabschnitt und einen lamellaren Stromabwärtsabschnitt im Verhältnis zu der Vorderkante der Trennstufe teilend die Höhe des Kanals an dem Stromabwärtsabschnitt unmittelbar nach der Trennstufe kleiner ist als die Höhe des Kanals an dem Stromaufwärtsabschnitt unmittelbar vor der Trennstufe.

7. Vorrichtung nach einem der Ansprüche 1 bis 6, wobei die Vorderkante eine Serpentinenform aufweist.

8. Vorrichtung nach einem der Ansprüche 1 bis 7, wobei die Trennstufe eine Stromaufwärtsseite auf ihrer Einlassseite aufweist, die senkrecht ist zu dem Abschnitt der Stufe, der den Stromabwärtsabschnitt definiert.

9. Vorrichtung nach einem der Ansprüche 1 bis 8, wobei das Trennelement (1) integral mit wenigstens dem Körper oder der Abdeckung ist.

10. Vorrichtung nach einem der Ansprüche 1 bis 9, wobei diese ferner einen Träger zum Aufrechterhalten der Höhe des Kanals umfasst, wobei der Träger in dem Kanal angeordnet ist und sich zwischen dem Trennelement und der Oberfläche des Zwischenraums erstreckt.

11. Vorrichtung nach einem der Ansprüche 1 bis 10, wobei die Vorrichtung eine Größe aufweist, die ungefähr der Größe eines üblichen Objektträgers entspricht.

12. Verfahren zum Trennen größerer und kleinerer Teilchen, wobei das Verfahren folgendes umfasst: das Bereitstellen einer Fluidsuspension größerer und kleinerer Teilchen an dem Einlass der Vorrichtung nach einem der Ansprüche 1 bis 11, das Drücken des Fluids durch den Kanal und das Trennen der Teilchen auf der Basis ihrer Fähigkeit, die Trennstufe zu passieren, und optional das Wiedergewinnen der so getrennten größeren Teilchen, die nicht zu dem Auslassbereich (58) verlaufen können.

13. Verfahren nach Anspruch 12, wobei das Verfahren ferner das Rückspülen von Fluid aus dem Auslassbereich in Richtung des Einlasses der Vorrichtung umfasst, um die größeren Teilchen wiederzugewinnen.

14. Verfahren nach Anspruch 12 oder 13, wobei das Fluid eine Blutprobe ist.

15. Verfahren nach einem der Ansprüche 12 bis 14, wobei die größere Teilchen zirkulierende Tumorzellen (CTCs) sind, und wobei die kleineren Teilchen Blutkörperchen sind.

16. Verfahren nach einem der Ansprüche 12 bis 14, wobei die größere Teilchen fötale Zellen sind, und wobei die kleineren Teilchen Blutkörperchen sind.

17. Verfahren nach einem der Ansprüche 12 bis 14, wobei die größere Teilchen zirkulierende Endothelzellen sind, und wobei die kleineren Teilchen Blutkörperchen sind.

18. Verfahren nach Anspruch 17, wobei die zirkulierenden Endothelzellen vergrößerte zirkulierende Endothelzellen kardialer Herkunft sind.

19. Verfahren nach einem der Ansprüche 15 bis 17, wobei die Blutkörperchen rote Blutkörperchen sind.

20. Verfahren zur Bewertung der Wirksamkeit einer Tumorbehandlung für ein an einem Tumor leidendes Subjekt, wobei

das Verfahren das Isolieren von CTCs aus von dem Subjekt vor und nach der Behandlung unter Verwendung des Verfahrens nach Anspruch 15 oder Anspruch 19 in Abhängigkeit von Anspruch 15 erhaltenen Blutproben umfasst sowie das Vergleichen wenigstens einer Eigenschaft der aus den Proben isolierten CTCs, wobei ein Unterschied in den Eigenschaften von aus den Blutproben isolierten CTCs eine Anzeige für die Wirksamkeit der Behandlung ist; wobei
optional die Eigenschaft die CTC-Konzentration in der Probe ist.

21. Verfahren zur Diagnose des Auftretens eines Tumors in einem Wirbeltier, wobei das Verfahren das Trennen zirkulierender Tumorzellen (CTCs) aus einer von dem Subjekt unter Verwendung des Verfahrens nach Anspruch 15 oder Anspruch 19 in Abhängigkeit von Anspruch 15 erhaltenen Blutprobe umfasst,
wobei die Höhe des Stromabwärtsabschnitts es verhindert, dass eine CTC dort hindurch verläuft;
wobei die Präsenz wenigstens einer getrennten zirkulierenden Tumorzelle eine Indikation dafür ist, dass in dem Subjekt ein Tumor auftritt.

22. Verfahren nach Anspruch 21, wobei das Verfahren ferner das Untersuchen des Abschnitts der Vorrichtung stromaufwärts der Vorderkante der Trennstufe in Bezug auf die Präsenz wenigstens einer getrennten CTC umfasst.

23. Verfahren nach Anspruch 21 oder Anspruch 22, wobei das Verfahren ferner danach das Durchführen eines diagnostischen Tests umfasst, der eine Eigenschaft einer Tumorzelle an der wenigstens einen getrennten zirkulierenden Tumorzelle bewertet, die vorhanden gewesen ist.

24. Verfahren nach Anspruch 23, wobei der diagnostische Test eine Bindung mit einem gewebespezifischen Antikörper vorsieht; UND/ODER wobei der diagnostische Test eine Bindung mit einem tumorspezifischen Antikörper vorsieht; UND/ODER,
wobei der diagnostische Test das Analysieren von Nukleinsäuren umfasst, die von der wenigstens einen getrennten zirkulierenden Tumorzelle erhalten werden, die vorhanden gewesen ist; UND/ODER
wobei der diagnostische Test die proliferative Fähigkeit der getrennten zirkulierenden Tumorzelle bewertet; UND/ODER
wobei der diagnostische Test eine mikroskopische Observation der Morphologie der getrennten zirkulierenden Tumorzelle ist.

25. Verfahren nach einem der Ansprüche 21 bis 24, wobei die wenigstens eine zirkulierende Tumorzelle von einem festen Tumor stammen, wie etwa einem Eierstockkrebstumor, einem Prostatakrebstumor oder einem Brustkrebstumor.

26. Verfahren zum Bestimmen einer Indikation der Präsenz oder des Vorkommens von Trauma in einem Individuum auf der Basis der Präsenz zirkulierender Endothelzellen in einer Blutprobe, wobei das Verfahren das Trennen zirkulierender Endothelzellen aus der von dem Individuum erhaltenen Blutprobe unter Verwendung des Verfahrens nach Anspruch 17 oder nach Anspruch 19 in Abhängigkeit von Anspruch 17 umfasst.

27. Verfahren zum Bestimmen einer Indikation, dass bei einem Patienten kürzlich das Risiko für das Auftreten eines Herzinfarkts aufgetreten ist, diese Gefahr aktuell besteht oder unmittelbar bevorsteht auf der Basis der Präsenz vergrößerter zirkulierender Endothelzellen kardialer Herkunft in einer Blutprobe, wobei das Verfahren das Trennen vergrößerter zirkulierender Endothelzellen aus der von dem Patienten unter Verwendung des Verfahrens nach Anspruch 18 erhaltenen Blutprobe umfasst.

Revendications

1. Dispositif pour la ségrégation de particules plus petites et plus grandes, le dispositif comprenant :

un corps (2) et
un couvercle (4) qui définit un vide (50) entre eux, le vide (50) contenant

un élément de séparation (1) qui ségrège une région d'entrée (52) et une région de sortie (58) du vide (50), l'élément de séparation (1) définissant, avec une surface du vide (50),
un canal qui relie de façon fluidique les régions d'entrée et de sortie au moyen d'une partie de séparation, le canal ayant

une largeur hors tout au niveau de la partie de séparation et
une hauteur définie par la distance entre l'élément de séparation (1) et la surface du vide (50),

au moins l'un du corps (2), du couvercle (4), et de l'élément de séparation (1) portant un gradin de ségrégation disposé en son sein et ayant un bord d'attaque s'étendant complètement à travers la partie de séparation du canal, moyennant quoi le canal est divisé en une partie amont sur le côté entrée du bord d'attaque et une partie aval lamellaire sur le côté sortie du bord d'attaque,
la hauteur de la partie amont étant suffisante pour permettre le passage au travers de particules plus grandes et plus petites,
la hauteur de la partie aval étant suffisamment grande pour permettre le passage au travers des particules plus petites et suffisamment petite pour empêcher le passage au travers des particules plus grosses, et
la longueur du bord d'attaque étant au moins 20 fois supérieure à la largeur hors tout du canal au niveau de la région de séparation,
la partie amont du canal étant lamellaire dans une région entre la région d'entrée (52) et l'élément de séparation (1), et
le bord d'attaque ayant une forme ondulante,
les particules pouvant être ségrégées en les faisant passer à travers le canal, et en récupérant les particules en fonction de leur capacité à traverser le gradin de ségrégation.

2. Dispositif selon la revendication 1, la hauteur de la partie aval empêchant le passage au travers de cellules tumorales circulantes et permettant le passage au travers des globules rouges humains.

3. Dispositif selon la revendication 2, les cellules tumorales circulantes provenant d'une tumeur solide telle qu'une tumeur d'un cancer de l'ovaire, de la prostate ou du sein.

4. Dispositif selon l'une quelconque des revendications 1 à 3, la hauteur de la partie aval empêchant le passage au travers d'au moins une cellule parmi les cellules suivantes :

cellules foetales humaines ; cellules de type cellules-souches foetales humaines ; cellules endothéliales circulantes ; et cellules endothéliales circulantes agrandies, et permettant le passage au travers des globules rouges humains.

5. Dispositif selon l'une quelconque des revendications 1 à 4, au moins l'un du corps (2), du couvercle (4) et de l'élément de séparation (1) portant un gradin de concentration disposé en son sein et s'étendant complètement à travers le canal sur le côté entrée du gradin de ségrégation, le canal ayant une plus grande hauteur sur le côté entrée du gradin de concentration que sur son côté sortie ; éventuellement,
le gradin de concentration s'étendant perpendiculairement à travers le canal.

6. Dispositif selon l'une quelconque des revendications 1 à 5, au moins l'un parmi le corps (2), le couvercle (4) et l'élément de séparation (1) portant une pluralité de gradins de ségrégation disposés en série dans la partie de séparation, chaque gradin de ségrégation :

a) ayant un bord d'attaque qui s'étend à travers la partie de séparation et a une longueur supérieure à la largeur hors tout du canal au niveau de la partie de séparation ; et
b) diviser le canal entre une partie amont et une partie aval lamellaire par rapport au bord d'attaque du gradin de ségrégation, la hauteur du canal au niveau de la partie aval suivant immédiatement le gradin de ségrégation étant plus petite que la hauteur du canal au niveau de la partie amont précédant immédiatement le gradin de ségrégation.

7. Dispositif selon l'une quelconque des revendications 1 à 6, le bord d'attaque ayant une forme de serpentin.

8. Dispositif selon l'une quelconque des revendications 1 à 7, le gradin de séparation ayant une face amont sur son côté entrée qui est perpendiculaire à la partie du gradin qui définit la partie aval.

9. Dispositif selon l'une quelconque des revendications 1 à 8, l'élément de séparation (1) faisant partie intégrante du corps et/ou du couvercle.

10. Dispositif selon l'une quelconque des revendications 1 à 9, comprenant en outre un support pour maintenir la hauteur

du canal, le support étant disposé au sein du canal et s'étendant entre l'élément de séparation et la surface du vide.

11. Dispositif selon l'une quelconque des revendications 1 à 10, le dispositif ayant une taille approximativement égale à une lame de microscope commune.

12. Procédé de ségrégation de particules plus grandes et plus petites, le procédé comprenant les étapes consistant à fournir une suspension fluide de particules plus grandes et plus petites à l'entrée du dispositif selon l'une quelconque des revendications 1 à 11, pousser le fluide à travers le canal et ségréger les particules en fonction de leur capacité à traverser le gradin de ségrégation, et éventuellement récupérer les particules les plus grandes ainsi ségrégées qui sont incapables de passer à la région de sortie (58).

13. Procédé selon la revendication 12, le procédé comprenant en outre l'étape consistant à contre-balayer le fluide provenant de la région de sortie en direction de l'entrée du dispositif pour récupérer les particules plus grandes.

14. Procédé selon la revendication 12 ou 13, le fluide étant un échantillon de sang.

15. Procédé selon l'une quelconque des revendications 12 à 14, les particules plus grandes étant des cellules tumorales circulantes (CTC) et les particules plus petites étant des cellules sanguines.

16. Procédé selon l'une quelconque des revendications 12 à 14, les particules plus grandes étant des cellules foetales et les particules plus petites étant des cellules sanguines.

17. Procédé selon l'une quelconque des revendications 12 à 14, les particules plus grandes étant des cellules endothéliales circulantes et les particules plus petites étant des cellules sanguines.

18. Procédé selon la revendication 17, les cellules endothéliales circulantes étant des cellules endothéliales circulantes agrandies d'origine cardiaque.

19. Procédé selon l'une quelconque des revendications 15 à 17, les cellules sanguines étant des globules rouges.

20. Procédé d'évaluation de l'efficacité d'un traitement tumoral pour un sujet atteint d'une tumeur, le procédé comprenant les étapes consistant à isoler les CTC des échantillons de sang obtenus auprès du sujet avant et après le traitement à l'aide du procédé selon la revendication 15 ou 19 lorsqu'elle dépend de la revendication 15 et comparer au moins une caractéristique des CTC isolés à partir des échantillons, moyennant quoi une différence dans les caractéristiques des CTC isolées à partir des échantillons de sang est une indication de l'efficacité du traitement ; éventuellement, la caractéristique étant la concentration en CCT dans l'échantillon.

21. Procédé de diagnostic de la présence d'une tumeur chez un sujet vertébré, le procédé comprenant l'étape consistant à ségréger les cellules tumorales circulantes (CTC) d'un échantillon de sang obtenu d'un sujet au moyen du procédé selon la revendication 15 ou 19 lorsqu'elle dépend de la revendication 15,
la hauteur de la partie aval empêchant le passage au travers d'une CCT,
moyennant quoi la présence d'au moins une cellule tumorale circulantes ségrégée est une indication qu'une tumeur est présente chez le sujet.

22. Procédé selon la revendication 21, comprenant en outre l'étape consistant à examiner la partie de l'appareil en amont du bord d'attaque du gradin de ségrégation pour détecter la présence d'au moins une CCT ségrégée.

23. Procédé selon la revendication 21 ou 22, comprenant en outre l'étape consistant à effectuer ensuite un test diagnostic qui évalue une caractéristique d'une cellule tumorale sur au moins une cellule tumorale circulante ségrégée qui était présente.

24. Procédé selon la revendication 23, le test de diagnostic étant liant avec un anticorps spécifique au tissu ; ET/OU,
le test de diagnostic étant liant avec un anticorps spécifique à la tumeur ; ET/OU,
le test de diagnostic comprenant l'analyse des acides nucléiques obtenus à partir de l'au moins une cellule tumorale circulante ségrégée qui était présente ; ET/OU,
le test de diagnostic évaluant la capacité de prolifération de la cellule tumorale circulante ségrégée ; ET/OU,
le test de diagnostic étant l'observation microscopique de la morphologie de la cellule tumorale circulante ségrégée.

25. Procédé selon l'une quelconque des revendications 21 à 24, l'au moins une cellule tumorale circulante provenant d'une tumeur solide telle qu'une tumeur d'un cancer de l'ovaire, de la prostate ou du sein.

26. Procédé de détermination de l'indication de la présence ou de la survenue d'un traumatisme chez un individu sur la base de la présence de cellules endothéliales circulantes dans un échantillon de sang, le procédé comprenant l'étape consistant à ségréger les cellules endothéliales circulantes de l'échantillon de sang obtenu auprès de l'individu à l'aide du procédé selon la revendication 17 ou 19 lorsqu'elle dépend de la revendication 17.

27. Procédé de détermination d'une indication selon laquelle un patient a subi récemment, est actuellement en train de subir ou court un risque imminent de subir un infarctus du myocarde sur la base de la présence de cellules endothéliales circulantes agrandies d'origine cardiaque dans un échantillon de sang, le procédé comprenant l'étape consistant à ségréguer les cellules endothéliales circulantes agrandies de l'échantillon de sang obtenu du patient à l'aide du procédé selon la revendication 18.

PRIOR ART

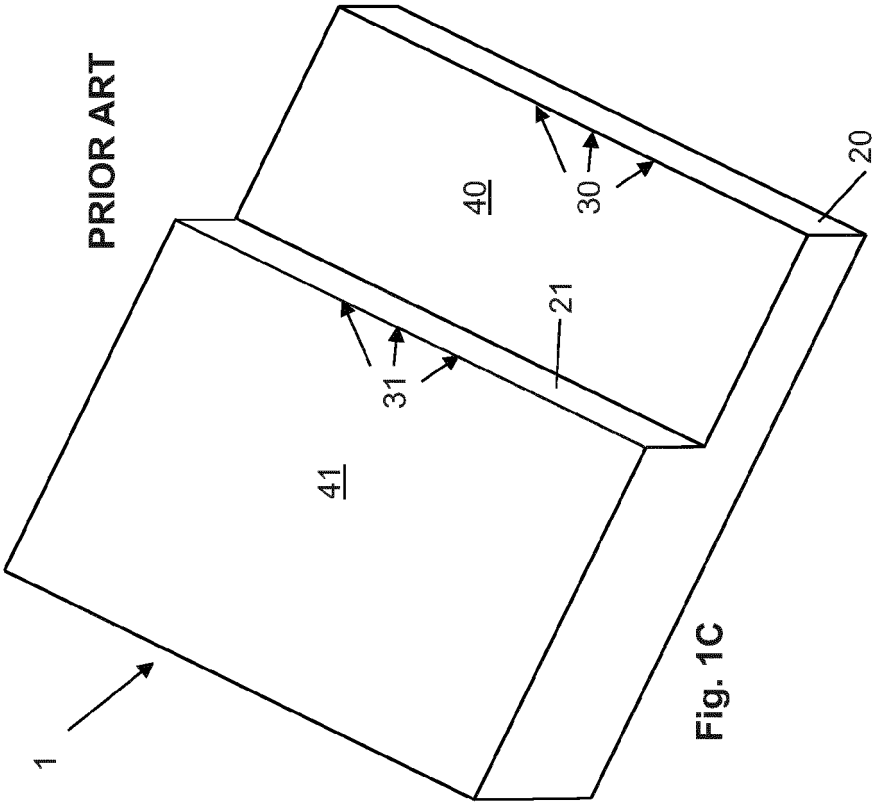


Fig. 1C

PRIOR ART

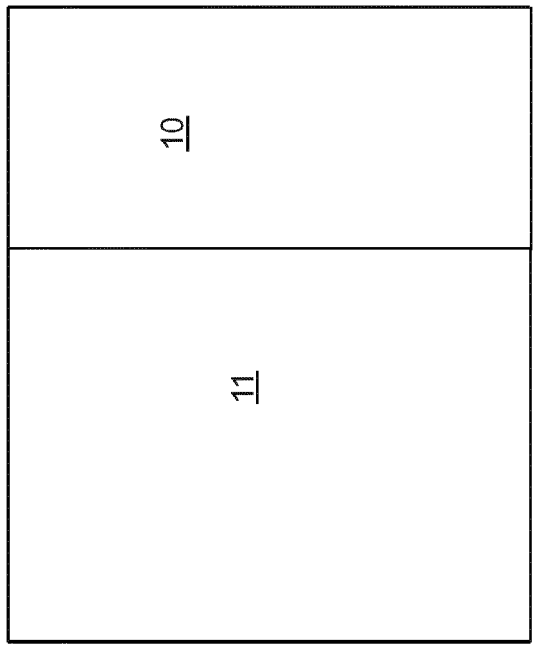


Fig. 1A

PRIOR ART

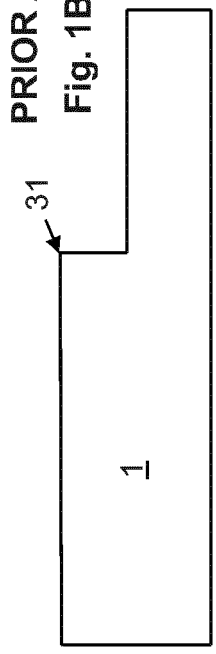


Fig. 1B

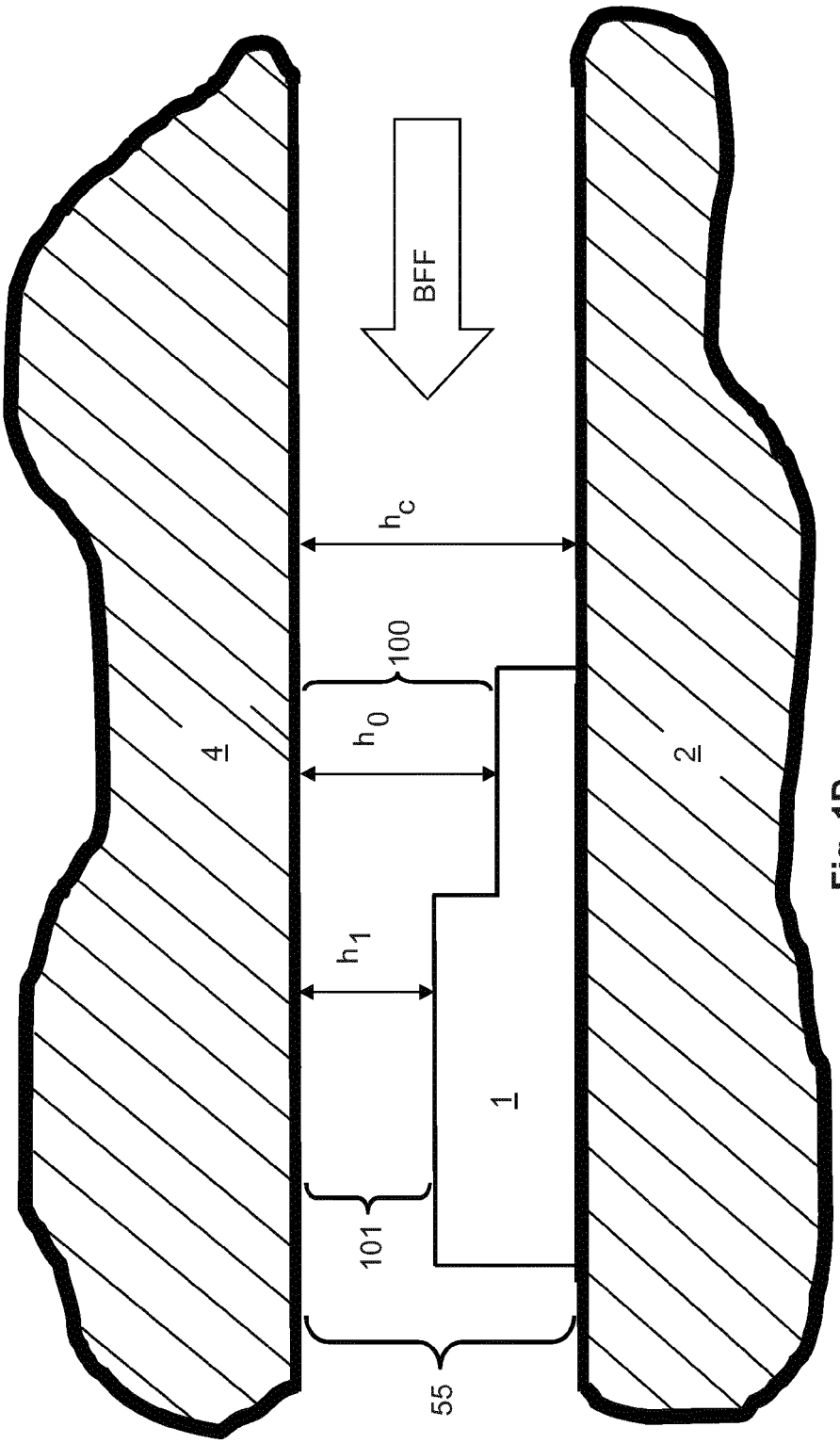


Fig. 1D

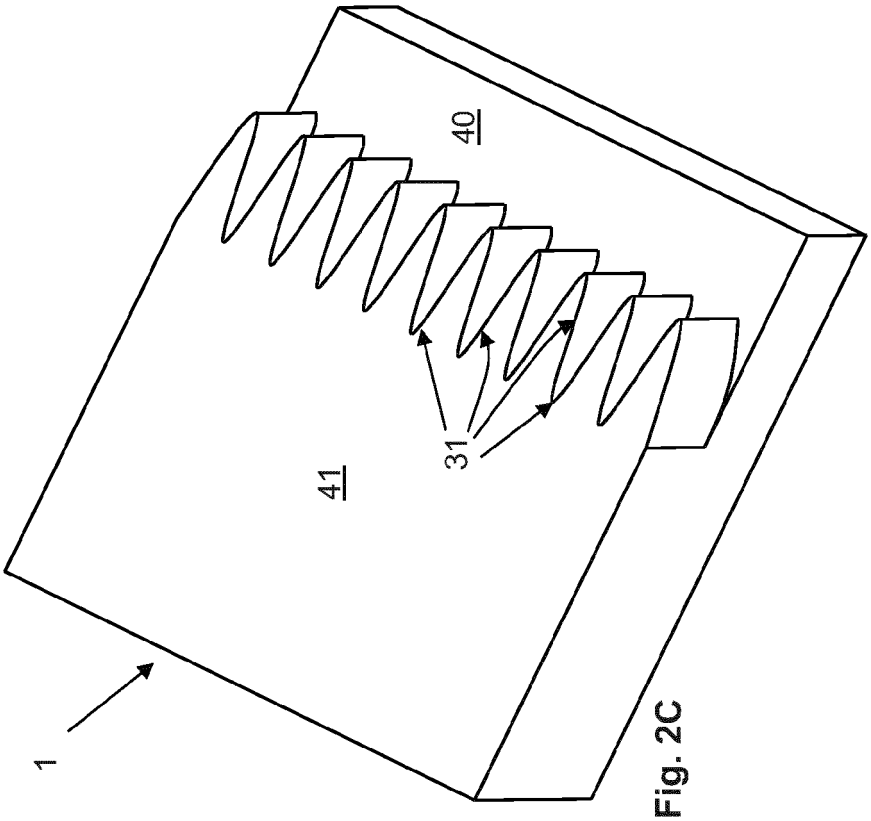


Fig. 2C

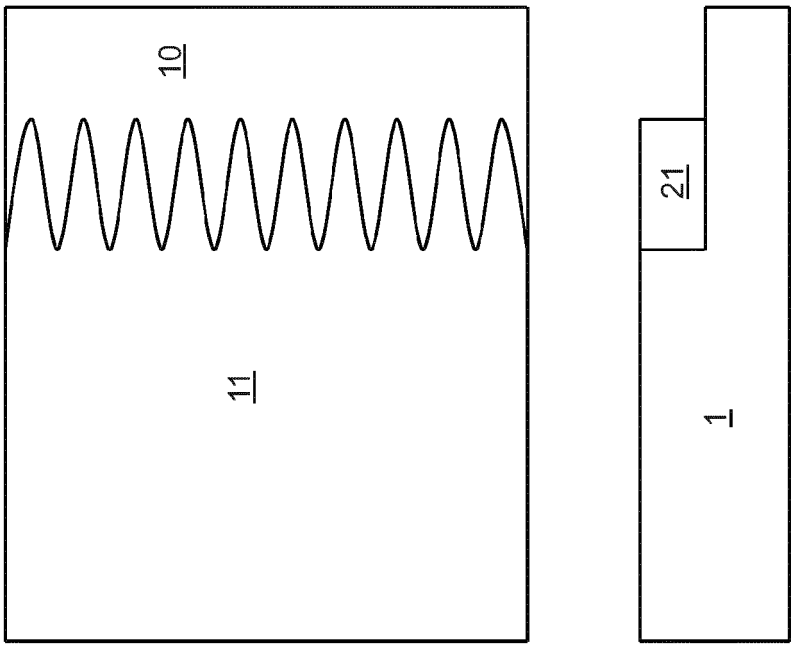


Fig. 2A

Fig. 2B

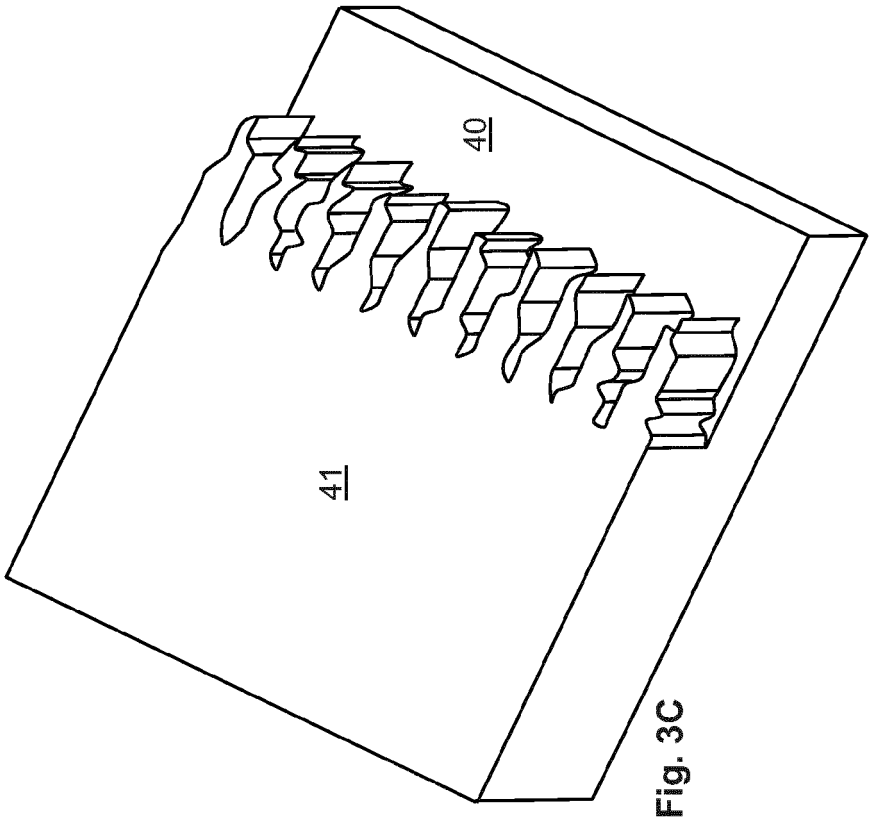


Fig. 3C

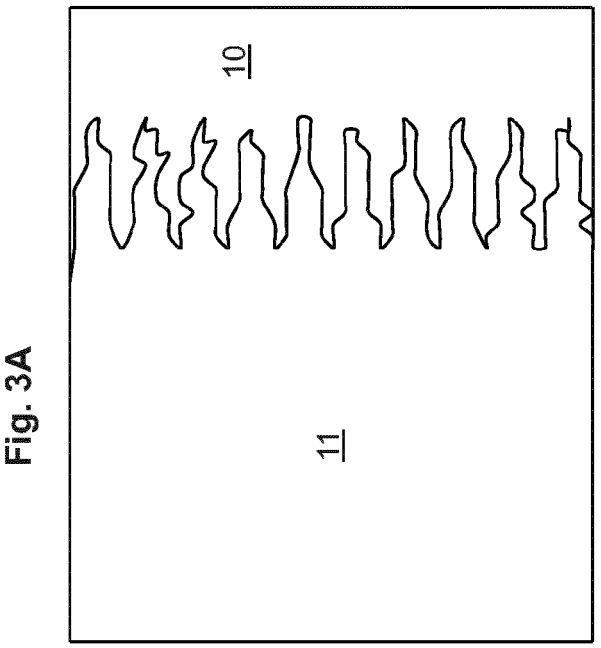


Fig. 3A

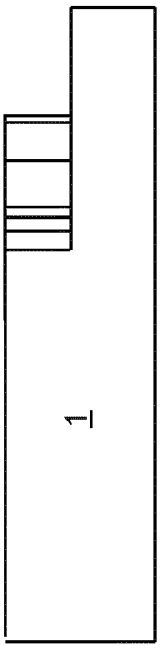


Fig. 3B

Fig. 4A

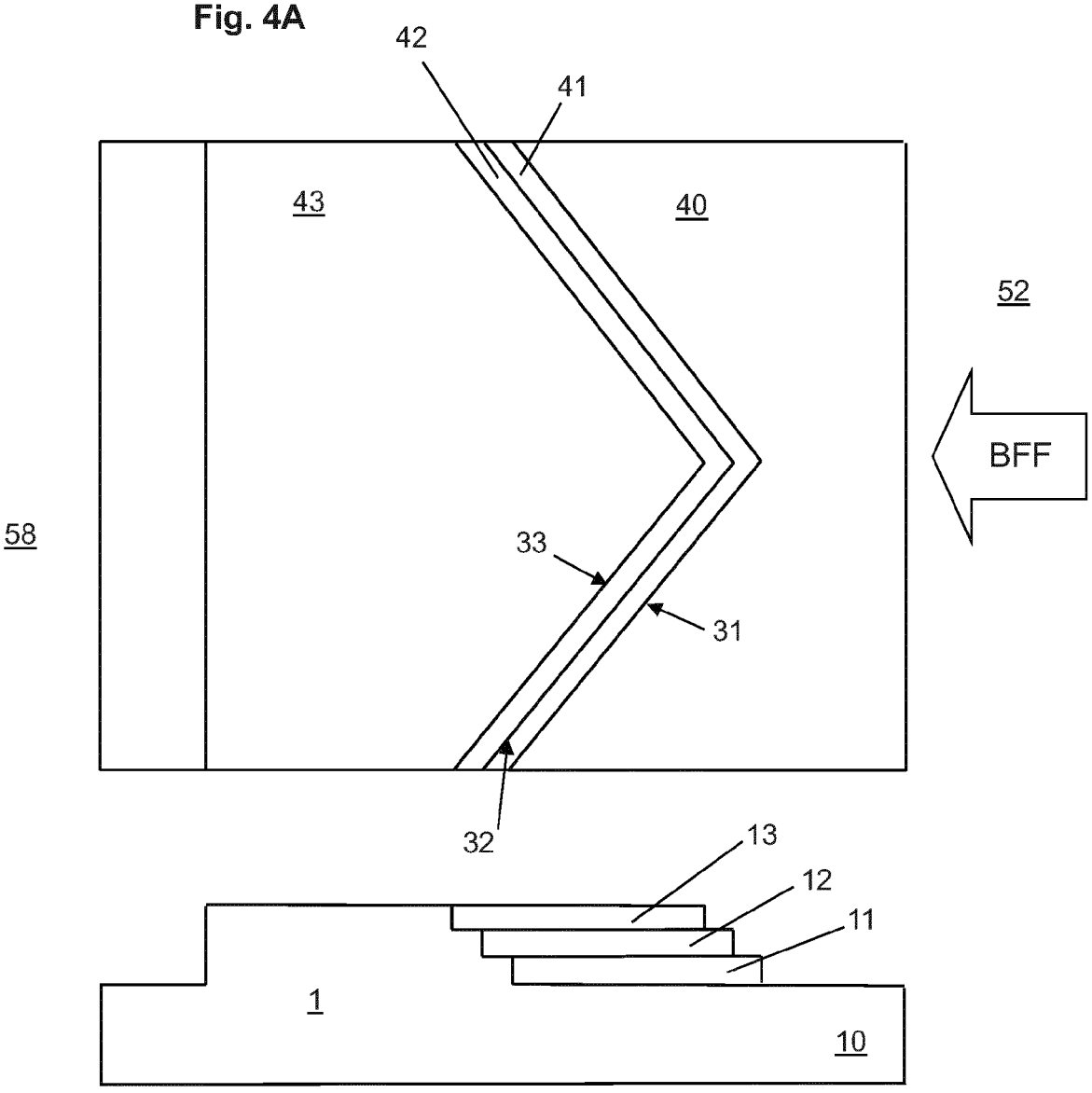


Fig. 4B

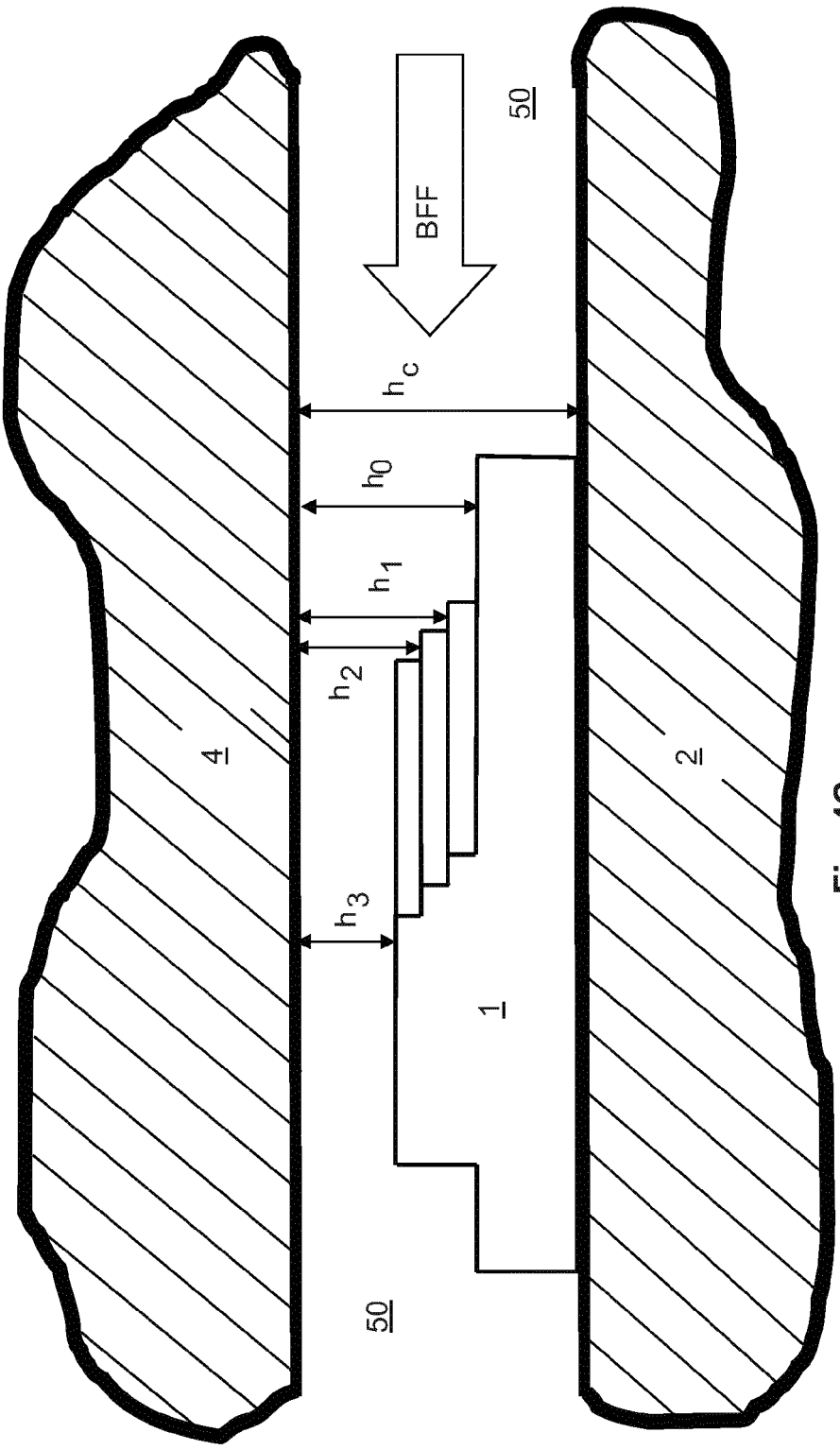
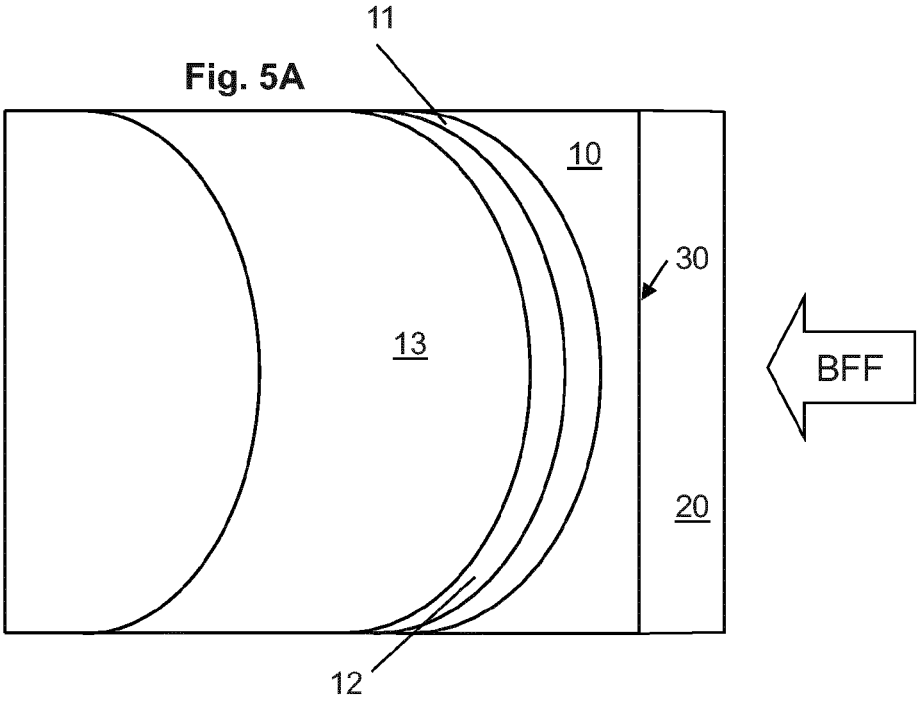


Fig. 4C

Fig. 5A



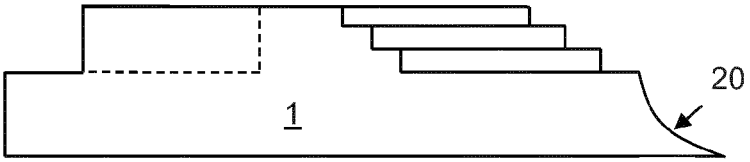
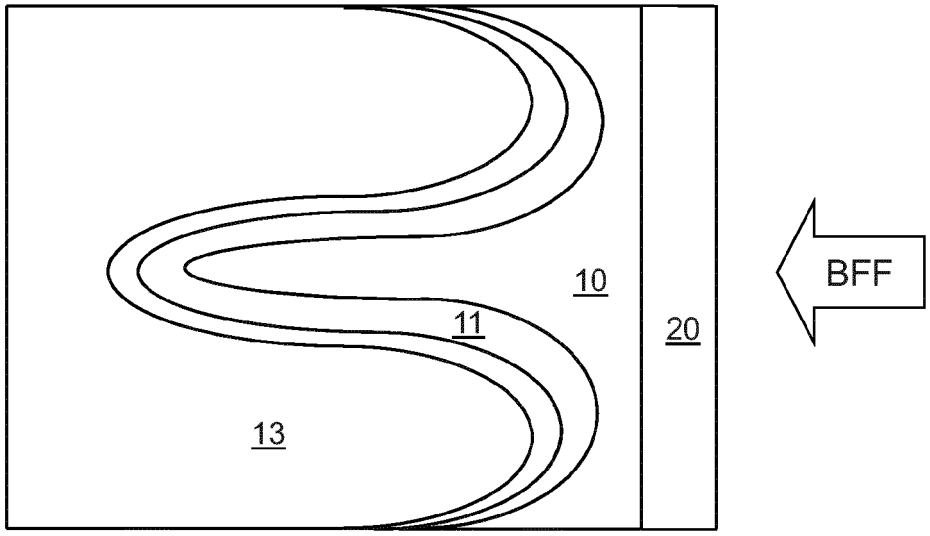


Fig. 5B

Fig. 6A



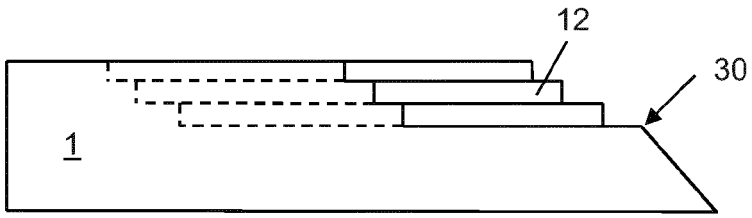


Fig. 6B

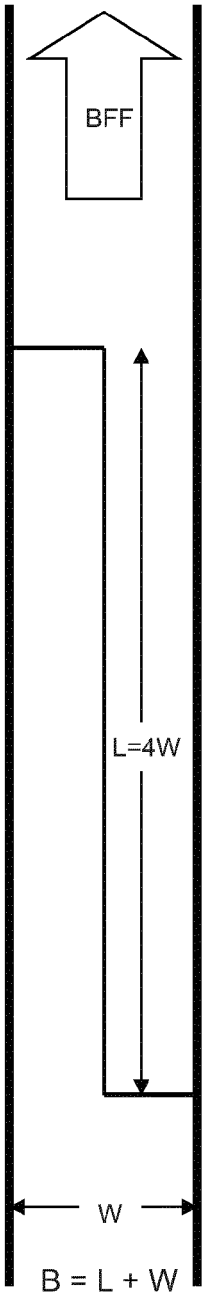


Fig. 7A

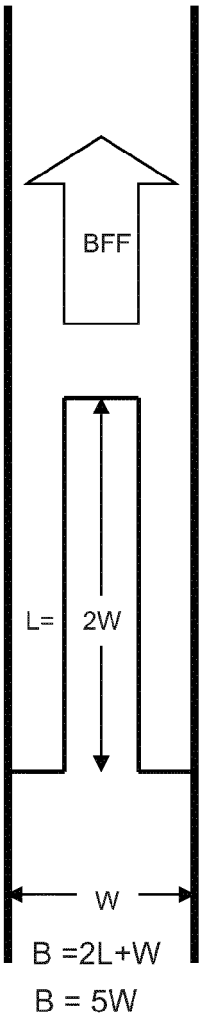


Fig. 7B

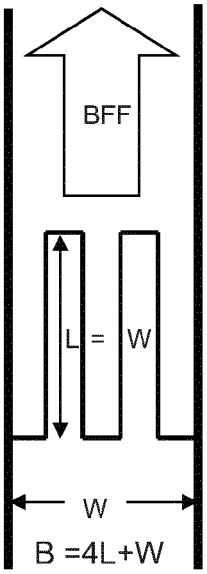


Fig. 7C

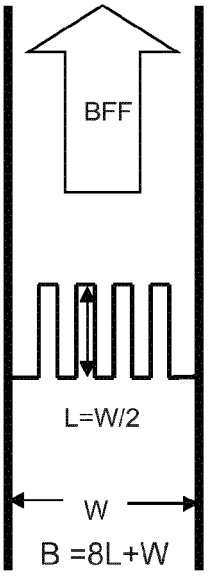


Fig. 7D

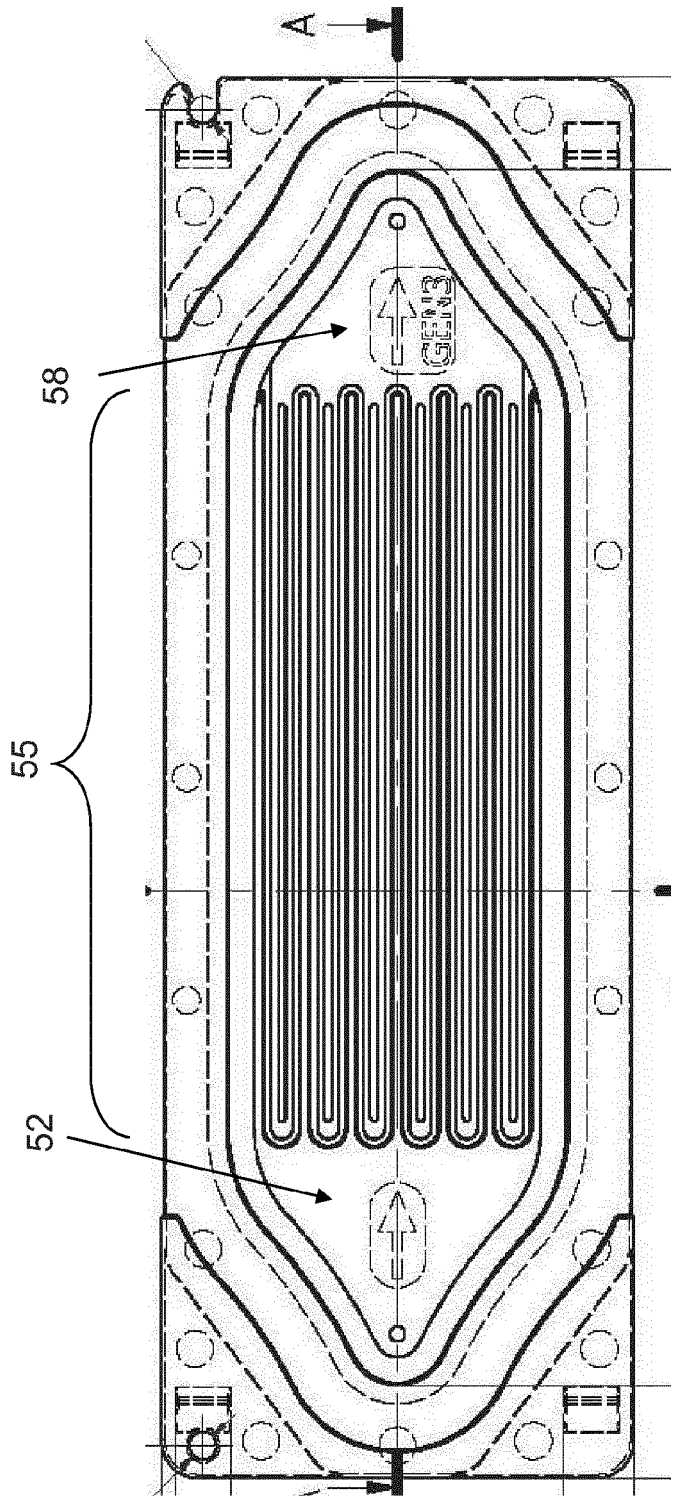


Fig. 8

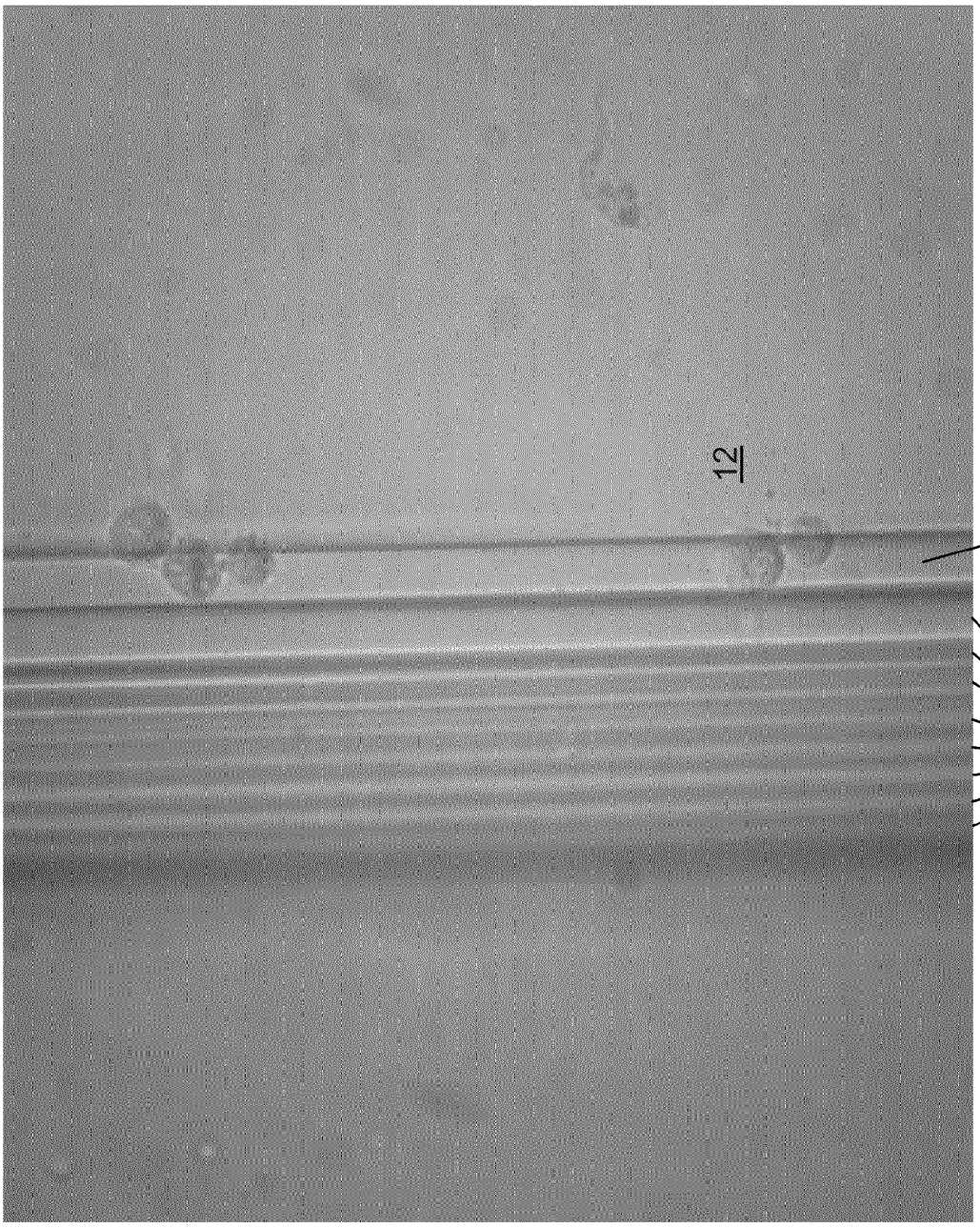


Fig. 9

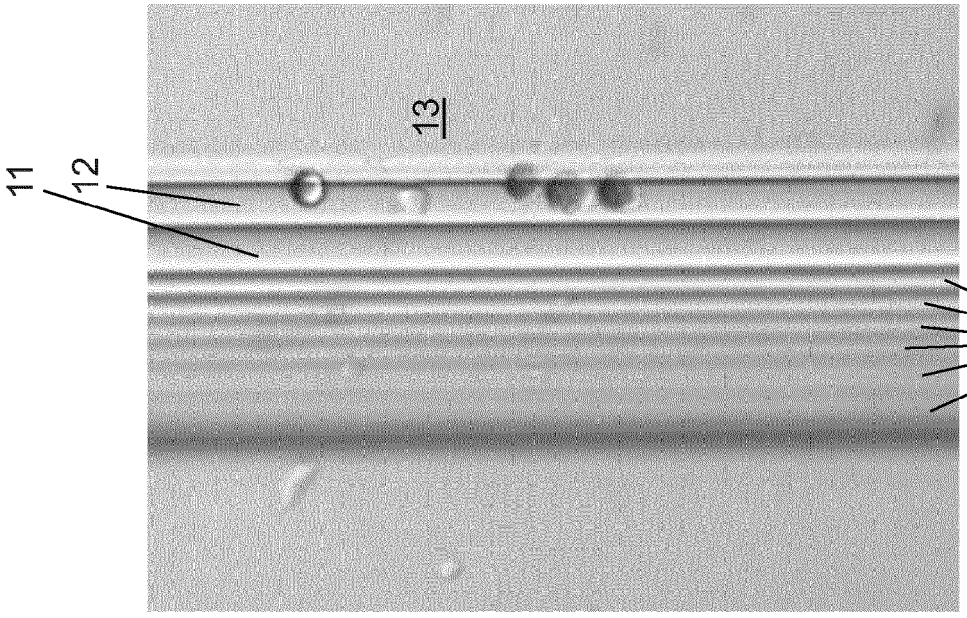


Fig. 10B

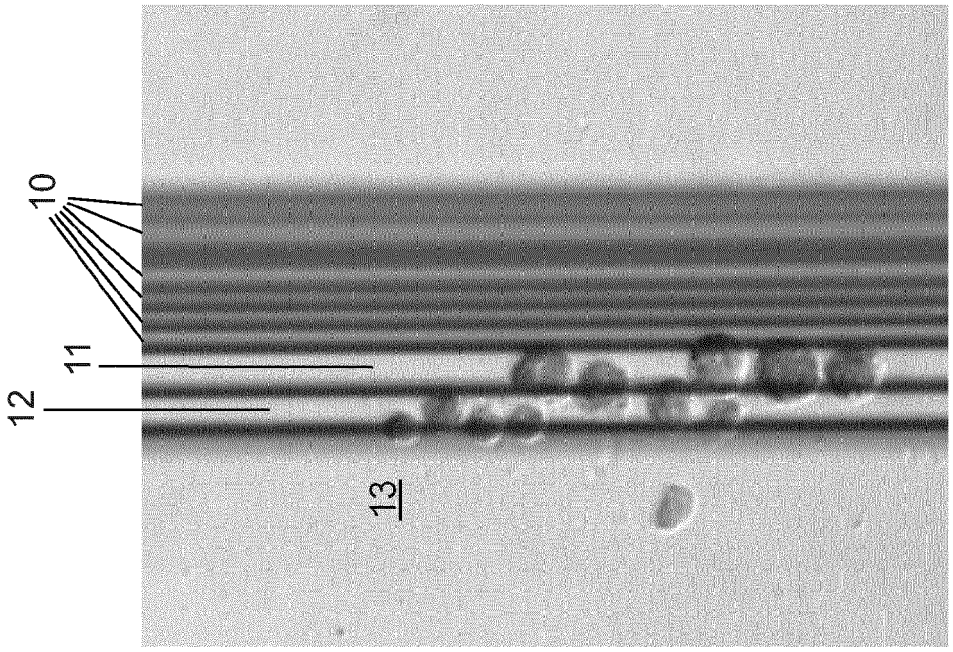


Fig. 10A

REFERENCES CITED IN THE DESCRIPTION

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

Patent documents cited in the description

- US 5928880 A **[0001]**
- US 5866345 A **[0001]**
- US 5744366 A **[0001]**
- US 5486335 A **[0001]**
- US 5427946 A **[0001]**
- US 7993908 B **[0002] [0017] [0029] [0063] [0077] [0078] [0083]**
- WO 03008931 A **[0002] [0040]**
- WO 2009131645 A **[0002] [0040]**
- WO 2011066497 A **[0002] [0040] [0063] [0077] [0078] [0083]**
- WO 2005036139 A **[0003]**
- US 2002022689 W **[0040]**
- US 2009002421 W **[0040]**
- US 2010046350 W **[0040]**
- US 2010058172 W **[0040]**
- WO 20111028483 A **[0040]**

Non-patent literature cited in the description

- **VANKRUNKELSVEN et al.** A novel microstep device for the size separation of cells. Electrophoresis, Wiley Interscience, 25 June 2004, 1714-1722 **[0003]**
- **DAMANI et al.** *Sci. Transl. Med.,* 2012, vol. 4, 126ra33 **[0082]**