HU000027402T2

(19) **HU** (11) Lajstromszám: **E 027 402** (13) **T2**

MAGYARORSZÁG
Szellemi Tulajdon Nemzeti Hivatala

EURÓPAI SZABADALOM
SZÖVEGÉNEK FORDÍTÁSA

(21) Magyar ügyszám: **E 12 167890**
(22) A bejelentés napja: **2012. 05. 14.**

(51) Int. Cl.: ***G01N 15/14*** *(2006.01)*

(96) Az európai bejelentés bejelentési száma:
EP 20120167890
(97) Az európai bejelentés közzétételi adatai:
EP 2522983 A2 **2012. 11. 14.**
(97) Az európai szabadalom megadásának meghirdetési adatai:
EP 2522983 B1 **2015. 12. 16.**

(30) Elsőbbségi adatok:
102011075711 **2011. 05. 12.** **DE**

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(54) **Fúvóka részecskeirányításhoz folyadékáramban**

EP2522983

Nozzle for particle alignment in fluid flows

Description

The present invention concerns a nozzle for orientation of particles in a liquid stream, which has a particle-containing core stream liquid within a sheath stream liquid or consists thereof, wherein the nozzle has an introduction tube for core stream liquid, a flow cytometer with the nozzle, and a method for orientation of particles in a liquid stream, preferably a method for producing at least one fraction of the particles with the step of orientation of the particles and of separating the particles into the at least one fraction and a further fraction. Preferred particles, which can be oriented by the nozzle in a liquid stream, have an altogether flat shape, and are symmetrical, for example, with respect to a section plane, so that the particles have a cross-section with a first and a second dimension that is perpendicular to its longitudinal axis, wherein the cross-section is smaller in the first dimension than in the second dimension. Preferred particles are biological cells, such as blood cells, in particular plate-shaped cells, human and non-human mammalian sperm, in particular sperm derived from a male bovine, pig, sheep, elephant, camel or horse.

Prior Art

US 6,149,867 describes a generic nozzle and a method with a nozzle for sorting of mammalian sperm in sex chromosome specific sperm fractions. The method uses a flow cytometer with a conically tapering nozzle, which contains in its volume a feed line for the core fluid containing sperm, in such a manner that a core stream surrounded by a sheath stream and containing sperm flows out at the nozzle outlet. The geometry of the feed line for sperm and more particularly the outlet opening of the feed tube are not described in more detail.

WO 99/05504 describes a nozzle for use in a flow cytometer for the sorting of sperm, which is characterized in that the nozzle in the section between the outlet opening of a feed tube for core stream liquid containing sperm has an essentially funnel-shaped tapering inner surface, which has a first elliptical cross-section, and an adjacent axial section with elliptical cross-section, the longitudinal axis of which is rotated by 90°. The feed tube has a cylindrical shape and can be slanted in the area of the outlet opening.

EP 1238261 B1 describes a nozzle for a flow cytometer, in which a feed tube for core stream liquid containing sperm arranged coaxially in a nozzle runs from a section with a cylindrical outer diameter to an essentially rectangular cross-section within a section in the shape of a truncated cone of the nozzle, wherein a tapering section of the nozzle with elliptical cross-section abuts downstream of this inlet tube. The outlet of the feed tube is slanted and has an essentially round opening in the cross-section.

US 2004/0095574 A1 describes a flow cell with a hollow needle for injection of a sample liquid in a liquid channel for sheath liquid, the cross-section of which tapers up to a flat cross-section of an observation section at which a microscope is directed. The hollow needle runs from a round cross-section to an elliptical cross-section at its outlet, which has a larger width than the observation area of the microscope, e.g. 2 mm, and a height of e.g. 50-400 µm. The focussing of the sample liquid in a plane is effected on the one hand through the tapering of the liquid channel for the sheath liquid, which is arranged downstream of the hollow needle and on the other hand through different flow velocities of sample liquid and sheath liquid.

WO 2009/151624 A1 describes a nozzle for orientation of particles, wherein an internally located small tube for sample liquid in sections can have a round outer cross-section, which passes over to a rectangular outer cross-section at the outlet end. The inner cross-section of this small tube is always cylindrical.

US 2004/0050186 A1 describes a nozzle, which is characterized by a tapered internal surface with elliptical cross-section adjacent to the nozzle outlet. The introduction tube arranged in the nozzle can have a rectangular outer cross-section.

EP 0288029 A2 describes a microfluidic chamber for optical analysis of cells, which has a stream channel for sheath liquid with an outlet, which tapers to a rectangular cross-section, wherein a nozzle for sample liquid is provided in the stream channel.

Kachel et al., The Journal of Histochemistry and Cytometry 774-780 (1977), describe the orientation of particles in a nozzle, which downstream of the round introduction tube for particle-containing core stream liquid has a tapered and stretched cross-section for hydrodynamic orientation of the particles.

These known nozzles for flow cytometers produce an orientation of the particles through the simple or double elliptical inner cross-section of the nozzle. This is attributed to a pressure increase in sections through the two opposite nozzle areas, which are closer to each other in the smaller ellipse radius than the nozzle areas, which are in the larger ellipse radius. Since these asymmetrical pressure and flow conditions have an effect on the sheath flow and the core stream arranged therein, this method is also referred to as hydrodynamic orientation of the particles.

Task of the invention

With respect to the prior art, the task of the present invention is to provide an alternative nozzle for particle orientation and a method for orientation of not rotationally symmetrical particles, in particular mammalian sperm, in a liquid stream.

General description of the invention

The invention solves the task through a nozzle according to claim 1 and a method according to claim 15, in particular through a nozzle, in which an introduction tube for core stream liquid discharges with its orifice into a nozzle and, preferably, the nozzle and introduction tube are arranged on a common axis, so that the introduction tube can also be said to run coaxially to the nozzle, optionally in its internal volume, and in perpendicular to its longitudinal axis has a cross-section, which is spanned by a first and a second dimension, wherein the cross-section is smaller in the first dimension than in the second dimension, so that the introduction tube has a stretched, not rotationally symmetrical inner cross-section at least in a section adjacent to its orifice. The section adjacent to the orifice of the introduction tube extends over the full length of the introduction tube, which is arranged within the nozzle, in particular over the section of the introduction tube between the first end of the nozzle, which is opposite its outlet opening, and its orifice. The introduction tube ends up in an orifice, which is arranged at a distance from the outlet opening of the nozzle, so that core stream liquid flowing out of the orifice of the introduction tube is surrounded by sheath stream liquid, accelerated and stretched through the inner cross-section tapering towards the outlet opening and flows out of the outlet opening. According to the invention, the orientation of the particles is effected by the inner cross-section of the introduction tube stretched in a dimension. In the section of the nozzle, which abuts to the orifice of the introduction tube, up to its outlet opening, the core stream liquid flowing out of the introduction tube is brought into contact with the particles of sheath stream liquid contained therein. Since the particles, due to their passage through the introduction tube, have a constant orientation with respect to the inner cross-section of the introduction tube resp. with respect to its flat shape, the particles are spaced apart from one another when using the nozzle by a higher speed of the sheath stream liquid with respect to the core stream liquid, wherein a hydrodynamic orientation through a sideward pressure gradient on the core stream liquid, which is produced by an elliptical nozzle, contributes only additionally to the orientation. Preferably, the nozzle is configured in such a manner that a pressure gradient acting laterally on the core stream liquid does not occur, e.g. through a rotationally symmetrical shape of the section of the nozzle at least between the orifice of the introduction tube and the outlet opening of the nozzle.

The inner cross-section of the introduction tube of the nozzle according to the invention allows an orientation of the particles, e.g. with their subsequent separation into fractions, preferably at high speeds resp. sorting rates, since the acceleration of the core stream liquid by a higher speed essentially leads only to acceleration along the nozzle axis without significantly modifying the uniformity of the orientation of the particle after outflow of the introduction tube. In contrast thereto, for conventional nozzles with rotationally symmetrical introduction tubes and a simple or double elliptical nozzle in the nozzle section between the orifice of the introduction tube and the outlet opening of the nozzle, higher speeds of the sheath stream liquid lead to change of the sideward pressure gradient on the core stream liquid and therefore to the exclusively hydrodynamic orientation of the particles. Therefore, for conventional nozzles, a higher speed leads to a less uniform orientation of the particles, while a good orientation is achieved for low speeds. For the purposes of the invention, the introduction tube can also be a circumferentially closed channel and referred to as such.

The nozzle according to the invention can have a simple or double elliptical inner cross-section and is preferably rotationally symmetrical, i.e. with a round, tapered inner cross-section, since the orientation of the particles takes place within the introduction tube, which forms the core stream. Since the orientation of the particles takes place only in the core stream, i.e. without any contact with the sheath stream liquid, the method according to the invention can also be referred to as mechanical orientation of the particles. It is assumed that the particles orientate themselves in the cross-section of the introduction tube that is stretched in a dimension due to the fact that, when the particle-containing core stream liquid flows through the introduction tube, a laminar stream with a speed gradient forms that orientates the particles.

Generally, the invention relates to a nozzle according to claim 1 for the orientation of particles for a flow cytometer having a first end and an opposite second end and an introduction tube for core stream fluid arranged in the inner volume of the nozzle, the introduction tube being arranged in a section adjacent to its orifice coaxially in the nozzle, wherein the introduction tube has a first inlet opening for core stream fluid and an orifice, which is spaced apart from an outlet opening arranged at the second end of the nozzle, and the nozzle has a second inlet opening for sheath stream fluid, wherein the inner cross-section of the introduction tube for core stream liquid at least in a section adjacent to its orifice is smaller in a first dimension than in a second dimension. Preferably, the introduction tube has a rectangular inner cross-section, the longitudinal extension of which is in the second dimension; in particular, the introduction tube has an oval inner cross-section auf, the longitudinal extension of which is in the second dimension. In particular, the first dimension is smaller than the second dimension by at least the factor 0.3 to 0.9. The nozzle can have an electric contact, which projects into the internal volume of the nozzle. Preferably, the nozzle has a piezo crystal at a distance from the first end of the nozzle on a wall, which closes the cross-section of the nozzle up to the introduction tube, so that the nozzle is configured for generation of a droplet stream.

The nozzle according to the invention can be divided into four sections I, II, III and IV. Section I comprises the first end of the nozzle, which is formed by a holder, and can essentially take a cylindrical, cubic, parallelepipedic or prismatic shape. Section II lies between Section I and Section III and has a tapered shape with e.g. an elliptical inner cross-section or with a rotationally symmetrical inner cross-section, in particular with a rotationally symmetrical cylindrical shape or truncated cone shape. Preferably, the inner cross-section in Section II is a rectangular inner cross-section tapering towards the nozzle outlet opening or an inner cross-section point-symmetrical towards the longitudinal axis of the introduction tube, in the internal volume of which the introduction tube for core stream liquid runs, preferably coaxially. The inner cross-section of Section II can be delimited e.g. by a support and a cover being parallel thereto, which are preferably plan and envelop the introduction tube. In this embodiment, the support and cover form between themselves, the introduction tube and walls, which are spaced apart from the introduction tube and connect the support and the cover, the inner cross-section for sheath liquid. The internal volume of the nozzle in Section II in this embodiment envelops the introduction tube and for the method according to the invention is essentially filled out by the sheath stream liquid.

Section III is arranged between Section II and Section IV and preferably has a symmetrical inner cross-section with respect to the longitudinal axis of the introduction tube, e.g. a fully rotationally symmetrical inner cross-section or a section of a rotationally symmetrical, preferably cylindrical form arranged between a support and an opposite cover, which are arranged at a distance from and parallel to the longitudinal axis of the introduction tube, which form is essentially formed by the section of a nozzle insert arranged at the second end the nozzle and facing towards the first end of the nozzle. Section III can e.g. have a fully rotationally symmetrical inner cross-section, e.g. in the shape of a truncated cone with a coaxial introduction tube, or an inner cross-section made by two sections with respectively rectangular cross-section spaced apart by the introduction tube, which form a section of the fully rotationally symmetrical inner cross-section, wherein the inner cross-section can taper towards Section IV. The sections with respectively rectangular cross-sections can be formed between the support and the cover and delimited on both sides at a distance from the introduction tube by the walls of the nozzle. In the inside of Section III of the nozzle, the introduction tube for core stream liquid, the orifice of which is arranged at the end of Section III facing towards the second end of the, runs preferably coaxially with respect to the nozzle. This Section III can have, e.g. for orientation of non-human mammalian sperm, a length of 1 to 200 mm, preferably of 5 to 100 mm or to 50 or to 20 mm.

Section IV is located at the second end of the nozzle, tapers towards the outlet opening arranged at the second end of the nozzle and is optionally rotationally symmetrical, preferably conical resp. in the shape of a truncated cone or can have a rectangular cross-section tapering towards the outlet opening of the nozzle. Section IV can be formed between a support and a cover that is spaced apart therefrom, which can each be plan, and delimited by the walls of the nozzle, which extend between the support and the cover. Generally, the outlet opening of the nozzle is preferably circular. Section IV can have a rectangular cross-section and converts preferably into a circular cross-section, which forms the outlet opening of the nozzle.

Optionally, Section II and/or Section III can be omitted, so that the nozzle consists of the introduction tube, which is arranged coaxially in a nozzle adjacent to its orifice, which nozzle is adjoining the orifice of the introduction tube, wherein the nozzle has a second inlet opening for sheath stream liquid, and the nozzle between its outlet opening and the orifice of the introduction tube comprises an inner cross-section, which preferably tapers towards the outlet opening. In this embodiment, the nozzle is delimited along its longitudinal axis and/or along the longitudinal axis of the introduction tube to the section between the outlet opening of the nozzle and the orifice of the introduction tube, e.g. by a wall, which extends adjacent to the orifice. Such a wall, which forms e.g. the first end of the nozzle, can extend evenly, convexly or concavely, in particular with a parabolic cross-section, about the introduction tube and abut e.g. to a conical nozzle wall or to supports and covers that are spaced apart from the axis of the introduction tube with walls arranged between them, which span over the internal volume of the nozzle, wherein a second inlet opening for sheath stream liquid can be arranged in the wall. The second inlet opening is in particular configured for laminar intake of sheath stream liquid, e.g. as inlet opening of a pipe for sheath stream liquid, whose longitudinal axis is arranged parallel to the longitudinal axis of the introduction tube, and/or as inlet opening, which is parallel to the orifice of the introduction tube and/or which is in the plane of the orifice of the introduction tube. The nozzle in particular can have an inner cross-section, as was described for each embodiment for the section of the nozzle between the orifice of the introduction tube and the outlet opening, e.g. with respect to Section IV.

The individual sections can be formed in one piece or welded with one another or detachably connected with one another, for example screw-fastened via threads with one another, so that simple cleaning of the individual parts can be performed.

Preferably, Sections I, II and III have a common longitudinal axis, on which the introduction tube for core stream liquid runs.

The nozzle according to the invention has an inner cross-section along a longitudinal axis, which connects a first end with a second end. Here, the inner cross-section continuously decreases in terms of area from the first to the second end of the nozzle, independently of its shape, wherein it can remain constant in sections. Section I of the nozzle is essentially formed by a holder, in which a first inlet opening for core stream liquid is embedded that is formed by the introduction tube for core stream liquid, so that the particles contained in the core stream liquid can be pumped directly into the introduction tube for core stream liquid. The introduction tube connects the first end of the nozzle along the longitudinal axis of the nozzle with an opposite second end of the nozzle.

The introduction tube for core stream liquid perpendicularly to its longitudinal axis has an outer cross-section and an inner cross-section, which are spanned in a first and a second dimension, respectively. In this process, the first dimension of the inner cross-section of the introduction tube for the particle containing core stream liquid has a smaller size than in the second dimension, so that the inner cross-section of the introduction tube is stretched. In a preferred embodiment, the inner cross-section in the first dimension is smaller by at least the factor 0.2 to 0.8 than in the second dimension. This inner cross-section stretched along the second dimension has the advantage that an orientation of the particles is already achieved up to the orifice of the introduction tube. This effect increases with a difference in terms of value of the first and second dimension spanning the inner cross-section of the introduction tube, so that the inner cross-section preferably in the first dimension is smaller by at least the factor 0.3 to 0.9, preferably by at least a factor 0.5 to 0.8 than in the second dimension.

The particles or cells passing through the introduction tube for core stream liquid through the geometry of the inner top surface of the introduction tube experience each the same orientation towards the introduction tube, wherein this orientation remains after the outflow of the particle-containing core stream liquid from the orifice of the introduction tube via the section between the orifice of the introduction tube and the outlet opening of the nozzle. For better orientation, it is preferred that the factor from the first to the second dimension of the cross-section through the particles along their longitudinal axis is equal to the factor from the first to the second dimension, which span over the inner cross-section through the introduction tube for core stream liquid.

In a first embodiment, the inner cross-section of the introduction tube is elliptical, in a second embodiment, it is rectangular. A rectangular inner cross-section can amount e.g. in the first dimension to 50 µm to 200 µm, preferably 50 to 150 µm, and in the second dimension to 75 µm to 300 µm, preferably up to 200 µm. For an elliptical inner cross-section, the small diameter, which is in the first dimension, can amount to 50 µm to 200 µm, preferably 50 to 150 µm, and the large diameter, which is in the second dimension is, can amount 75 µm to 300 µm, preferably up to 200 µm.

The wall thickness of the introduction tube for core stream liquid can be constant over the whole length, increase from the first to the second end of the nozzle or decrease from the first to the second end of the nozzle.

The outer cross-section of the introduction tube for core stream liquid is spanned perpendicular to the longitudinal axis of the introduction tube by a first and a second dimension, which is preferably parallel to the first resp. second dimension of the inner cross-section, wherein the outer cross-section is likewise smaller in its first dimension than in its second dimension. The first and the second dimension, which span over the outer cross-section of the introduction tube, therein can alternatively have different sizes or have identical values, so that the outer cross-section can subsequently be round or stretched independently of the geometry of the inner cross-section.

The inner cross-section spanned perpendicularly to the longitudinal axis of the introduction tube can decrease from the first end of the nozzle to the second end of the nozzle along the stream channel or remain constant. In a preferred embodiment, the inner cross-section is constant over the whole length of the introduction tube. In embodiments, in which the inner cross-section decreases, it is preferred that the ratio of first to second dimension, which span over the inner cross-section, remains approximately constant.

Also the outer cross-section can decrease along the stream channel or remain constant. In a preferred embodiment, the outer cross-section tapers over the length of the introduction tube, preferably with a constant wall thickness over the length.

The introduction tube for core stream liquid can optionally be electrically conductive in order to transmit an electric pulse to the core stream liquid.

Arranged on the holder is a second inlet opening for sheath liquid connected to a sheath liquid supply. The second inlet opening is preferably embedded in the holder and preferably arranged closer to the first end of the nozzle than to the second end of the nozzle, for example on the shell resp. in a wall of the holder. By feeding the sheath liquid into the nozzle, the sheath liquid sheaths the introduction tube for core stream liquid, at least in Section IV, which is facing towards the second end of the nozzle. Preferably, Section IV for sheath liquid tapers in the shape of a truncated cone from the end facing towards the first end of the nozzle to its end facing towards the second end of the nozzle, wherein the introduction tube is arranged coaxially therein.

Section IV of the nozzle can be formed by a nozzle insert, which is liquid-tight and preferably releasably connects to Section III. The sheath liquid as well as the introduction tube discharge into Section IV resp. the nozzle insert, which is arranged at the second end of the nozzle and is preferably rotationally symmetrical. Section IV, resp. the nozzle insert in the section facing towards the second end of the nozzle has a tapering section or consists thereof. The outlet opening of the nozzle insert, which forms the outlet opening of the nozzle, is preferably designed circular. The outlet opening of the nozzle is preferably smaller than the orifice of the introduction tube. According to a preferred embodiment, the outlet opening of the nozzle insert is designed essentially circular perpendicular to its longitudinal axis.

The introduction tube for core stream liquid can be fixed on a section of the nozzle opposite its outlet opening, in particular on the holder.

In a preferred embodiment, the introduction tube is formed by a support, two blocks spaced apart and arranged on the support, which can also be referred to as stems, and a cover that is spaced apart from the support. Preferably, the support and the cover are approximately plan and parallel, respectively. The blocks can be formed in one piece with the support. Between the support and the cover, at a distance from the blocks, walls that form the wall of the nozzle are arranged. Therein, the support and the cover extend on both sides of the introduction tube up to the walls and between themselves and the blocks and the walls form the inner cross-section of the nozzle, which extends around the introduction tube, e.g. in Section III and/or Section II of the nozzle. Section IV of the nozzle between the orifice of the introduction tube and the outlet opening of the nozzle can be formed by walls arranged between the support and the cover or by a nozzle insert, which abuts liquid-tightly to the walls and tapers with an elliptical or a circular inner cross-section towards the outlet opening. The cover and the support in the section, in which they delimit the introduction tube between the blocks, can be arranged at the same, a larger or smaller distance than in the section, in which they extend between the blocks and the walls and delimit the inner cross-section of the nozzle. The walls in the section of the nozzle between the orifice of the introduction tube and the outlet opening of the nozzle can be attached to the support and/or the cover, in particular formed in one piece with the support and/or the cover, and the support and the cover optionally extend up to the outlet opening.

In each embodiment, the inner cross-section of Section II of the nozzle between the introduction tube and its inner wall, which is spaced apart therefrom, can be constant along the longitudinal axis of the nozzle or taper towards the nozzle outlet. Preferably, the blocks have external surfaces facing towards the walls of the nozzle, which taper towards the orifice of the introduction tube, preferably external surfaces, which are arranged in Section III, in which the walls of the nozzle form a cross-section tapering towards the outlet opening, approximately parallel to the walls of the nozzle.

In this embodiment, the nozzle can have a support and a preferably parallel cover, walls arranged between them, which delimit the inner cross-section of the nozzle, and blocks that are spaced apart and arranged between the support and the cover or consist thereof, wherein the section of the nozzle between the orifice of the introduction tube and the outlet opening of the nozzle is optionally formed by the walls or a nozzle insert abutting liquid-tightly to the walls, the support and the cover.

The manufacturing a nozzle according to this embodiment can be performed by arrangement resp. one-piece formation of the walls delimiting the nozzle on a support, in particular including the section of the nozzle between the orifice of the introduction tube and the outlet opening of the nozzle, arrangement resp. one-piece formation of the blocks at a distance from one another on the support and/or on the cover and arrangement of the support and the cover against each other. Since the walls and blocks respectively extend liquid-tightly between the support and the cover, the support, the cover and the blocks form the introduction tube between themselves. The walls and the top surfaces of the blocks facing towards them form the stream channel for the sheath liquid. The outlet opening of the nozzle is formed at a distance from the orifice of the introduction tube by the walls, the support and the cover. The walls of the blocks and the section of the support and/or cover arranged on the blocks, which span the inner cross-section of the introduction tube, can be produced by removing the material, which is arranged in the inner cross-section of the introduction tube, e.g. by milling or laser cutting of the big block along the longitudinal axis of the introduction tube, from a big block arranged on the support and/or cover, in particular formed in one piece with the support and/or the cover. Alternatively, a nozzle according in particular to this embodiment can be manufactured by injection molding. The inlet opening for sheath stream liquid can be arranged in a wall, which connects the support and the cover. Preferably, at least an oscillation generator, generally referred to as piezo crystal, is arranged in or adjacent to a wall resp. holder opposite the outlet opening of the nozzle.

Preferably, the support, the blocks and the cover, and the walls optionally arranged between the support and the cover, which form the section of the nozzle between the orifice of the introduction tube and the outlet opening of the nozzle form, are made of plastics.

The method according to the invention according to claim 15 for fractionating particles, preferably for producing a fraction of particles, in particular using the nozzle, has the following steps:

providing a particle-containing core stream liquid,

providing a sheath stream liquid,

pumping the particle-containing core stream liquid through a first inlet opening for core stream liquid of a nozzle, which is preferably embedded in a holder arranged on the nozzle,

pumping the sheath stream liquid through a second inlet opening of the nozzle for sheath stream liquid,

flowing of core stream liquid through the nozzle in a core stream, which perpendicularly to its longitudinal axis has a cross-section, which is spanned in a first dimension and a second dimension, wherein the cross-section is smaller in the first dimension than in the second dimension, by means of pumping the core stream liquid through an introduction tube, which has a cross-section, which is smaller in a first dimension than in a second dimension,

flowing sheath stream liquid through the nozzle, wherein this is conducted by a sheath liquid supply, which is arranged around the introduction tube for core stream liquid and is coaxial to it, wherein the core stream is directly enveloped resp. contacted by the sheath flow in a section, which is located between the orifice of the introduction tube and the outlet opening of the nozzle,

optionally introducing the core stream liquid and the sheath stream liquid into a nozzle insert,

exiting the core stream liquid sheathed by the sheath stream liquid to through the outlet opening of the nozzle.

Optionally, the method contains the step of detecting a property of the particle and,

further optionally, the step of treating (e.g. through laser irradiation) and/or of deflecting the particle-containing liquid into separate containers in dependence on a detected property, e.g. by means of an electric field, through which the particle-containing liquid passes after impingement with an electric charge depending on the detected property. Preferably, the nozzle is made to vibrate, e.g. by means of excitation of an oscillation generator, which leads to formation of a stream of particle-containing droplets.

Particularly preferred is the method for producing sex-chromosome-specifically sorted sperm fractions, e.g. with the step of detecting the sex chromosome, directly or indirectly through detection of the total DNA content, of the mammalian sperm exiting the nozzle and deflecting the mammalian sperm according to the detected sex chromosome into separate fractions, as is generally known, e.g. from US 5,135,759 with electric charging of the sperm-containing liquid droplets exiting the nozzle and their deflection between charged plates or according to WO2010/149739 by means of deflection of sperm-containing liquid droplets exiting the nozzle by means of their superficial vaporization by laser radiation.

The method can further be characterized in that the particles are non-human mammalian sperm, which, after exiting the outlet opening of the nozzle in liquid droplets with core stream liquid and sheath stream liquid , are deflected sex-chromosome-specifically into separate fractions, in which the sperms are enriched with respect to the existence of a sex chromosome. The method can be characterized in that liquid droplets are irradiated depending on a sex-chromosome-specific detection signal with laser radiation, which is directed in a second position at the fluid stream, which is arranged at a larger distance from the nozzle than the first position of the fluid stream, in which a sex-chromosome-specific signal is detected, wherein the laser radiation is absorbed only superficially by the liquid droplet and the liquid droplet is vaporized only superficially and accelerated and deflected in the direction opposite the superficial vaporization. In particular, the laser radiation can be focused onto the top surface of one of the liquid droplets for induction of a superficial photodisruption and/or the laser radiation can have a wavelength, which is absorbed by the liquid droplet. Alternatively, the method can enrich the sperm in sex-chromosome-specific fractions by electrically charging liquid droplets depending on a sex-chromosome-specific detection signal through impingement of the nozzle with an electric charge, and subsequently deflecting them into separated fractions by means of passage through an electric field. The method is preferably a manufacturing method for a preparation of particles, which have a cross-section perpendicular to their longitudinal axis, which cross-section is smaller in a first dimension than in a second dimension, which particles are in particular non-human mammalian sperms, wherein the detected property is the presence of the X-chromosome or of the Y-chromosome in a sperm, and the method comprises the fractionation of the sperm depending on the detected property into at least two different fractions, in particular for producing a fraction, in which the sperm is enriched with X-chromosome or Y-chromosome, e.g. to at least 90%, preferably to at least 95%, preferably to at least 98% or at least 99%.

The flow of sheath stream liquid through the nozzle preferably takes place in Section III in a rotationally symmetrical inner cross-section or an inner cross-section of two sections spaced apart by the introduction tube each having a rectangular cross-section, wherein the inner cross-section can taper between the introduction tube and the nozzle wall towards Section IV arranged between Section III and the outlet opening. Sections III and IV can be formed with a respectively rectangular cross-section between a support and a cover, which extend spaced apart on both sides and parallel to the longitudinal axis of the core stream liquid, and can be delimited by the walls of the nozzles at a distance on both sides of the introduction tube. In Section IV, which is located at the second end of the nozzle, the cross-section of the sheath stream liquid preferably tapers towards the outlet opening arranged at the second end of the nozzle and is formed optionally rotationally symmetrical, preferably conical resp. in the shape of a truncated cone or can have a rectangular cross-section, which decreases towards the outlet opening of the nozzle. As described with reference to Section III, Section IV can also have sections with a respectively rectangular cross-section, which extend on both sides of the introduction tube and are formed between the support and the cover and are delimited at a distance on both sides of the introduction tube by the walls of the nozzle. In Sections I, II and III, the core stream liquid flows through the introduction tube and flows out of its orifice into the adjacent Section IV, in which the core stream liquid is directly enveloped by the sheath stream liquid. In Sections I, II and III, the sheath stream liquid preferably flows between the introduction tube and the walls of the nozzle, and in Section IV between the core stream liquid flowing out of the orifice of the introduction tube and the nozzle wall. Leading the core stream liquid through the introduction tube results in a core stream, the cross-section of which is smaller in a first dimension than in a second dimension, wherein this cross-section produces the regular orientation of particles having a flat cross-section, which is smaller in a first dimension than in a second dimension. Preferably, the core stream liquid sheathed by the sheath stream liquid, which is formed by the outflow of the core stream liquid from the orifice of the introduction tube, has a cross-section, which is smaller in a first dimension than in a second dimension.

Section IV of the nozzle can be formed by a nozzle insert. The core stream liquid flows into the nozzle section IV abutting to the orifice of the introduction tube resp. into the nozzle insert essentially as a jet having the geometry, which corresponds to the inner cross-section of the introduction tube. A core stream liquid that is sheathed by a sheath stream liquid flows out of the outlet opening of the nozzle insert. For a circular outlet opening of the nozzle, the sheath stream liquid flows out of the outlet opening in a cylindrical geometry. This is due to the fact that, because of the orientation of the particles in the core stream liquid by the stretched inner cross-section of the introduction tube, the nozzle can have a simple, rotationally symmetrical Section IV in the shape of a truncated cone. In the sheath stream liquid, the core stream liquid is arranged with a stretched cross-section, in which the particles are oriented in an essentially uniform manner, e.g. with the longitudinal sides of their cross-section being parallel to the longitudinal sides of the cross-section of the core stream liquid and with the narrow sides of their cross-section being parallel to the narrow sides of the cross-section of the core stream liquid, wherein preferably the longitudinal axis of the particles, e.g. of sperms, is arranged parallel to the longitudinal axis of the core stream liquid in the introduction tube.

In a preferred embodiment, in the end facing towards the second end of the nozzle in the shape of a truncated cone, the nozzle section IV runs toward the outlet opening, whereas the cross-section of the introduction tube remains constant over its whole length, at least in its Section III. In particular at a flow velocity of the sheath liquid in the cross-section at the level of the orifice of the introduction tube equal to or greater than the flow velocity of the core liquid, the decreasing inner cross-section of the nozzle in Section IV results in an acceleration of the sheath stream liquid with respect to the core stream liquid, which effects singling of particles of the core stream liquid along the longitudinal axis of the nozzle insert.

In an embodiment, the core stream liquid is pumped into the first inlet opening with approximately the same volumetric flow as for the sheath stream liquid pumped into the second inlet opening. Alternatively, the core stream liquid can also be pumped into the first inlet opening with a volumetric flow, which is greater or is smaller than the volumetric flow, at which the sheath stream liquid is pumped into the second inlet opening.

Preferably, the nozzle has an oscillation generator, e.g. a piezoelectric element, which is configured to produce pressure waves that are perpendicular to the longitudinal axis of the nozzle in order to create a droplet stream in the liquid stream after the outlet opening. The oscillation generator can be attached e.g. in a wall at the first end of the nozzle, in particular be run in, e.g. in a holder.

The nozzle can be arranged on a flow cytometer, which has at least one first radiation source, which is oriented towards a first section of the liquid stream exiting the outlet opening, e.g. a laser, and a first detector oriented opposite the radiation source towards the first section of the liquid stream, wherein optionally the detector generates a signal, which controls a deflection apparatus in order to deflect sections of the liquid stream depending on the detection by means of the signal, e.g. to fractionate. Optionally, a second radiation source is oriented e.g. towards a second section of the liquid stream between the first radiation source and the nozzle insert, while a second detector is oriented towards this second section. Preferably, the second detector generates a second signal, which controls the deflection apparatus, so that sections of the liquid stream are deflected additionally depending on the second signal.

The nozzle can have a metal connection and more particularly a stainless steel connection for charge transfer, which is preferably arranged in Section I of the nozzle on the support on both sides of the longitudinal axis. The stainless steel connection has an electric contact, which can impinge an electric charge onto the feed line for core stream liquid and/or the feed line for sheath stream liquid . Preferably, the electric contact is controlled depending on a first and/or second signal, which is obtained through measurement of the singled droplets below the outlet opening. Positive or negative charging of the droplets takes place depending on the first and/or second signal. According to their charge, the drops can be deflected in different directions, so that at least two fractions are obtained.

Precise description of the invention

The invention will now be described more precisely with reference to the figures and by means of examples, which show schematically

in Figure 1 a cross-section of a nozzle according to the invention,

in Figures 2 to 5 sections along the line A-A in Figure 1,

in Figure 6 a top view of a preferred embodiment of the nozzle without its cover,

in Figure 7 a cover for the nozzle according to Figure 6,

in Figure 8 a top view of a further preferred embodiment of the nozzle without its cover,

in Figure 9 a cover for the nozzle according to Figure 8 and

in Figure 10 a further embodiment of the nozzle.

In the figures, the same reference numerals designate and refer to elements having the same function. Figure 1 shows a nozzle according to the invention for particle orientation for a flow cytometer with a first end 10 and a second end 11. Located at the first end 10 of the nozzle is Section I, which is formed cylindrical in the present case and is essentially formed by a holder 2, along the longitudinal axis of which the introduction tube 5 for core stream liquid runs and in which a second inlet opening 4 for sheath stream liquid is embedded. The adjacent Section II is arranged coaxial to Section I and tapers conically towards the second end 11 of the nozzle. The adjacent cylinder-shaped Section III is formed by a nozzle insert 6, which is arranged at the second end 11 of the nozzle. The introduction tube 5 for core stream liquid runs coaxially to Section III formed by the nozzle insert 6 in its internal volume. The orifice 12 of the introduction tube 5 lies e.g. between Section III and Section IV. Section IV is also formed by the nozzle insert 6, is in the shape of a truncated cone and tapers towards the second end 11 of the nozzle up to an outlet opening 7 having a round cross-section.

A first inlet opening 1 for core stream liquid is located at the first end 10 of the nozzle and is embedded in the holder 2. In the embodiment shown, the first inlet opening 1 for core stream liquid is embedded coaxially in the cylinder-shaped section of the nozzle and is formed by the introduction tube 5. This introduction tube 5 runs from the first end 10 of the nozzle towards the second end 11 of the nozzle, where it leads into the nozzle insert 6 and has an orifice 12 at a distance from the outlet opening 7. The introduction tube 5 for core stream liquid and the support 2 are arranged coaxial.

A second inlet opening 4 for sheath stream liquid is arranged on the holder 2, spaced apart from the first inlet opening 1 for core stream liquid. This second inlet opening 4 is located in the embodiment shown in the wall of the holder 2. Connected to the second inlet opening 4 is the sheath liquid supply 9, which connects the holder 2 with the nozzle insert 6 arranged at the second end 11 of the nozzle. The sheath liquid supply 9 is formed in the shape of a truncated cone in the area between the holder 2 and the nozzle insert 6. The introduction tube 5 runs coaxial to the sheath liquid supply 9 in its internal volume.

Both the sheath liquid supply 9 and the introduction tube 5 for core stream liquid discharge into a nozzle insert 6, which is arranged at the second end 11 of the nozzle. The nozzle insert 6 has a taper section adjacent to its outlet opening 7, which is designed circular. The introduction tube 5 for core stream liquid is arranged coaxial to the nozzle insert 6 and ends at a distance from the outlet opening 7. The intermediate space between the introduction tube 5 and the wall of the nozzle insert 6 is filled with sheath stream liquid.

Arranged in addition on the nozzle are a piezo crystal 3 for generation of a droplet stream as well as a metal connection resp. stainless steel connection 8 for charge transfer to the sheath stream liquid.

Figure 2 shows a cut along the line A-A shown in Figure 1 and the outlet opening 7 of the nozzle arranged at a distance from the orifice 12. The inner cross-section 5A of the introduction tube 5 for core stream liquid is spanned in a first dimension and a second dimension, which differ clearly from each other in terms of value, here approximately by the factor 0.5. According to this embodiment, the inner cross-section 5A has the shape of a rectangle. The other cross-sections are essentially circular.

The sheath stream liquid 9 fills the room between the inner wall of the nozzle insert 6 and the outer wall of the introduction tube 5 and contacts the core stream liquid in the section between the orifice 12 and the outlet opening 7, which is spaced apart.

Figure 3 shows a cut along the line A-A of Figure 1. The inner cross-section 5A of the introduction tube 5 is spanned in a first dimension and a second dimension, which differ clearly in terms of value, here approximately by the factor 0.3. According to this embodiment, the inner cross-section 5A has the shape of an ellipse. The outer cross-section 5B of the introduction tube 5, the nozzle insert 6, the sheath liquid supply 9 and the outlet opening 7 arranged at a distance from the orifice 12 have a circular cross-section.

Figure 4 shows a cut A-A of Figure 1 and the outlet opening 7 of the nozzle arranged at a distance from the orifice 12. The inner cross-section 5A of the introduction tube 5 is smaller in a first dimension by approximately the factor 0.3 than in a second dimension and has the shape of an ellipse. Also the outer cross-section 5B of the introduction tube 5 is smaller in a first dimension than in a second dimension and has the shape of an ellipse. The longitudinal axis of the elliptical inner cross-section 5A lies parallel to the longitudinal axis of the elliptical outer cross-section 5B.

Figure 5 shows a cut A-A of Figure 1 and the outlet opening 7 of the nozzle arranged at a distance from the orifice 12. The inner cross-section 5A of the introduction tube 5 has the shape of an ellipse and is spanned in a first dimension and a second dimension, which differ here approximately by the factor 0.3. The outer cross-section 5B has likewise the shape of an ellipse and is spanned in a first and a second dimension. Both ellipses are arranged in such a way that their longitudinal axes are at right angles to each other.

Figure 6 shows a preferred embodiment, in which the nozzle has a support 13 and two blocks 14, 15 arranged at a distance from each other thereupon. The blocks 14, 15 delimit the introduction tube 5 with the support 13 and a cover 16, which is shown in Figure 7, arranged opposite the support 13. The top surfaces of the support 13 and of the cover 16 facing each other are preferably parallel and plan. The support 13 and the cover 16 can be plate-shaped, respectively.

The top surfaces of the blocks 14, 15 opposite the introduction tube 5 form the portion of the outer cross-section 5B of the introduction tube 5, which together with the walls 17, 18 delimits the flow path for the sheath stream liquid. The walls 17, 18 can generally be parallel to one another and the top surfaces of the blocks 14, 15, which are facing towards the walls 17, 18 can be parallel to the walls. The section of the nozzle between the orifice 12 and the outlet opening 7 can be formed by a nozzle insert 6, which abuts liquid-tightly to the walls 17, 18. According to Figure 6, the nozzle insert 6 can be arranged in sections within the walls 17, 18 or outside, wherein preferably a protrusion 20 is provided on the internal surface of the walls 17, 18 resp. on their external surface, respectively, wherein the protrusion 20 preferably extends over the support 13 and the cover 16. The walls 17, 18 are arranged on support 13.

The cover 16 extends up to the liquid-tightly arranged nozzle insert 6.

Optionally, a gasket 19 can be arranged between the walls 17, 18 and the support 13 resp. cover 16. Furthermore, a gasket 19 can be arranged between the blocks 14, 15 and the cover 16, the cover 16 is preferably plan and has an elastic top surface as the gasket 19 auf, which extends over the portion of its top surface, which is arranged against the front side of the walls 17, 18 facing towards the cover 16 and the portion of its top surface, which is arranged against the blocks 14, 15, in particular extending over the entire top surface of the cover 16, which extends over front sides of the walls 17, 18 facing towards it.

Figure 8 shows a generally preferred embodiment of the nozzle, in which the introduction tube 5 is formed by two parallel blocks 14, 15 spaced apart between the plan top surface of the support 13 and the parallel and plan spaced-apart top surface of the cover 16, in which spaced-apart walls 17, 18 extend between the support 13 and the cover 16 to the blocks 14, 15, which walls taper towards an outlet opening 7 spaced apart from the orifice 12 of the introduction tube 5. As can be seen in Figure 8, the section of the nozzle, which tapers from the orifice 12 of the introduction tube 5 to the outlet opening 7, can be formed by the walls 17, 18, which extend liquid-tightly between the support 13 and the cover 6. The second inlet opening 4 for sheath stream liquid is arranged in one of the walls 17, 18, the first inlet opening 1 for core stream liquid at the first end 10 of the nozzle. The cover 16 shown in Figure 9 through arrangement against the abutting surfaces of the blocks 14, 15 and the walls 17, 18, in particular with an intermediate gasket 19, can delimit the cross-section between the cover 16 and the support 13 as well as between the walls 17, 18 and the blocks 14, 15 to two rectangular inner cross-sections for the sheath liquid, e.g. up to a point adjacent to a nozzle insert arranged in Section IV.

Figure 10 shows an embodiment, in which the orifice 12 of the introduction tube 5 is arranged in a nozzle, which is limited in the longitudinal axis of the introduction tube 5 to Section IV between the orifice 12 of the introduction tube 5 and its outlet opening 7. Section IV tapers from the orifice 12 to the outlet opening 7. Second inlet openings 4 for sheath stream liquid are arranged in the plane of the orifice 12 of the introduction tube 5. The inner cross-section 5A of the introduction tube 5 ends in the orifice 12.

Example 1: Detection of Y-chromosome-containing sperm in fresh semen and sex-specific sorting

Freshly obtained bull semen was diluted in the usual manner in a diluent and incubated with DNA-specific dye, e.g. Bisbenzimid H 33342 (Hoechst), for 30 to 60 min at a temperature of 20°C to 40°C and subsequently irradiated in a flow cytometer according to

US 5125759 or DE 10 2005 044 530 with light of the appropriate excitation wavelength for the dye. The respective emission was measured.

The orientation of the sperms was determined with a detector, which was oriented directly downstream the outlet opening towards the liquid stream consisting of individual droplets flowing out. The total DNA content was determined with a further detector, which was oriented further downstream towards the liquid stream. The deflection apparatus had two oppositely charged plates on both sides of the liquid stream and a contact for electrically charging the liquid in the nozzle. This charge was fed, as is known, depending on the signal of the detector, which determines the orientation of the sperm, and the polarity of the charge depending on the signal of the detector determining the total DNA content. In this manner, the spermatozoa were deflected depending on the detected signal through an electric field into sex chromosome specific fractions.

Optionally, a fluoride was added to immobilize the sperm, e.g. into the sheath liquid or transport liquid used in the course of the sorting process, and/or before or during the addition of the dye in order to increase the penetration of the dye into the spermatozoa. Fluoride ions were added in the range from 0.1 to 100 mM, preferably from 10 nM to 10 mM. It was found that the optimal concentration of the fluoride, e.g. NaF or KF, diverged between different species and for individuals. The optimal concentration for the species is specific and could generally be determined as the concentration, which during microscopic observation resulted in an immobilization of at least 90% of the spermatozoa, preferably of essentially all spermatozoa.

Accordingly, the present invention also refers to compositions of the sperm fractions prepared by the method according to the invention, and to methods for producing sex-specific sperm fractions and subsequent preservation of the sperm fractions of non-human mammals, each preferably in the presence of fluoride and/or anti-oxidants.

List of reference signs

1	first inlet opening
2	holder
3	piezo crystal
4	second inlet opening
5	introduction tube
5A	inner cross-section of the introduction tube
5B	outer cross-section of the introduction tube
6	nozzle insert
7	outlet opening
8	stainless steel connection for charge transfer
9	sheath liquid supply
10	first end of the nozzle
11	second end of the nozzle
12	orifice
13	support
14	block
15	block
16	cover
17	wall
18	wall
19	gasket
20	projection

EP2522983

Fúvóka részecskeirányításhoz folyadékáramban

Szabadalmi igénypontok

1. Porlasztó átfolyásos citométerhez első véggel (10), és szembefekvő második véggel (11), és csatlakozásával (12) a porlasztó belső volumenjében részecsketartalmú folyadékáram számára elrendezett bevezető csövecskével (5), ami a csatlakozásával (12) határos szakaszban a fúvókával közös tengelyen van elrendezve, ahol a bevezető csövecskének (5) egy első bevezető nyílása (1) van a részecsketartalmú folyadékáram számára, és csatlakozása (12) egy a fúvóka második végén (11) elrendezett kieresztő nyíláshoz (7) képest bizonyos távolságban van, és a fúvóka második bevezető nyílással (4) rendelkezik lamináris áramlású folyadék (fedőáramlású folyadék) számára, ahol a bevezető csövecske (5) csatlakozására határos szakasz a bevezető csövecske (5) teljes hosszán át kiterjed, ami a fúvókán belül van elrendezve és a bevezető csövecske (5) belső keresztmetszete (5A) részecsketartalmú folyadékáram számára a bevezető csövecske (5) hossztengelyére merőleges, **azzal jellemezve, hogy** a bevezető csövecske (5) belső keresztmetszete (5A), legalább a saját csatlakozásával határos szakaszban, egy első dimenzióban legalább 0,3 faktor körüli mértékben kisebb, mint egy második dimenzióban, és **hogy** a bevezető csövecske (5) belső keresztmetszete derékszögű, vagy elliptikus, és az első dimenzióban 50µm-200µm, és a második dimenzióban 75µm-300µm mértékű.

2. Az 1. igénypont szerinti fúvóka, **azzal jellemezve, hogy** a bevezető csövecskének (5) derékszögű belső keresztmetszete (5A) van, melynek hosszanti kiterjedése a második dimenzióban van.

3. Az 1. igénypont szerinti fúvóka, **azzal jellemezve, hogy** a bevezető csövecskének (5) ovális belső keresztmetszete (5A) van, melynek hosszanti kiterjedése a második dimenzióban van.

4. Az előző igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a fúvóka belső volumene, hossztengelye mentén, a fúvóka kiömlő nyílása (7) és a bevezető csövecske (5) csatlakozása (12) közötti szakaszra korlátozódik.

5. Az előző igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** piezokristály (3) van elrendezve a fúvóka első végéhez (10) képest bizonyos távolságban egy falon, amely lezárja a fúvóka keresztmetszetét a bevezető csövecskéig (5).

6. Az előző igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a fúvóka villamos csatlakozással rendelkezik, amely a fúvóka belső volumenét érintkezésbe hozza.

7. Az előző igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a bevezető csövecske (5) a fúvókán belül állandó falvastagsággal rendelkezik.

8. Az előző igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a bevezető csövecske (5) belső keresztmetszete a fúvóka első végétől (10) saját csatlakozásáig (12) csökken.

9. Az előző igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a bevezető csövecskét (5) egy tartó (13), egy a tartótól (13) bizonyos távolságban levő fedő (16), és két, tartó (13) és fedő (16) között elrendezett, egymástól távolievő blokk (14, 15) képezi.

10. Az előző igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a fúvóka belső volumenét egy tartó (13) és egy, attól bizonyos távolságban levő fedő (16) és falak (17, 18) határolják, amelyek tartó (13) és fedő (16) között a bevezető csövecskétől bizonyos távolságban terjednek ki.

11. A 10. igénypont szerinti fúvóka, **azzal jellemezve, hogy** a falak (17, 18) a bevezető csövecske (5) mentén, a blokkok (14, 15) felületéhez képest párhuzamosan vannak elrendezve, amelyek a falakhoz (17, 18) kapcsolódnak.

12. A 9.-11. igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a tartó (13) és fedő (16) kapcsolódó felületei párhuzamosak és síkfelületek.

13. A 9.-12. igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a falak (17, 18), a fúvóka egy szakaszában, amely a bevezető csövecske (5) csatlakozása (12) és a kilépő nyílás (7) között nyúlik ki, egymáson összefutnak, és maguk között képezik a kieresztő nyílást (7).

14. Az 1.-12. igénypontok egyike szerinti fúvóka, **azzal jellemezve, hogy** a fúvóka második végén (11) elrendezett kieresztő nyílás (7) egy szakaszában (III), a fúvóka összeszűkül és a fúvókát egy folyadéktömítetten elrendezett fúvókabetét (6) képezi.

15. Eljárás részecskeirányításhoz egy lamináris áramlású folyadék (fedőáramlású folyadék) által körülvett, részecskéket tartalmazó részecsketartalmú folyadékáram irányításához, a következő lépésekkel: részecsketartalmú folyadékot és lamináris áramlású folyadékot készítünk elő, a részecsketartalmú folyadékáramot átszivattyúzzuk egy első bemenő nyíláson (1) keresztül egy első bevezető csövecskébe (5), és átszivattyúzzuk egy második bevezető nyíláson (4) keresztül a lamináris áramlású folyadékot, és egy lamináris áramlású folyadék által körülölelt részecsketartalmú folyadékot egy fúvókának a fúvóka első végével (10) szemben levő második végén (11) kiléptetjük egy kieresztő nyíláson (7) keresztül, ahol a részecsketartalmú folyadékot a visszavezető csövecskében (5) vezetjük, amely a csatlakozásával (12) határos szakaszban a fúvókával közös tengelyen van elrendezve, és a bevezető csövecske (5) belső keresztmetszete (5A) a bevezető csövecske (5) hossztengelyére merőleges, ahol a bevezető csövecske (5) csatlakozására határos szakasz a bevezető csövecske (5) teljes hosszán keresztül terjed ki, ami a fúvókán belül van elrendezve, **azzal jellemezve, hogy** a bevezető csövecske (5) belső keresztmetszete (5A), legalább a csatlakozásával (12) határos szakaszban, egy első dimenzióban, legalább 0,3-as faktor körül kisebb, mint egy második dimenzióban, és **hogy** a bevezető csövecske (5) belső keresztmetszete négyszögletű, vagy elliptikus, és az első dimenzióban 50µm-200µm, a második dimenzióban 75µm-300µm értéket ér el.

16. Eljárás a 15. igénypont szerint, **azzal jellemezve, hogy** a részecskéknek hossztengelyükre merőlegesen olyan keresztmetszetük van, amely egy első dimenzióban kisebb, mint egy második dimenzióban.

17. Eljárás a 15.-16. igénypontok egyike szerint, részecske preparátum előállításához, amelyek hossztengelyükre merőlegesen olyan keresztmetszettel rendelkeznek, amely egy első dimenzióban kisebb, mint egy második dimenzióban, **azzal jellemezve, hogy** a részecskék nem-emberi emlős spermiumok, a részecske egy tulajdonsága detektálásának lépésével, és a részecske kezelésének lépésével a detektált tulajdonság függvényében, és/vagy a részecske eltérítésének lépésével a detektált tulajdonság függvényében elválasztott tartályba, legalább kettős különböző frakció képzéséhez.

Fig. 1

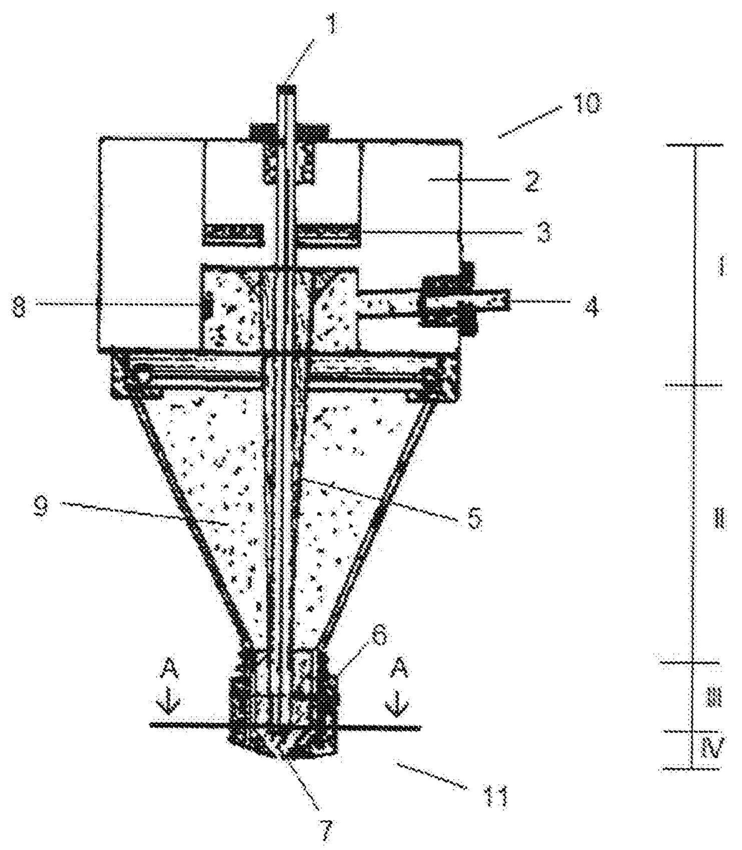

Fig. 2

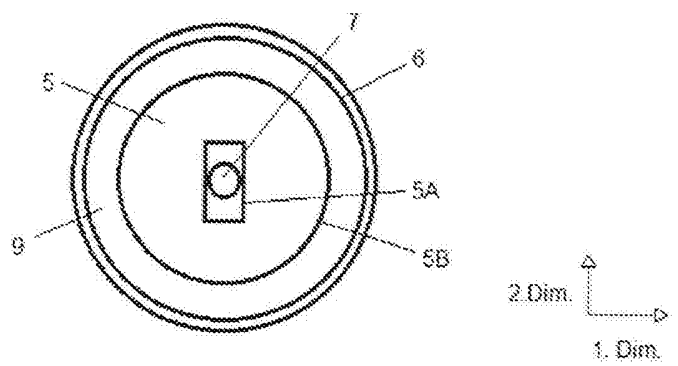

Fig. 3

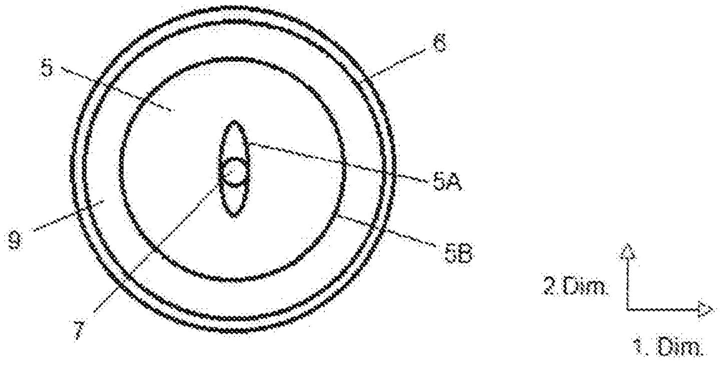

Fig. 4

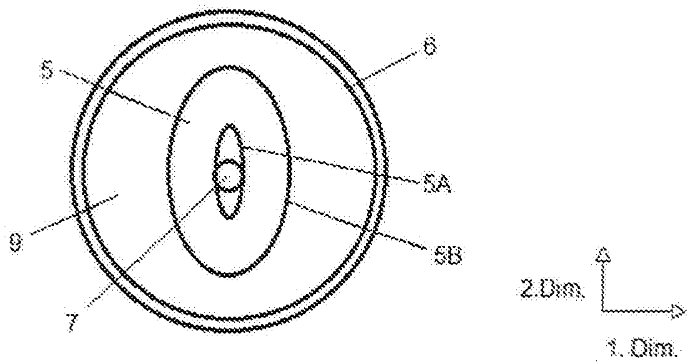

Fig. 5

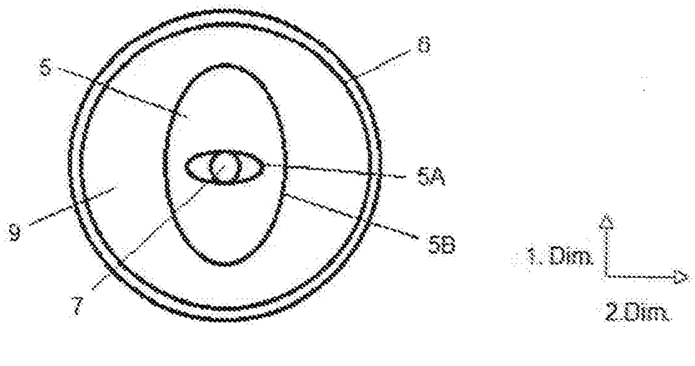

Fig. 6

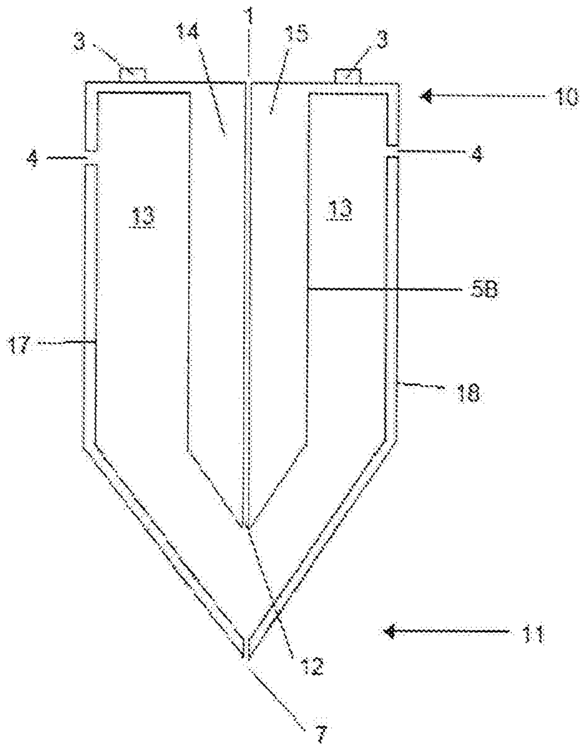

Fig. 7

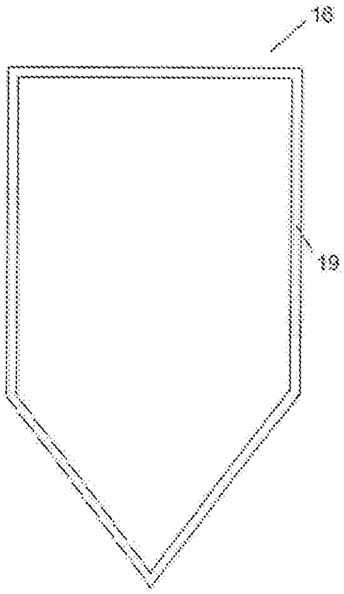

Fig. 8

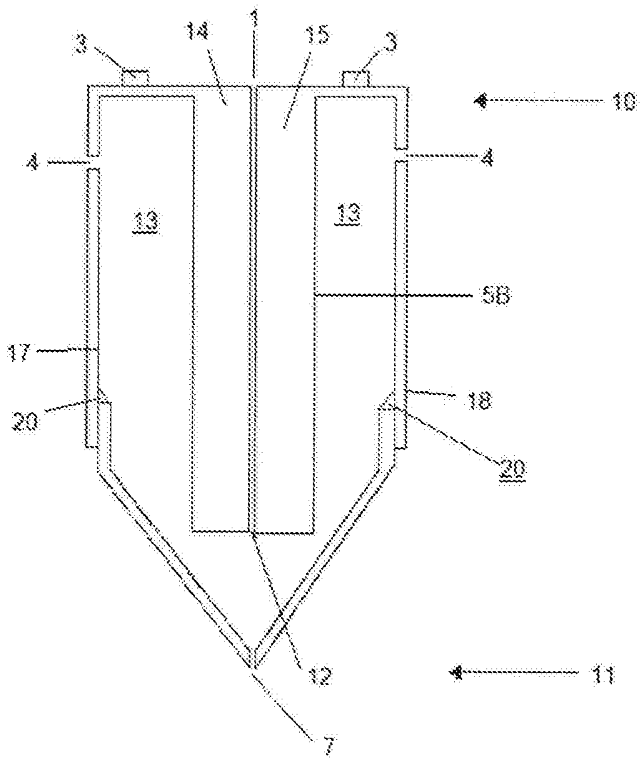

Fig. 9

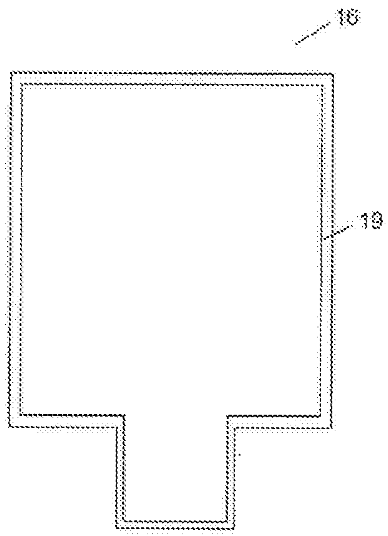

Fig. 10