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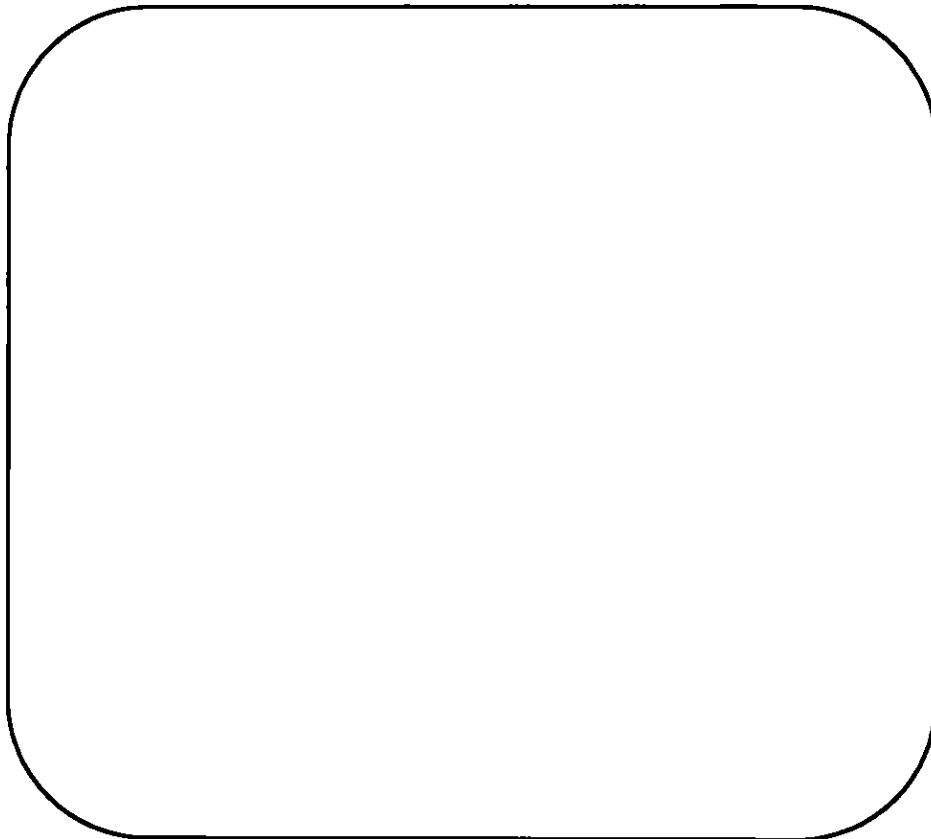
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Anexos

- ☒ Comprobante de pago de la tasa de presentación de la solicitud por \$ 378 000 oo
- ☐ Comprobante de pago por reivindicación de prioridad
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica
- ☐ Poderes si fuere el caso
- ☐ Documento de cesión del inventor al solicitante o a su causante
- ☐ Declaraciones
- ☒ Copia de la solicitud internacional (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano (Resumen descripción reivindicaciones dibujos)
- ☒ Copia del examen preliminar efectuado por la IPEA.
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- ☐ Otros

11

FIGURA CARACTERISTICA.



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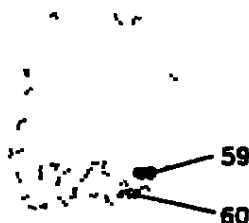
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(54) Title HIGH PURITY X-CHROMOSOME BEARING AND Y-CHROMOSOME BEARING POPULATIONS OF SPERMA TOZOA



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(57) Abstract Isolated non naturally occurring populations of spermatozoa (15) having high purity and technologies to differentiate spermatozoa (28) based on characteristics such as mass volume orientation or emitted light including methods of analysis and apparatus such as beam shaping optics (30) and detectors (32).



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HIGH PURITY X-CHROMOSOME BEARING AND Y-CHROMOSOME BEARING
POPULATIONS OF SPERMATOOZA

I TECHNICAL FIELD

Isolated high purity X-chromosome bearing or Y-chromosome bearing
5 populations of spermatozoa and technologies to isolate spermatozoa, particles, or events
based upon differentiation characteristics such as mass, volume, DNA content, or the like.

II BACKGROUND

Isolated high purity X-chromosome bearing or Y-chromosome bearing
populations of spermatozoa can be utilized to accomplish in vitro or in vivo artificial
10 insemination of or fertilization of ova or oocytes of numerous mammals such as bovids,
equids, ovids, goats, swine, dogs, cats, camels, elephants, oxen, buffalo, or the like See
also, United States Patent 5,135,759, hereby incorporated by reference

However, conventional technologies for separating spermatozoa into X-
chromosome bearing and Y-chromosome bearing populations can result in spermatozoa
15 populations having low purity Regardless of the separation method spermatozoa have
not been routinely separated into X-chromosome bearing and to Y-chromosome bearing
sperm samples having high purity, such as 90%, 95%, or greater than 95%

A number of techniques, directly or indirectly based on differences in size, mass,
or density have been disclosed with respect to separating X-chromosome bearing from Y-
20 chromosome bearing spermatozoa As disclosed by United States Patent No 4,474,875, a
buoyant force is applied to all sperm cells simultaneously and X-chromosome bearing and
Y-chromosome bearing spermatozoa may then be isolated at different locations in the
separation medium United States Patent No 5,514,537 discloses a technique whereby
spermatozoa traverse a column packed with two sizes of beads The larger X-chromosome
25 bearing spermatozoa become isolated in the layer containing the larger beads, while the
smaller Y-chromosome bearing spermatozoa become isolated in the layer containing the
smaller beads United States Patent No 4,605,558 discloses that spermatozoa may be

made differentially responsive to a density gradient and United States Patent No 4,009,260 exploits the differences in migration-rate, or swimming-speed, between the Y-bearing spermatozoa, and the X-chromosome bearing spermatozoa, through a column of retarding medium

- 5 A problem common to each of the above-mentioned technologies may be that they each act on all the spermatozoa in a 'bulk-manner', meaning that all the spermatozoa undergo the same treatment at the same time, and the Y-chromosome bearing sperm cells come out faster, earlier, or at a different position than X-chromosome bearing sperm cells. As such, individual sperm cells may not be assessed and there may be no actual
- 10 'measurement' of volume, mass, density, or other sperm cell characteristics. One-by-one assessment of sperm cells can provide advantages in that the actual separation process can be monitored, and objective quantitative data can be generated even during the separation process, and separation parameters altered as desired. Furthermore, these technologies may not be coupled with flow cell sorting devices
- 15 Flow cytometer techniques for the separation of spermatozoa have also been disclosed. Using these techniques spermatozoa may be stained with a fluorochrome and made to flow in a narrow stream or band passing by an excitation or irradiation source such as a laser beam. As stained particles or cells pass through the excitation or irradiation source, the fluorochrome emits fluorescent light. The fluorescent light may be
- 20 collected by an optical lens assembly, focused on a detector, such as a photomultiplier tube which generates and multiplies an electronic signal, which may then be analyzed by an analyzer. The data can then be displayed as multiple or single parameter chromatograms or histograms. The number of cells and fluorescence per cell may be used as coordinates. See United States Patent No 5,135,759, hereby incorporated by
- 25 reference. However, with respect to this type of technology a variety of problems remain unresolved and isolating highly purified populations of X-chromosome bearing or Y-chromosome bearing sperm cells be difficult

A significant problem with conventional flow cytometer technologies can be the

orientation of objects, particles, or cells in the sheath fluid stream. This can be particularly problematic when the object or cell is irregular in shape with respect to more than-one axis, such as spermatozoa for example. One aspect of this problem may be establishing the initial orientation of the object within the sheath fluid stream. A second
5 aspect of this problem may be maintaining the orientation of the object with respect to the detector (photomultiplier tube or otherwise) during the period that emitted light from the object is measured.

Another significant problem with conventional flow cytometer technologies can be the failure to encapsulate the objects or cells in a droplet of liquid. Especially, when
10 droplets are formed around irregularly shaped objects the droplet may not be of sufficient size to completely surround all the features of the objects or cells. For example, during flow cytometry operation as above-described droplets can be formed at very high speed, even as many as 10,000 to 90,000 droplets per second and in some applications as many as 80,000 droplets per second. When spermatozoa are encapsulated into droplets,
15 especially at these high rates of speed, a portion of the tail or neck may not be encapsulated in the droplet. That portion of the tail or neck not encapsulated in the droplet may then be responsive with the nozzle or may be responsive to the environment surrounding the droplet in a manner that interferes with subsequent droplet formation or with proper deflection of the droplet. As a result some of the spermatozoa may not be
20 analyzed at all reducing the efficiency of the procedure, or may not be resolved sufficiently to be assigned to a population, or may be deflected in errant trajectories, or a combination of all may occur.

Another significant problem with conventional flow cytometer technologies, as
25 well as other technologies, can be a coincidence of measurable events. One aspect of this problem can be that the incident light flux from a first event continues to produce signals after the incident light flux from a second event starts to generate a signal. As such, the two events remain at least partially unresolved from one another. Another aspect of this problem can be that two or more events are simultaneously initiated and the incident light
30 flux comprises the contribution of all the events. As such, the multiplicity of events may

- not be resolved at all and the objects corresponding to the multiplicity of events can be incorrectly assigned to a population or not assigned to a population at all, or both. Specifically, with respect to flow cytometry, individual particles, objects, cells, or spermatozoa in suspension flow through a beam of light with which they interact
- 5 providing a measurable response, such as fluorescent emission. In conventional flow cytometry, Hoechst stained spermatozoa traverse a laser beam resulting in a fluorescent light emission. The fluorescent light emission from the excited fluorochrome bound to the DNA can be bright enough to produce an electron flow in conventional photomultiplier tubes for a period of time after the actual emission event has ended.
- 10 Moreover, in a conventional flow cytometer, the laser beam can produce a pattern having a height of 30 μm while the width can be approximately 80 μm . The nucleus of a bovine spermatozoa which contains fluorochrome bound DNA can be about 9 μm in length making the height of the laser beam some three (3) times greater than the nucleus. This difference can allow for the laser excitation of the bound fluorochrome in more than one
- 15 spermatozoa within the laser beam pattern at one time. Each of these conventional flow cytometry problems decreases the ability to resolve individual events from one another.

Another significant problem with conventional flow cytometer technologies, and other technologies, can be that irregularly shaped objects, such as spermatozoa, generate differing signals (shape, duration, or amount) depending on their orientation within the

20 excitation/detection path. As such, individuals within a homogenous population can generate a broad spectrum of emission characteristics that may overlap with the emission characteristics of individuals from another homogenous population obviating or reducing the ability to resolve the individuals of the two populations.

Another significant problem with conventional flow cytometer technologies, and

25 other technologies, can be that objects are not uniformly exposed to the excitation source. Conventional beam shaping optics may not provide uniform exposure to laser light when the objects are close to the periphery of the beam.

Another significant problem with conventional flow cytometer technologies can

be that objects, such as spermatozoa, can be exposed to the excitation source for unnecessarily long periods of time. Irradiation of cells, such as spermatozoa, with laser light may result in damage to the cells or to the DNA contained within them.

Another significant problem with conventional flow cytometer technologies can be that there may be a disruption of the laminar flow within the nozzle by the injection tube. Disruption of the laminar flow can change the orientation of irregularly shaped objects within the flow and lower the speed of sorting and the purity of the sorted populations of X-chromosome bearing sperm or Y-chromosome bearing spermatozoa.

There may be additional problems with technologies that utilize stain bound to the nuclear DNA of sperm cells. First, because the DNA in the nucleus is highly condensed and flat in shape, stoichiometric staining of the DNA may be difficult or impossible. Second, stained nuclei may have a high index of refraction. Third, stain bound to the DNA to form a DNA-stain complex may reduce fertilization rates or the viability of the subsequent embryos. Fourth, the DNA-stain complex is typically irradiated with ultra-violet light to cause the stain to fluoresce. This irradiation may affect the viability of the spermatozoa. Due to these various problems, it may be preferable to use a method that requires less or no stain, or less or no ultra-violet radiation, or less or none of both.

With respect to generating high purity samples of X-chromosome bearing sperm cell or Y-chromosome bearing sperm cell populations (whether live, fixed, viable, non-viable, intact, tailless, or as nuclei), or generally, with respect to detecting small differences in photo-generated signal between serial events having relatively high incident light flux, or with respect to orienting irregularly shaped objects in a fluid stream, or eliminating coincident events within an optical path, or removing undesirably oriented objects from analysis, the instant invention addresses every one of the above-mentioned problems in a practical fashion.

III. DISCLOSURE OF THE INVENTION

A broad object of the invention can be to provide isolated high purity X-chromosome

bearing and Y-chromosome bearing populations of spermatozoa. Isolated non-naturally occurring populations of spermatozoa that have high purity have numerous applications including sex selection of offspring from mammals, various in vitro protocols for the fertilization of ova, various in vivo protocols such as artificial insemination, business
5 methods involving the production of prize animals or meat animals, or preservation of rare or endangered animals, to recite but a few of the applications for high purity populations of spermatozoa.

Another broad object of the invention involves both devices and methods for the production of high purity X-chromosome bearing and Y-chromosome bearing sperm samples.

10 Particular embodiments of the invention are described, which may be used in numerous applications as above-mentioned, that can be used to achieve the specific objects of differentiating between bright photoemissive events having small measurable differences in total light flux, orienting irregularly shaped objects in a fluid stream, the minimization of coincident events within an optical path, the removal of signal contributed by undesired
15 unoriented objects within an optical path (including the removal of the object itself), and the encapsulation of irregularly shaped objects within a droplet. As such, the specific objects of the invention can be quite varied

Another broad object of the invention can be to provide X-chromosome bearing or Y-chromosome bearing spermatozoa samples (live, fixed, viable, non-viable, intact, tailless,
20 or sperm nuclei) having a graded level of high purity in the range of 80%, 85%, 90%, 95%, or even greater than 95%

Another significant object of particular embodiments of the invention can be to sort spermatozoa into X-chromosome bearing and Y-chromosome bearing populations having high purity even at high separation rates. The high speed separation can produce live sperm
25 of each sex at rates of about 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8,000, 9,000 or even 10,000 per second, or higher

Another significant object of particular embodiments of the invention can be to substantially eliminate or remove spermatozoa (live, fixed, viable, non-viable, intact, tailless, or sperm nuclei) having undesired orientation in the excitation/detection portion of the flow path of a flow cytometer

- 5 Another significant object of particular embodiments of the invention can be to provide artificial insemination samples of X-chromosome bearing or Y-chromosome bearing spermatozoa having a high level of purity

- Another significant object of particular embodiments of the invention can be to provide in vitro insemination samples of X-chromosome bearing or Y-chromosome bearing
10 spermatozoa having a high level of purity

Another significant object of a particular embodiment of the invention can be to preselect the sex of offspring of females inseminated with high purity artificial insemination samples, the sex of offspring of ova fertilized with high purity artificial insemination samples, with selection success rates of 80%, 85%, 90%, 95%, or greater than 95%

- 15 Another significant object of particular embodiments of the invention can be to differentiate between photoemissive events having small differences in total emitted light flux

- Another significant object of particular embodiments of the invention can be to substantially eliminate or reduce the amount of background noise generated by a
20 photomultiplier tube, even in the absence of light, during the period after exposure to high incident light flux.

Another significant object of particular embodiments of the invention can be to substantially eliminate saturation of the photocathode of photomultiplier tube(s) used in conjunction with flow cytometry, or otherwise

Another significant object of particular embodiments of the invention can be to reduce the number electrons migrating from the photocathode of a photomultiplier tube to the first dynode.

Another significant object of particular embodiments of the invention can be to
5 reduce the total flow of electrons to the N electrode of a photomultiplier tube.

Another significant object of particular embodiments of the invention can be to allow increased light flux to the photocathode of the photomultiplier tube without proportionately increasing the amount of background signal generated by the photomultiplier tube.

Another significant object of particular embodiments of the invention can be to
10 increase the signal to background signal ratio from measured photoemissive events.

Another significant object of particular embodiments of the invention can be to allow increased amplification of the signal generated from the photomultiplier tube during high incident light flux events or serial high incident light flux events without saturating the photocathode of the photomultiplier tube.

15 Another significant object of particular embodiments of the invention can be to increase the apparent resolution of chromatograms or histograms resulting from sorting fluorochrome stained sperm, or other cells, or other objects, having small differences in emitted light flux upon excitation of the bound fluorochrome(s)

Another significant object of particular embodiments of the invention can be to
20 improve the calibration of sorting flow cytometer instruments when used for sorting spermatozoa.

Another significant object of particular embodiments of the invention can be to increase the sperm sorting rate of flow cytometer systems

14

Another significant object of particular embodiments of the invention can be to increase the purity of the sperm samples sorted by flow cytometry

Another significant object of particular embodiments of the invention can be to provide techniques for the sorting of X-chromosome bearing sperm from Y-chromosome bearing sperm where there is a small difference in the amount of Y chromosome DNA to the amount of X chromosome DNA relative to the total amount of nuclear DNA.

Another significant object of particular embodiments of the invention can be to provide techniques which improve the apparent resolution of histograms generated during the process of sorting X-chromosome bearing sperm from Y-chromosome bearing sperm with a flow cytometer

Another significant object of particular embodiments of the invention can be to provide beam shaping optics which minimizes coincidence of objects within the excitation/detection path

Another significant object of particular embodiments of the invention can be to provide beam shaping optics that minimizes the total lumens an object is exposed to traversing the excitation beam. One aspect of this object can be to decrease the total lumens an object is exposed to. A second aspect of this object can be to increase the power of the light source without increasing the total lumens the object is exposed to

Another significant object of particular embodiments of the invention can be to provide beam shaping optics that allow for uniform exposure of objects that pass through the optical path.

Another significant object of particular embodiments of the invention can be to provide a nozzle that orients irregularly shaped objects in a fluid stream. One aspect this object can be to orient elongated objects in the same direction. A second aspect of this object can be to orient dorso-laterally flattened objects in the same direction.

Another significant object of particular embodiments of the invention can be to increase the purity of the sperm samples sorted by flow cytometry

Another significant object of particular embodiments of the invention can be to provide techniques for the sorting of X-chromosome bearing sperm from Y-chromosome
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10 with a flow cytometer

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25 object can be to orient elongated objects in the same direction. A second aspect of this object can be to orient dorso-laterally flattened objects in the same direction.

Another significant object of particular embodiments of the invention can be to fully encapsulate irregularly shaped objects within a drop of fluid.

Another significant object of particular embodiments of the invention can be to
5 differentiate undesirably oriented objects from desirably oriented objects in a fluid stream.

Another object of an embodiment of the invention can be to provide differential interference contrast technology, whereby the object-plane consists of a fluid stream carrying the objects of interest, and whereby the image-plane can be used to measure the signal from the passing objects

10 Another object of an embodiment of the invention can be to provide optics that form two laterally separated images from each object in such a way that one can be used to measure the actual volume, and one to determine the orientation. This way, objects that were not orientated properly to allow an accurate measurement of its volume can be discarded. This can be accomplished by modifications so that the light pulses, resulting from these two
15 images can be detected independently using two pinholes in the image plane. Optics are tuned in such a way that a first image can give rise to a light pulse proportional to the volume of the object, and that a second image can give rise to a light pulse dependent on the orientation the object had when it was measured

Another object of an embodiment of the invention can provide a manner of
20 compensating for the fact that the objects are contained inside a fluid stream. The fluid stream can be a cylinder of water, for example, which acts as a cylindrical lens, thus distorting the image of the object. Optically, this corresponds to cylinder of higher refractive index (water) than its surroundings (air). The compensation disclosed in this invention can consist of, for example, a cylinder having a refractive index lower than its surroundings,
25 although other compensating elements of various shapes and refractive index may also be designed as the need requires. By making sure the light passes through this compensation element, the optical effect of the fluid stream can be compensated by the exactly opposite behavior of the compensation element

17

Naturally further objects of the invention are disclosed throughout other areas of the specification and claims

IV BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a generalized flow cytometer

5 Figure 2 shows a second view of a generalized flow cytometer

Figure 3 shows a comparison of univariate histograms from flow cytometers (#1, #2, and #3) without the amplifier invention (Figure 3A) with univariate histograms for the same flow cytometers using a particular embodiment of the amplification invention (Figure 3B) illustrating the improved resolution between X-chromosome bearing and Y-chromosome bearing populations of bovine spermatozoa.

Figure 4 shows univariate and bivariate histograms illustrating the conventional resolution between X-chromosome bearing and Y-chromosome bearing populations of bovine spermatozoa.

Figure 5 shows univariate and bivariate histograms illustrating improved resolution between X-chromosome bearing and Y-chromosome bearing populations of bovine spermatozoa using a particular embodiment of the amplification invention

Figure 6 shows a second example of univariate and bivariate histograms illustrating the conventional resolution between X-chromosome bearing and Y-chromosome bearing populations of bovine spermatozoa.

Figure 7 shows a second example of univariate and bivariate histograms illustrating the improved resolution between X-chromosome bearing and Y-chromosome bearing populations of bovine spermatozoa using a particular embodiment of the amplification invention

13

Figure 8 shows univariate and bivariate histograms illustrating the conventional resolution between X-chromosome bearing and Y-chromosome bearing populations of equine spermatozoa.

Figure 9 shows univariate and bivariate histograms illustrating the improved
5 resolution between X-chromosome bearing and Y-chromosome bearing populations of equine spermatozoa using a particular embodiment of the amplification invention.

Figure 10 shows univariate and bivariate histograms illustrating the improved resolution between X-chromosome bearing and Y-chromosome bearing populations of equine spermatozoa nuclei using a particular embodiment of the amplification invention.

10 Figure 11 shows a particular embodiment of the circuit board modification to make the amplification invention with respect to a MoFlo® flow cytometer

Figure 12 shows an electrical schematic diagram of a particular embodiment of the amplification invention with respect to a MoFlo® flow cytometer

Figure 13 shows the laser beam pattern using conventional beam shape optics (Figure
15 13A) and the laser beam pattern using the reduced height beam shape optics (Figure 13B)

Figure 14 shows a bar graph that compares the purity of separated X-chromosome bearing spermatozoa (Figure 14A) and Y-chromosome bearing spermatozoa (Figure 14B) using conventional technology or using the amplification invention independently or in conjunction with reduced height beam shaping optics

20 Figure 15 shows a front view of the reduced height beam shaping optics

Figure 16 shows a top view of the reduced height beam shaping optics

Figure 17 shows a perspective and two cross sections of the object orienting nozzle

invention.

Figure 18 shows a graded series of cross sections of the object orienting nozzle invention.

Figure 19 shows a front view and an end view of an embodiment of the beveled injection tube invention.

Figure 20 illustrates the removal of undesired unoriented spermatozoa (RUUS) invention by comparison of signal(s) from the oriented spermatozoa (Figures 20A and 20B) and the signal(s) from the unoriented spermatozoa (Figures 20C and 20D)

Figure 21 shows a perspective of another embodiment of the beveled injection tube invention having a paddle shaped beveled blade

Figure 22 shows a conventional optics technology coupled to a flow cytometer

Figure 23A shows the shape and size of a typical spermatozoon and Figure 23B shows the difference between correctly and non-correctly orientated spermatozoa

Figure 24 shows an embodiment of the invention having construction allowing the measurement of two signals, for example volume and orientation.

Figure 25A and B shows an embodiment of the invention having two halves with a pinhole corresponding to each half, Figure 25C shows an image plane of an embodiment of the invention, Figure 25D shows an embodiment of the invention having two independently rotatable polarizers

Figures 26A and 26B illustrates the compensation method for the fluid stream for an embodiment of the invention, Figure 26C shows an embodiment of a compensation element, 26 D shows another embodiment of a compensation element where images of a fluid stream

and from the compensation element fall on top of each other in the image plane.

Figure 27 shows an embodiment of the interference optics invention.

Figure 28 shows an a second view of the interference optics invention.

V MODE(S) FOR CARRYING OUT THE INVENTION

- 5 The invention involves isolated high purity X-chromosome bearing and Y-chromosome bearing populations of spermatozoa or sperm cells. High purity X-chromosome bearing and Y-chromosome bearing populations of spermatozoa can comprise populations of intact live spermatozoa, and may also comprise populations of
- 10 tailless spermatozoa (sperm nuclei), or populations of other viable or non-viable forms of spermatozoa, as may be desired. While particular examples are provided that describe the invention in the context of separating intact live sperm cells each having a sperm cell head, necks, and tail, it should be understood that the technologies described can have various applications with respect to sperm nuclei as well. X-chromosome bearing and Y-chromosome bearing populations of spermatozoa should further be understood to
- 15 encompass spermatozoa from any male of a species of mammal including, but not limited to, spermatozoa from humans and spermatozoa from commonly known animals such as bovids, equids, ovids, canids, felids, goats, or swine, as well as less commonly known animals such as elephants, zebra, camels, or kudu. This list of animals is intended to be exemplary of the great variety of animals from which spermatozoa can be routinely sorted
- 20 at 90% or greater purity, and is not intended to limit the description of the invention to the spermatozoa from any particular species of mammals.

High purity separated spermatozoa from the various species of mammals can be incorporated into products that can be used with artificial insemination protocols or as part of commercial business methods such as those as described in United States Patent

25 Application Nos. 60/211,093, 60/224,050, or Patent Cooperation Treaty Application No. US99/17165, or be used with low dose insemination protocols as described in Patent Cooperation Treaty Application No. US98/27909, or used in vitro fertilization of oocytes.

from animals, including humans, as described in United States Patent Application No 60/253,785, each of the above-mentioned references are hereby incorporated by reference

The use of the term purity or high purity should be understood to be the percent of the isolated spermatozoa population bearing a particular differentiating characteristic or
5 desired combination of characteristics. For example, where a population of spermatozoa are separated based upon bearing an X-chromosome as opposed to a Y-chromosome, a X-chromosome bearing population having 90% purity comprises a population of spermatozoa of which 90% of the individual spermatozoa bear an X-chromosome while 10% of such population of spermatozoa may bear a Y-chromosome. As such, high purity
10 with respect to X-chromosome bearing populations or Y-chromosome bearing populations can comprise a purity selected from the group consisting of between 90% to about 100%, between about 91% to about 100%, between about 92% to about 100%, between about 93% to about 100%, between about 94% to about 100%, between about 95% to about 100%, between about 96% to about 100%, between about 97% to about
15 100%, between about 98% to about 100%, between about 99% to about 100%

Importantly, while numerous embodiments of the invention describe isolated high purity X-chromosome and Y-chromosome bearing populations of spermatozoa, and while the description further discloses high purity spermatozoa separation devices and methods of how to isolate and how to use isolated high purity populations of spermatozoa, the
20 basic concepts of the invention should be understood to be applicable to other types of particles or events having particle differentiation characteristics or event differentiation characteristics. It should be understood that the invention can be applicable to a variety of circumstances in which resolving small differences in photogenerated signal may be necessary, such as product defect detection, field flow fractionation, liquid
25 chromatography, electrophoresis, computer tomography, gamma cameras, time of flight instruments, or the like as would be readily understood by those skilled in those arts

Moreover, while this disclosure provides descriptions of embodiments of apparatus and methods for flow separation of X-chromosome bearing spermatozoa from

Y-chromosome bearing spermatozoa, the description of these embodiments of the invention is not meant to reduce the scope of the invention to only flow separation of spermatozoa or only to high purity flow cytometer spermatozoa separation systems but rather these examples are intended to exemplify the basic concepts of the invention in a
5 practical manner so that they may be applied to the wide variety of applications

Now referring to Figures 1 and 2, a flow cytometer embodiment of the invention is shown which includes a particle or cell source (1) which acts to establish or supply particles or cells stained with at least one fluorochrome for analysis. The particles or cells are deposited within a nozzle (2) in a manner such that the particles or cells are introduced
10 into a fluid stream or sheath fluid (3). The sheath fluid (3) is usually supplied by some sheath fluid source (4) so that as the particle or cell source (1) supplies the particles or cells into the sheath fluid (4) they are concurrently fed through the nozzle (2).

In this manner it can be easily understood how the sheath fluid (3) forms a sheath fluid environment for the particles or cells. Since the various fluids are provided to the
15 flow cytometer at some pressure, they flow out of nozzle (2) and exit at the nozzle orifice (5). By providing some type of oscillator (6) which may be very precisely controlled through an oscillator control (7), pressure waves may be established within the nozzle (2) and transmitted to the fluids exiting the nozzle (2) at nozzle orifice (5). Since the oscillator (6) acts upon the sheath fluid (3), the stream (8) exiting the nozzle orifice (5)
20 eventually and regularly forms drops (9). Because the particles or cells are surrounded by the fluid stream or sheath fluid environment, the drops (9) may entrain within them individually isolated particles or cells, and can be sperm cells with respect to some embodiments of the invention.

Since the drops (9) can entrain particles or cells, the flow cytometer can be used to
25 separate particles, cells, sperm cells or the like based upon particle or cell characteristics. This is accomplished through a particle or cell sensing system (10). The particle or cell sensing system involves at least some type of detector or sensor (11) which responds to the particles or cells contained within fluid stream (8). The particle or cell sensing system

(10) may cause an action depending upon the relative presence or relative absence of a characteristic, such as fluorochrome bound to the particle or cell or the DNA within the cell that may be excited by an irradiation source such as a laser exciter (12) generating an irradiation beam to which the particle can be responsive. While each type of particle, cell, or the nuclear DNA of sperm cells may be stained with at least one type of fluorochrome different amounts of fluorochrome bind to each individual particle or cell based on the number of binding sites available to the particular type of fluorochrome used. With respect to spermatozoa, the availability of binding sites for Hoechst 33342 stain is dependant upon the amount of DNA contained within each spermatozoa. Because X-chromosome bearing spermatozoa contain more DNA than Y-chromosome bearing spermatozoa, the X-chromosome bearing spermatozoa can bind a greater amount of fluorochrome than Y-chromosome bearing spermatozoa. Thus, by measuring the fluorescence emitted by the bound fluorochrome upon excitation, it is possible to differentiate between X-bearing spermatozoa and Y-bearing spermatozoa.

15 In order to achieve separation and isolation based upon particle or cell characteristics, emitted light can be received by sensor (11) and fed to some type of separation discrimination system or analyzer (13) coupled to a droplet charger which differentially charges each droplet (9) based upon the characteristics of the particle or cell contained within that droplet (9). In this manner the separation discrimination system or analyzer (13) acts to permit the electrostatic deflection plates (14) to deflect drops (9) based on whether or not they contain the appropriate particle or cell.

As a result, the flow cytometer acts to separate the particle or cells (16) by causing them to be directed to one or more collection containers (15). For example, when the analyzer differentiates sperm cells based upon a sperm cell characteristic, the droplets entraining X-chromosome bearing spermatozoa can be charged positively and thus deflect in one direction, while the droplets entraining Y-chromosome bearing spermatozoa can be charged negatively and thus deflect the other way, and the wasted stream (that is droplets that do not entrain a particle or cell or entrain undesired or unsortable cells) can be left uncharged and thus is collected in an undeflected stream into a suction tube or the like as

discussed in United States Patent Application 09/001,394, hereby incorporated by reference herein. Naturally, numerous deflection trajectories can be established and collected simultaneously

To routinely separate particles, cells, sperm cells, or spermatozoa (intact, live, 5 fixed, viable, non-viable, or nuclei) into high purity X-chromosome bearing and Y-chromosome bearing populations, the particle differentiation apparatus or methods used must provide high resolution of the differentiation characteristics that are used as the basis of analysis and separation.

With respect to spermatozoa, differentiating between the light emitted by the 10 fluorochrome bound to the nuclear DNA of X-chromosome bearing sperm cells and the light emitted by the fluorochrome bound to the nuclear DNA of Y-chromosome bearing sperm cells may be difficult as discussed above.

In many applications, the total emitted light from photoemissive events incident to the detector, which can be a photomultiplier tube, can be high while the difference 15 between the emitted light of each photoemissive events to be differentiated can be small. The problem can be exacerbated when the photoemissive events happen serially at high rate of speed and the time period between photoemissive events is short, such as with high speed cell sorting using flow cytometers. When separating particles, cells, or sperm cells based upon the difference in bound fluorochrome the cells flow past an excitation 20 source and a high number of emissive events per second can be established. As a result, the amount of emitted light generated in the stream of particles, cells, or sperm cells, can be enormous. As the speed of the stream is increased, the intercept point with the excitation source becomes very bright. This high level of incident light upon the photocathode of the photomultiplier tube can cause a very low signal to background 25 signal ratio. The amount of background signal can be further exacerbated when fluorochrome such as Hoechst 33342 can be used to label the nuclear DNA of sperm cells.

Most solutions to the problem have focused on decreasing the total amount of light flux upon the photocathode tube by placing optical filters in front of the photomultiplier tube. This approach does not change the proportion of signal to background signal and subsequent attempts to increase the sensitivity of the photomultiplier tube generates additional background signal as the photomultiplier tube saturates from the amount of background signal.

Typically, photomultiplier tubes have an operation voltage range of about 400 volts to about 900 volts. The lower limit of linear operation of standard photomultiplier tubes, such as the R928 and R1477 photomultiplier tubes available from Hamamatsu Corporation, may be about 300 volts. As such, equipment or instruments which employ photomultiplier tubes are configured to operate such photomultiplier tubes at or above 400 volts. Even where reduction of the number of electrons at the anode is desired, as disclosed in United States Patent Nos. 4,501,366 and 5,880,457 the voltage between the photocathode and the first dynode is maintained at a high voltage and reduction of the electrons at the anode is accomplished by either decreasing the voltage to the remaining dynodes, or the inherent dark noise or shot noise is filtered out electromagnetically.

Unexpectedly, reducing the amount of voltage to the photomultiplier tube below 400 volts to about 280 volts, or about 250 volts, or even to 0 volts can allow small differences in photoemissive light to be differentiated even when the total light emitted from each photoemissive event is high, or even when there are a high number of bright serial events per second. With respect to the rate of photoemissive events generated from the irradiation of fluorochromes bound to the nuclear DNA of spermatozoa, the invention allows the rate of photoemissive events that can be achieved during separation of spermatozoa into X-chromosome bearing and Y-chromosome bearing populations to be increased to a separable event rate of at least 5000 separable events per second, at least 6000 separable events per second, at least 7000 separable events per second, at least 8000 separable events per second, at least 9000 separable events per second, at least 10,000 separable events per second, at least 11,000 separable events per second, at least 12,000 separable events per second, at least 13,000 separable events per second, at least 14,000

separable events per second, at least 15,000 separable events per second, at least 16,000
 separable events per second, at least 17,000 separable events per second, at least 18,000
 separable events per second, at least 19,000 separable events per second, at least 20,000
 separable events per second, at least 25,000 separable events per second, at least 30,000
 5 separable events per second, and at least 35,000 separable events per second, or greater

As a specific example, existing Cytomation SX MoFlo® sorting flow cytometers
 are configured to operate the photomultiplier tube at 400 volts minimum. The gain can be
 adjusted to operate the photomultiplier tube at higher voltages but not lower voltages. SX
 MoFlo® flow cytometers can be converted by reconfiguring the photomultiplier
 10 controllers. The R16C resistor (2.49 kilohms) on channel three can be replaced by a 2.0K
 resistor to alter the gain of the amplifier that controls the photomultiplier tube. This
 conversion allowed the photomultiplier tube to be operated at about 280 volts. Similar
 conversion of SX MoFlo® flow cytometers with two 3.75 kilohm resistors in parallel, or
 a 1.3 kilohm resistors can allow the photomultiplier tube to be operated at voltages of
 15 about 200 volts, or just above zero volts, respectively. Also with respect to this
 conversion, the neutral density filter in front of the photocathode can also be removed as a
 result of operating the photomultiplier tube outside of the typical operation voltage range.

This conversion unexpectedly increases the signal to noise ratio of the
 photoemissive event as it is translated to an electronic signal by the photomultiplier tube.
 20 The cleaner signal may then be amplified by increasing the gain amplifier to the analog to
 digital converter of the analyzer (13) to the appropriate level and output may be generated
 as univariate or bivariate histograms.

Now referring to Figure 3, a comparison of univariate histograms generated on
 three different SX MoFlo® flow cytometers (#1, #2, #3) prior to the use of the invention
 25 (Figure 1A), and using the invention (Figure 1B) with respect to the separation of intact
 live ejaculated bovine sperm are shown. As can be understood from the univariate
 histograms, the resolution (the apparent differentiation of the X-chromosome bearing
 population from the Y-chromosome bearing population represented by the valley between

peaks) of intact live X-chromosome bearing spermatozoa (17) from live Y-chromosome bearing spermatozoa (18) can be substantially improved by use of the invention

The mean separation rate or sort rates of intact live spermatozoa prior to use of this embodiment of the invention with the SX MoFlo® flow cytometers was about $1.79 \times 10^4 / 4.5$ hours of both X-chromosome bearing spermatozoa and Y-chromosome bearing spermatozoa (i.e. about 1,100 separations or sorts per second in each of two streams — the first stream X-chromosome bearing spermatozoa and the second stream Y-chromosome bearing spermatozoa) at about 87% purity with a range of 84% to 93% purity. The separable event rate was 22,000, 23,000, and 20,000 respectively for the three sorts

10 The mean sort rates of live spermatozoa after the above-mentioned conversion was about $4.03 \times 10^4 / 4.5$ hour sort (i.e. about 2,500 sorts per second per stream) at about 90.8% purity with a range of 89% to about 92%. The events per second were 13,000, 15,000, and 19,500 respectively for the three sorts

As can be understood from the data not only did this embodiment of the invention
15 result in increased purity of the separated spermatozoa populations but also allowed the separation rate or sort rate to be more than doubled while the separable events rate was actually decreased.

Similarly, referring now to Figures 4 and 5, which show bivariate histograms from sorting of intact live bull spermatozoa with the SX MoFlo® flow cytometer #1 prior
20 to using the invention (Figure 4) and after the above-mentioned conversion (Figure 5). Prior to using the invention, the SX MoFlo® flow cytometer was initially operated at 440 volts at the photocathode with the laser adjusted to 135 mW, a gain of 1X and with a neutral density filter of 1.0 (1/10th amplitude) at about 10,000 events per second. Upon
25 using the invention, the SX MoFlo® flow cytometer was operated at about 262 volts at the photocathode, with the laser adjusted to about 100mW, a gain of 4X, without the neutral density filter, at about 10,000 separable events per second. As can be understood from this data there is a large increase in resolution as evidenced by the increased depth of the

valley between the X-chromosome bearing population (19) and the Y-chromosome bearing population (20)

Similarly, referring now to Figures 6 and 7, which show bivariate histograms from sorting of intact live bull spermatozoa with the SX MoFlo[®] flow cytometer #2 before
5 using this embodiment of the invention (Figure 6) and upon using this embodiment of the invention (Figure 7) operated at the same parameters as shown in Figures 3 and 4 respectively. Again, there can be a large increase in resolution as evidenced by the depth of the valley between the X-chromosome bearing population (21) and the Y-chromosome bearing population (22)

10 Now referring to Figures 8 and 9, which show bivariate histograms from separation or sorting of intact live equine spermatozoa with the SX MoFlo[®] flow cytometer before using this embodiment of the invention (Figure 8) and upon using this embodiment of the invention (Figure 9). When using this embodiment of the invention, live equine spermatozoa were separated or sorted with the laser power at 100mW with the
15 photomultiplier tube voltage below 300 volts. The separation rates or sort rates exceeded 4,800 sorts per second average at 12,000 events per second. The increased resolution of the X-chromosome bearing population (23) and the Y-chromosome bearing population (24) is dramatic. The data shows that about 8 to about 9 channels separation can be achieved with this embodiment of the invention as compared to 5 channels of separation
20 between the peaks without the use of this embodiment of the invention. The purity of both the sorted X-chromosome bearing population and the sorted Y-chromosome bearing population was about 93%.

Now referring to Figure 10, which shows a univariate histogram and a bivariate dot plot from sorting of Hoechst 33342 stained stallion sperm nuclei (S-05400) separated
25 using this embodiment of the invention. The nuclei were prepared from freshly ejaculated stallion sperm. The sperm were washed by centrifugation, sonicated and the resultant heads and tails separated using Percoll density gradient centrifugation. The isolated heads were washed, fixed with 2% formalin and then stained with Hoechst 33342. The stained

nuclei were stabilized using sodium azide (0.5%). The sample was run at 5000 events per second to produce the histograms. The stained nuclei were then used to calibrate an SX MoFlo® flow cytometer was converted as above-mentioned to incorporate the photomultiplier tube embodiment of the invention. Compensation was used to level the two populations (X stained nuclei and Y stained nuclei) in the bivariate plot. Note that the two populations of equine sperm nuclei are nearly fully resolved to baseline as shown by the univariate plot.

Now referring to Figure 11, a modification specifically for SX MoFlo® flow cytometer includes the use of two resistors in parallel to provide the correct value of 1.8K. Two 3.57K resistors (25) and (26) are equal to about 1.785K which can be sufficiently close to the value to be effective. With this modification the photomultiplier tube on this particular instrument can then be run at about 200 volts. Naturally, a similar modification can be made to other flow cytometer instruments or other instruments which use a photomultiplier tube to measure the amount of light emitted from particular events. Figure 12, provides a electrical schematic diagram for this particular embodiment of the invention.

Another important embodiment of the invention can be a reduced height irradiation beam pattern optics. As shown by Figure 13A, conventional irradiation beam shaping optics generate a beam pattern (27) that can have a height can be greater than much greater than the height of the sperm cell head(s) (28) passing through it. As a result, more than a single sperm cell head containing fluorochrome bound DNA can enter the irradiation beam pattern at the same time. In that case, the fluorochrome(s) bound to the DNAs contained within the multiple sperm heads can be excited simultaneously and fluoresce within a single emissive event. As such, the prior or subsequent emissive event can include coincident light flux contributed from other sperm head(s) in the beam pattern (27). This results in a reduced difference in mean light flux between light emissive events which distinguish between X-chromosome bearing spermatozoa and Y-chromosome bearing spermatozoa. It can also decrease the difference in mean light flux between events that compare light emissions of X-chromosome bearing spermatozoa or

Y-chromosome bearing spermatozoa. Importantly, coincident excitation of fluorochrome bound to multiple DNAs increases the mean brightness of the events making the measurable difference in light flux between events an even smaller percentage of the total light flux emitted. This makes quantification of the differences between events even more
5 difficult

By reducing the height of the beam shape as shown by Figure 13B, the coincidence of multiple sperm heads being within the reduced height beam (29) pattern during the same measured event is reduced. This results in an increased mean difference between light emissive events which distinguish between X-chromosome bearing
10 spermatozoa and Y-chromosome bearing spermatozoa. It can also reduce the mean total light flux for each measured emissive event. For particular embodiments of the invention used for sorting bovine sperm which have a nucleus of about 9 μ m, it has been found that the height of the beam can be about 20 μ m. In this application, it has been found that vertical beam heights of less than 20 μ m did not provide an additional gain in resolution.

15 Referring to Figure 14, it can be understood that the use of reduced height irradiation beam pattern optics can improve the purity of sorted populations of X-chromosome bearing bovine spermatozoa (Figure 14A) and sorted populations of Y-chromosome bearing bovine sperm (Figure 14B) that have been stained with Hoechst 33342 stain. This is true for both 25% and 40% sort gates of the univariate peak. As can
20 be further understood from Figure 14, the reduced height beam pattern optics can improve purity of separated spermatozoa independent of any other aspect of the invention, such as modification of photomultiplier circuitry embodiment of the invention (new PMT) as described above, or can be used in conjunction with the modified photomultiplier embodiment of the invention to increase the purity of separated spermatozoa samples
25 even further

Another advantage of the reduced height beam pattern optics can be that the transit time of the spermatozoa in the excitation laser beam or irradiation beam can be reduced. A reduced amount of irradiation time within the excitation laser beam may

result in less stress or damage to the spermatozoa.

Again referring to Figure 14B, it can be understood that the reduced height beam pattern can be used in conjunction with a irradiation beam pattern having greater area than conventionally used. For example, conventional beam patterns (27), such as that shown 5 in Figure 14A, have an elliptical pattern of about 30 um X 80 um while the invention when used for sorting bovine sperm generates optimal resolution between X-chromosome bearing and Y-chromosome bearing populations when the beam has a 20 um X 160 um beam pattern (29). The 20 um X 160 um beam pattern has approximately 1.3 times the area of the 30 um X 80 um beam pattern. As such, there can be an inverse proportion in 10 loss of energy at the incident point. This makes it possible to increase the excitation laser power without concern for increasing the irradiation damage to the spermatozoa. For example, if an instrument has conventional beam shaping optics that produce a 30 um X 80um irradiation beam pattern and the excitation laser is conventionally powered at 150mW, then particular embodiments of the invention with a 20 um X 160 um beam 15 pattern can have an excitation laser powered at 300mW without increasing the total amount of power at the incident point. Alternately, the excitation laser can be run at 150mW to take advantage of the lower per unit area irradiation energy, decreased irradiation damage, longer laser life, and the like.

In comparison to conventional beam shaping optics and conventional 20 photomultiplier tube amplification devices, the reduced height beam pattern optics invention and the photomultiplier tube amplification invention can increase the purity of X-chromosome bearing and Y-chromosome bearing populations of spermatozoa by about 4%, or more.

The beam shaping optics invention (30) can be installed to a flow cytometer as 25 shown in Figures 15 and 16. As can be understood, the light emitted (31) by laser excitation of fluorochrome(s) bound to the DNA contained within spermatozoa can be detected by photomultiplier tubes (32) situated at 0 and 90 degrees relative to the flat surface of the sperm head (28) as it flows through the excitation laser beam pattern.

As can be understood, stained spermatozoa must be pumped through the excitation beam or irradiation beam in a precise manner so that each sperm head is oriented with the flat surface of the sperm head directed toward the photomultiplier tube that is the 0 degree detector. Accurate measurement of the DNA content of the spermatozoa can only be measured from the flat surface of the paddle-shaped sperm head(28). Thus, only that proportion of the spermatozoa that enter the excitation beam in the proper orientation can be measured accurately and sorted based upon DNA content.

Now referring to Figures 17, 18, and 19, particular embodiments of the invention can also have an particle or sperm cell orienting nozzle (33) that hydrodynamically forces the flattened sperm head into the proper orientation as they pass in front of the photomultiplier(s). As shown by Figure 17, the orienting nozzle has interior surfaces (34) that form a cone-like shape. The internal cone gradually changes from circular at the inlet end (35) into a highly elliptical shape near the orifice (36) where the stream exits the tip. The orifice (36) can be circular rather than elliptical. Thus, the internal aspect of the orienting nozzle (34) goes from a round entrance to a narrow ellipse to a round exit shortly before the orifice(36). This internal shape is further clarified by the cross sections of the orienting nozzle shown by Figure 18.

As shown by Figure 19 and 21, the injection tube (37)(which may be about 0.061 inches in diameter) can be used with the orientation nozzle (or with a conventional nozzle) (33) which can be beveled near the tip to form a blade (38). The flattened blade (38) can be oriented at an angle 90 degrees from the greatest dimension of the ellipse in the orientation nozzle (33). The internal diameter of the injection needle can be about 0.010 inch in diameter forming a rounded orifice (39) in the center of the flattened needle tube blade (38).

In particular embodiments of the beveled injection tube the beveled blade can be configured in the paddle shape illustrated by Figure 21. The paddle shaped beveled blade can assist in maintaining laminar flow of the sheath fluid within the nozzle (whether conventional nozzle or orienting nozzle). As such, the laminar flow of liquid maintained

by the paddle shaped beveled blade presents less disruption of the objects injected into it. Spermatozoa introduced into the laminar flow of sheath fluid maintained by an embodiment of the injector tube invention having the paddle shaped beveled blade allows for a 20%, 30%, 40%, 50% or even greater increase in spermatozoa sorting rates over
 5 conventional injection tube technology. High speed sorting of spermatozoa at rates of about 4,000 to about 10,000 sorts of each sex per second can be accomplished. High purity (90% or greater) of the X-chromosome bearing and Y-chromosome bearing populations can be established at even these high sort rates. The injector tube invention with the beveled paddle shaped tip can be used independently of or in combination with
 10 the other inventions described herein or other technology such as that described in United States Patent Application No. 09/454,488 or International Patent Application No. PCT/US00/42350, each hereby incorporated by reference.

As shown by Figure 21, certain embodiments of the beveled blade injector tube invention or beveled blade paddle shape invention can further include laminar flow
 15 enhancement grooves (40). The laminar flow enhancement grooves (40) assist in maintaining a laminar flow to the orifice of the injector tube. Again, the enhanced laminar flow allows for more spermatozoa to maintain the correct orientation in the laminar sheath fluid flow resulting in higher numbers of sortable event rates which in turn leads to higher sort rates for each sex or spermatozoa.

20 In another embodiment of the invention, the orienting nozzle orifice (39) or other conventional can be sized to form droplets which encapsulate intact live sperm as they exit the orifice (39). Encapsulation of the sperm cells does not occur in conventional sperm cell entrainment technology. Rather a portion of the sperm cell tail resides outside of the droplet. For example, bovine sperm cells have a length of about 13.5 microseconds
 25 when the fluid stream has a pressure of about 50 pounds per square inch (i.e. the length of time for the entire length of the sperm cell to pass through the irradiation beam at about 50 pounds per square inch fluid stream pressure). Conventional droplet formation techniques for entraining bovine sperm cells establish a 1.4 microsecond droplet (i.e. the time it takes to form a single droplet waveform in a fluid stream) from a nozzle having an

orifice with a diameter of about 70 micrometers which can be made responsive to an oscillator operated at about 35 kilohertz. As such, a portion of the sperm cell tail readily protrudes from the droplet. To prevent the sperm cell tail from protruding from the droplet, one embodiment of the droplet encapsulation invention provides an orifice of
5 about 100 micrometers that can produce a droplet of about 28 microseconds at about 50 pounds per square inch at about 30 kilohertz. By entirely encapsulating the intact live sperm cell, including the tail portion, the sperm cell interacts with the nozzle less upon charging of the droplet and the deflection of the droplet can be more accurate. This leads to less cross contamination of X-chromosome bearing sperm with Y-chromosome bearing
10 sperm and also allows deflected spermatozoa to be more uniformly collected. Sperm that are uniformly deflected can be directed to collection surfaces that are cushioned by various liquids. Cushioning the separated spermatozoa can be important in reducing stress as described in United States Patent Application No. 09/001,394, hereby incorporated by reference. With respect to spermatozoa from other species of mammals,
15 the invention can be varied to produce droplet sizes to encapsulate the varying lengths of sperm cells. Depending on the length of the spermatozoa and the pressure of the fluid stream the droplet encapsulation invention can still achieve droplet formation rates of at least 10,000 droplets per second, at least 20,000 droplets per second, at least 30,000 droplets per second, at least 40,000 droplets per second, at least 50,000 droplets per
20 second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80,000 droplets per second, at least 90,000 droplets per second, at least 100,000 droplets per second and so on up to about 200,000 droplets per second in some embodiments of the droplet encapsulation invention.

Even with the orienting nozzle invention there will be a certain number of
25 spermatozoa, or particles, which are not properly oriented in the beam pattern. As described above, if the orientation of a sperm head is not proper then the DNA content cannot be measured accurately based upon the emitted light. Particular embodiments of the present invention provide for the removal of undesired unoriented spermatozoa (RUUS) or particles within a fluid stream.

Referring now to Figures 16 and 20A, it can be understood that accurate measurement of the DNA content of a spermatozoa depends upon the flat surface of the paddle-shaped sperm head (28) being oriented properly with the detector. Thus, only that proportion of the spermatozoa that enter the excitation beam in the proper orientation as shown by Figures 16 and 20A can be measured accurately and sorted in to X-chromosome bearing and Y-chromosome bearing populations based upon DNA content. As shown by Figures 20A and 20B, spermatozoa which transit through the excitation beam in proper orientation generate an oriented emission signal plot (40) that can be shaped differently than the unoriented emission signal plot (41) that is generated by unoriented spermatozoa shown by Figure 20D. Naturally, the shape of the unoriented emission signal plot (41) generated by unoriented spermatozoa will vary depending on the degree of improper orientation in the excitation beam. These improper orientations can include the orientation shown in Figure 20C but can also include all manner of orientations that rotate the sperm head any portion of a rotation that orients the surface of the paddle-shaped head out of alignment with the detector (proper alignment shown by Figure 16), or any portion of a rotation that orients the axis of the sperm head (42) out of alignment with the direction of flow. Naturally, proper orientation may be defined differently from species to species. For some species, in which the sperm head is not paddle-shaped, the proper orientation within the excitation beam, or relative to the detectors or otherwise, may be defined by other anatomical characteristics or signal characteristics. Nonetheless, an optimized signal for the properly oriented spermatozoa of various species within the excitation window can be generated as the standard emission signal plots for subsequent comparison with serial emission events.

By comparing the shape (or the integrated area or both) of each emission signal plot with the standard emission signal plot (or standard integrated area or both) established for an oriented spermatozoa of a species of mammal, unoriented sperm can be identified, the signal subtracted from univariate or bivariate histograms, and the unoriented sperm can be affirmatively removed, if desired, so that unoriented sperm are not collected into either the X-chromosome bearing population or the Y-chromosome bearing population.

Importantly, as the invention(s) improve(s) resolution between the two spermatozoa populations being separated which increase the rate at which the populations can be separated from one another, and improves the purity of the populations that are separated. As such, it is now possible to sort spermatozoa at remarkably high speeds

5 Sortable or separable event rates can be as high as about 35,000 per second (not including coincident events – multiple spermatozoa within the excitation/detection window at the same time) Sortable or separable event rates correlate with high separation or sort rates which can be about 5000 to about 11,000 intact live sperm of each sex per second with a purity of 90%, 92%, 93%, 95%, or greater. The above-described

10 inventions also allow for even higher purity X-chromosome bearing and Y-chromosome bearing populations to be obtained of about 97% to about 98% or even higher by reducing the sort or separation rates to around 2000 live sperm of each sex per second.

As can be understood, the above inventions described are particularly important in achieving the highest possible sortable or separable event rates and highest possible

15 resulting separation rates which can be at least 1,000 separations per second, at least 2,000 separations per second, at least 3,000 separations per second, at least 4,000 separations per second, at least 5,000 separations per second, at least 6,000 separations per second, at least 7,000 separations per second, at least 8,000 separations per second, at least 9,000 separations per second, or at least 10,000 separations per second of each sex

20 per second, or greater

The invention allows for high speed sorting, as set forth above, of spermatozoa even when they are difficult to stain, or have other anatomical or chemical features, that make differentiation between the X-bearing chromosome and Y-bearing chromosome populations more difficult. Even in these difficult cases, high purity X-chromosome

25 bearing and Y-chromosome bearing populations of bovine spermatozoa can be isolated at high purity of 92% to 93% by achieving sortable event rates of about 15,000 - 20,000 sortable events per second or higher as described above, and sort or separation rates of intact live spermatozoa of each sex (X-chromosome bearing and Y-chromosome bearing) of 2000 intact live sperm of each sex per second

37

Now referring to Figures 23 and 24, an embodiment of the invention utilizes differential interference contrast technology to measure the volume of a particle or capsule. A basic embodiment of the invention can comprise particles that have a difference volume, such as sperm cell heads (28) that have a difference in volume between X-chromosome bearing and Y-chromosome bearing sperm cells. An electromagnetic radiation source (43) generates electromagnetic radiation or a beam of electromagnetic radiation (44) having initial waveform characteristics differentially responsive to the difference in volume between the particles or sperm cell heads (28). The electromagnetic radiation which can be laser light, but could also be numerous types of electromagnetic radiation including, but not limited to, microwave radiation, ultraviolet radiation, or the like. Upon traversing the particle or capsule or sperm head volume containing phase shifting material the electromagnetic radiation can be focused through an objective lens (45) onto a detector (46) responsive to the waveform characteristics of the electromagnetic radiation. The detector can be coupled to an analyzer (47). The analyzer can differentiate between particles based on the change in the waveform characteristics prior to traversing the volume of the particle and after traversing the volume of the particle and can analyze the signal based on integrated areas or signal shape or both. In certain embodiments the invention analyzing waveform characteristics can comprise superimposing initial waveform characteristics with altered waveform characteristics upon traversing the volume of the particle, capsule, or sperm cell head. Superimposing the initial waveform characteristics and the phase shifted waveform characteristic can differentially modulate the intensity of the beam of electromagnetic radiation in manner that correlates to the amount of phase shift media the electromagnetic radiation traverses. The invention may also include additional filters (48), such as color filters.

Now referring to Figure 24, an embodiment of the optics invention involves using differential interference contrast optics that increase the actual distance over which the light is split up compared to conventional DIC microscopy which corresponds to the resolution limit of the microscope. In this embodiment of the invention, the induced split is larger than the size of the objects, thus giving rise to two individual images, separated

laterally, originating from one object. The second modification involves using plates of birefringent material, such as Savart plates, at a location away from the objective lens. This embodiment of the invention is easier to construct since the birefringent materials do not have to be located inside the objective housing. In conventional DIC microscopes the
5 birefringent material is used in the form of so-called Wollaston prisms, that have to be located inside the objective housing, making it necessary to use expensive objective lenses that have been manufactured specifically for this purpose.

Components of an embodiment of the invention may be arranged in line with each other and consist of a source of electromagnetic radiation (43), for example, a Mercury
10 arc lamp, a spectral adjustment element, for example, a bandpass filter, a polarization adjustment element (49), for example, a sheet polarizer (53) and a waveplate (54) responsive to a rotatable mount, a light condenser (51) allowing the light to be condensed onto the particle or sperm cell, for example, a condenser lens, or set of lenses, or microscope objective, a fluid stream (8) that may contain particles or sperm cells (28),
15 for example a fluid jet ejected under pressure, a light collector (45) to collect the light from the particle or cell, for example a 50x high working distance microscope objective and a tube lens, a beam splitter (50) to split up the beam into two, or more, components, for example, a piece of birefringent material in the form of a Savart plate, mounted in such a way that its orientation and location can be controlled accurately; image light
20 selector (55) to select only the light corresponding to the particle or sperm cell, for instance a set of pinholes, one pinhole (53) for each of the images formed

In one embodiment of the invention, the components may be arranged in such a way that the light source (43) or its image are located at the back focal plane of the light condenser (45) often referred to as Köhler type illumination. The image of the object
25 plane may best coincide with the object light selector (55) or pinhole(s) (53), in order to capture the light from individual particles or sperm cells. As shown in Figures 27 and 28, components can be mounted on a sturdy optical table, or bench, using mountings, posts, and holders. Components can be mounted in such a way that focusing of the object plane can be done accurately. This can be done by equipping the fluid stream with a stream

39

position controller, such as micrometers, in order to turn the stream in and out of focus. In addition it may be necessary to equip the light condenser (51) with a light condenser position controller (61) allowing it to be focused onto the object plane. It may be necessary to take special care about the mounting of the birefringent elements or beam splitter (50), a three axis rotation element may be preferable.

Now referring to Figure 25, embodiments of the present invention may also include the use of both generated images, in order to determine the orientation of a asymmetrical particles the fluid stream, including, but not limited to, spermatozoa such as bull sperm cells. An orientation assessment embodiments of the invention can include an optical system that allows for control of the polarization state of the light entering the system for both generated images independently. The interference optics invention may further provide polarization adjustment element (56) that controls the polarization state of light entering the system. For the orientation detection invention the polarization adjustment element (56) may be selected in such a way that it consists of two parts, that are imaged onto image light selector (55) that in one embodiment of the invention contains the pinholes (53). This can be accomplished by locating the polarization adjustment element (56) in the conjugate plane of the image plane (55), or by using other optics, to accomplish the same thing. A simple example of this component may be a 'half-shade' piece, for instance consisting of two hemi-circular parts of polarizing material, such as sheet-polarizer, the orientation angle of which may be chosen independently. Each pinhole in the image plane can fall in one of the halves of said hemisphere. The polarization angles can be chosen in such a way that the signal of one pinhole (53) corresponds to the volume, and is relatively independent from the orientation angle of the passing object, and the other pinhole (53) has a signal that depends, to a great degree, upon this orientation angle. The two signals may be processed by analyzer (47) in a manner similar to a conventional multi-channel flow cytometry, as but one example. With respect to this example, bivariate dotplots can be made, and also allow the user to select windows on this plot.

An improvement of the 'half-shade' piece described above may be the

construction shown by Figure 25D. The same said two hemispherical parts are projected onto the image plane but the way they are generated is different. A mirror (57) breaks up the light (44) into the hemi-circular parts, and recombines them back to back. Each of the halves traverses a separate means to control its polarization state. An advantage of this embodiment is that the polarization angles can be controlled continuously and independently, thus facilitating the adjustment of the set-up. Materials used in this embodiment can be supplied by standard optical supply firms, and can be mounted in the set-up using similar mounting materials as used for the interferometric optics.

Now referring to Figure 26, in order to correct for artifacts introduced by having light pass through a non-flat region of transparent material, such as a substantially cylindrical fluid stream but including other geometries as well, embodiments of the present invention disclose the incorporation of a component similar in shape to the non-flat region, but opposite in terms of relative refractive indices. In the specific case of a flow cytometer this shape approximates a cylinder. To correct for artifacts introduced by the fact that the objects to be assessed are located within a cylindrical stream of water, is the incorporation of an optical component (58) which can be in the shape of a transparent cylinder, located inside transparent material (59) of a higher refractive index. It may be preferred that the image of the stream and of the compensation element fall on top of each other in the image plane. This can be done by locating the compensation element between the objective lens and the image plane, and by incorporating auxiliary lenses.

An embodiment of the optical component (58) can be located within a thin slice of transparent material of higher refractive index (59), for instance glass, or perspex, with a cylindrical hole drilled across it. Perspex has the advantage that it can be easier to drill a round channel into it. The cylindrical hole may be filled by a transparent material, the refractive index of which is lower than that of the surrounding material. The difference in refractive index between the substance and the surrounding material can be the same as but opposite to the difference in refractive index between the water in the stream and the surrounding air for certain applications. It may not be necessary to have the cylinder the same size as the stream of water, as long as magnification by the lenses used, makes the

41

resulting images in the image plane the same size. In some applications, it may be desired or necessary to adjust the refractive index difference to compensate for this magnification. Manufacturing of such element out of perspex can be quite simple, and can be done by most mechanical workshops that have experience with machining perspex or the selected material. It may be made in such dimensions that it fits in a standard optical mounting hardware, to facilitate incorporation into the optics.

Exactly matching the refractive indices may be difficult. An embodiment of the invention that facilitates adjustment can be to make the substance inside the perspex, or other selected material, a transparent refractive index fluid (58), as but one example, an organic oil, or mixture of oils that have a refractive index close to the desired one. Due to the fact that the refractive index of most fluids changes with temperature, much more so than solids, or glasses, it may be possible to fine-tune the difference in refractive index by temperature. This may be done by incorporating a temperature controller (60).

Optical component (58) of transparent fluids or refractive index fluids can be supplied by chemical supply firms. These firms often have data regarding the refractive index of their fluids readily available. Some firms even offer fluids that are specially made to serve as refractive index fluids, and have a guaranteed and stable refractive index. Temperature controllers and thermostats are supplied by many firms. A practical way to apply heat to the refractive index fluid can be to use a hollow mounting made of heat conducting material, a metal as but one example, containing the refractive index fluid. Using a conventional immersion thermostat cycler, found in many laboratories, water can be pumped through the mounting, thus keeping the element at a fixed and controllable temperature.

The discussion included in this PCT application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible, many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of

42

alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in functionally-oriented terminology, each aspect of the function is accomplished by a device, subroutine, or program. Apparatus claims may not only be included for the devices described, but also method or process claims may be
5 included to address the functions the invention and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims which now be included.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. This disclosure should be understood to encompass
10 each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms – even if only the function or result is the same. Such equivalent, broader,
15 or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed
20 should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a “droplet separator” should be understood to encompass disclosure of the act of “separating droplets” – whether explicitly discussed or not – and, conversely, were there only disclosure of the act of “converting liquid-gas”, such a disclosure should be understood to
25 encompass disclosure of a “droplet separator” and even a means for “separating droplets”. Such changes and alternative terms are to be understood to be explicitly included in the description.

Additionally, the various combinations and permutations of all elements or applications can be created and presented. All can be done to optimize the design or

performance in a specific application

Any acts of law, statutes, regulations, or rules mentioned in this application for patent, or patents, publications, or other references mentioned in this application for patent are hereby incorporated by reference. Specifically, United States Patent

- 5 Application Nos 60/267571, 60/239,752, and 60/203,089 are each hereby incorporated by reference herein including any figures or attachments, and each of references in the following table of references are hereby incorporated by reference.

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	Woods, J and Glutcher, O.J., "Recent studies related to the collection of multiple embryos in mares" Theriogenology 19 101 - 108 (1983)
	Woods, J, et al, "Effects of time of insemination relative to ovulation on pregnancy rate and embryonic-loss rate in mares" Eq. Vet. J 22(6):410-415 (1990)
35	XP-002103478, File Biotis, (1988), one page

In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with such interpretation, common dictionary definitions

56

should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary, second edition are hereby incorporated by reference. However, as to each of the above, to the extent that such information or statements incorporated by reference might be

5 considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s)

In addition, unless the context requires otherwise, it should be understood that the term "comprise" or variations such as "comprises" or "comprising", are intended to imply the inclusion of a stated element or step or group of elements or steps but not the

10 exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible in countries such as Australia and the like

Thus, the applicant(s) should be understood to have support to claim at least i) each of the liquid to gas conversion devices described herein, ii) the related methods

15 disclosed and described, iii) similar, equivalent, and even implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and

20 independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, ix) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, and the x) the various combinations and permutations of each of the elements disclosed

25 In addition, unless the context requires otherwise, it should be understood that the term "comprise" or variations such as "comprises" or "comprising", are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should

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be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible in countries such as Australia and the like

The claims set forth in this specification by are hereby incorporated by reference as part of this description of the invention, and the applicant expressly reserves the right
5 to use all of or a portion of such incorporated content of such claims as additional description to support any of or all of the claims or any element or component thereof, and the applicant further expressly reserves the right to move any portion of or all of the incorporated content of such claims or any element or component thereof from the description into the claims or vice-versa as necessary to define the matter for which
10 protection is sought by this application or by any subsequent continuation, division, or continuation-in-part application thereof, or to obtain any benefit of, reduction in fees pursuant to, or to comply with the patent laws, rules, or regulations of any country or treaty, and such content incorporated by reference shall survive during the entire
15 pendency of this application including any subsequent continuation, division, or continuation-in-part application thereof or any reissue or extension thereon.

58

VI CLAIMS

We claim

- 1 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells, comprising the steps of
- 5 a. collecting sperm cells from a male of a species of mammal,
- b. determining a sex differentiation characteristic of said sperm cells,
- c. differentiating between said sperm cells based upon said sex differentiation characteristic,
- d. separating differentiated sperm cells into a X-chromosome bearing population and a Y-chromosome bearing population, and
- 10 e. producing separate X-chromosome bearing and Y-chromosome bearing populations of sperm cells each having a purity of greater than 90%
- 2 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 1, wherein said step of differentiating
- 15 between said sperm cells based upon said sex differentiation characteristic comprises assessing an amount of DNA within a nucleus of said sperm cells
- 3 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 1, wherein said step of differentiating
- between said sperm cells based upon said sex differentiation characteristic
- 20 comprises assessing a volume of a capsule containing said amount of DNA.
- 4 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 3, wherein said step of assessing said
- volume of said capsule containing said amount of DNA comprises determining a
- volume of a nucleus within said sperm cells /
- 25 5 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 3, wherein said step of assessing said

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volume of a capsule containing said DNA comprises determining the volume of a sperm cell head.

- 6 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claim 5, wherein said step of assessing said
5 volume of said capsule containing said amount of DNA further comprises the
steps of
- a. generating a beam of electromagnetic radiation having initial wave form
characteristics,
 - b. traversing said volume of said capsule containing said amount of DNA
10 with said beam of electromagnetic radiation,
 - c. altering said initial wave form characteristics by traversing said volume of
said capsule, and
 - d. analyzing altered wave form characteristics.
- 7 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
15 bearing sperm cells as described in claim 6, wherein said step of altering said
initial wave form characteristics by traversing said volume of said capsule
comprises shifting phase of said initial wave form of said beam of electromagnetic
radiation.
- 8 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
20 bearing sperm cells as described in claim 6, further comprising the step of
superimposing said beam of electromagnetic radiation having shifted phase on
said beam of electromagnetic radiation having said initial wave form
characteristics
- 9 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
25 bearing sperm cells as described in claim 8, further comprising the step of
comparing electromagnetic radiation intensity of superimposed beams of
electromagnetic radiation with intensity of said beam of electromagnetic radiation

60

having initial wave form characteristics

- 10 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claim 9, further comprising the step of
determining said volume of said capsule containing said amount of DNA based
5 upon a difference in said light intensity between said superimposed beams of
electromagnetic radiation and said beam of electromagnetic radiation having
initial waveform characteristics
- 11 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claim 2, further comprising the steps of
- 10 a. introducing said sperm cells into a fluid stream,
b. analyzing said sperm cells entrained in said fluid stream,
c. forming droplets a plurality having one of said sperm cells entrained,
d. charging each of said droplets differentially based upon said sex
differentiation characteristic of said sperm cells entrained in said droplets,
- 15 e. deflecting each of said droplets, and
f. differentially collecting each of said droplets based upon said sex
differentiation characteristic of said sperm cells entrained in said droplets
- 12 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claim 11, further comprising the step of
20 maintaining said amount of DNA within said nucleus of said sperm cell unstained.
- 13 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claim 11, further comprising the step of
staining said amount of DNA within said nucleus of said sperm cell.
- 14 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
25 bearing sperm cells as described in claim 13, further comprising the step of
irradiating stained DNA within said nucleus of said sperm cell

- 15 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 14, further comprising the step of detecting fluorescent light emitted from irradiated stained DNA within the nucleus of said sperm cell
- 5 16 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 15, wherein said step of detecting fluorescent light from irradiated stained DNA within said nucleus of said sperm cell comprises generating a signal with a photomultiplier tube.
- 17 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 16, further comprising the step of operating said photomultiplier tube outside a typical operation voltage range.
- 10 18 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 14, wherein said step of irradiating stained DNA within the nucleus of said sperm cell further comprises generating an irradiation beam pattern having a reduced height.
- 15 19 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 18, wherein said reduced height comprises a height about equal to the length of said sperm cells along the longitudinal axis to about three times the length of said sperm cells along the longitudinal axis
- 20 20 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 19, wherein said sperm cell has a longitudinal axis of about nine micrometers in length, and said height of said irradiation beam pattern is about 20 micrometers
- 25 21 A method of isolating X-chromosome bearing sperm cells and Y-chromosome

bearing sperm cells as described in claim 18, wherein said step of forming droplets comprises forming droplets having sufficient size to encapsulate said sperm cell.

22 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
5 bearing sperm cells as described in claim 21, further comprising the step of
ejecting said fluid stream from a nozzle having an orifice, wherein said orifice has
a diameter of 70 micrometers

23 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claim 22, further comprising the steps of
10 a. orienting said sperm cells with respect to a detector;
b. detecting light having characteristics differentially responsive to sperm cell
orientation to said detector;
c. converting said light having characteristics differentially responsive to said
sperm cell orientation into at least one signal containing sperm cell
15 orientation information, and
d. determining orientation of said sperm cell with respect to said detector

24 a method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claim 23, further comprising the step of
20 differentially collecting said droplets based upon determined orientation of said
sperm cell with respect to said detector

25 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claims 1, 6, 12, 13, 17, 18, 21, or 23, wherein
said purity of said X-chromosome bearing and said Y-chromosome bearing
25 populations of sperm cells is selected from the group consisting of between 90%
to about 100%, between about 91% to about 100%, between about 92% to about
100%, between about 93% to about 100%, between about 94% to about 100%,
between about 95% to about 100%, between about 96% to about 100%, between

67
63

about 97% to about 100%, between about 98% to about 100%, between about 99% to about 100%

- 26 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claims 1, 6, 12, 13, 17, 18, 21, or 23, further
5 comprising the step of establishing a separable event rate from the group
consisting of at least 5000 separable events per second, at least 6000 separable
events per second, at least 7000 separable events per second, at least 8000
separable events per second, at least 9000 separable events per second, at least
10,000 separable events per second, at least 11,000 separable events per second, at
10 least 12,000 separable events per second, at least 13,000 separable events per
second, at least 14,000 separable events per second, at least 15,000 separable
events per second, at least 16,000 separable events per second, at least 17,000
separable events per second, at least 18,000 separable events per second, at least
19,000 separable events per second, at least 20,000 separable events per second, at
15 least 21,000 separable events per second.
- 27 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claims 1, 6, 12, 13, 18, 21, or 23, wherein said
step of separating said sperm cells comprises a separation rate selected from the
group consisting of at least 500 separations per second, at least 1,000 separations
20 per second, at least 2,000 separations per second, at least 3,000 separations per
second, at least 4,000 separations per second, at least 5,000 separations per
second, at least 6,000 separations per second, at least 7,000 separations per
second, at least 8,000 separations per second, at least 9,000 separations per
second, at least 10,000 separations per second.
- 25 28 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
bearing sperm cells as described in claims 1, 6, 12, 13, 18, 21, or 23, wherein said
step of forming droplets each having one of said sperm cells entrained comprises a
droplet formation rate selected from the group consisting of at least 10,000

64

droplets per second, at least 20,000 droplets per second, at least 30,000 droplets per second, at least 40,000 droplets per second, at least 50,000 droplets per second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80,000 droplets per second, at least 90,000 droplets per second, at least
5 100,000 droplets per second.

29 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 1, wherein said species of mammal comprises a bovine mammal.

30 A method of isolating X-chromosome bearing sperm cells and Y-chromosome
10 bearing sperm cells as described in claim 1, wherein said species of mammal comprises an equine mammal.

31 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 1, wherein said species of mammal comprises an ovine mammal.

15 32 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells, comprising
a sperm cells having a sperm heads, wherein said sperm heads have a difference in volume between X-chromosome bearing sperms cells and Y-chromosome bearing sperm cells,
20 b a beam of electromagnetic radiation having mutual waveform characteristics differentially responsive to said difference in volume between X-chromosome bearing sperms cells and Y-chromosome bearing sperm cells,
c a detector responsive to altered waveform characteristics of said beam of
25 electromagnetic radiation,
d an analyzer coupled to said detector, wherein said analyzer differentiates between said difference in volume between said X-chromosome bearing

86
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sperms cells and said Y-chromosome bearing sperm cells based upon said altered waveform characteristics

- 33 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 32, further comprising a fluid stream
5 into which said sperm cells are introduced.
- 34 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 33, further comprising droplets breaking off from said fluid stream a plurality having one of said sperm cells entrained.
- 10 35 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 34, wherein said droplets breaking off from said fluid stream have sufficient size to encapsulate said one of said sperm cells
- 36 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 35, further comprising a nozzle having
15 an orifice of about 100 micrometers in diameter
- 37 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 36, further comprising a droplet charger coupled to said analyzer, wherein said droplets receive a charge differentially
20 based upon said difference in volume between X-chromosome bearing sperms cells and Y-chromosome bearing sperm cells
- 38 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 37, further comprising a droplet separator, wherein said droplet separator separates said droplet based upon charge
25 of said droplet.

C6

- 39 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 38, further comprising at least one collection container in which droplets containing said X-chromosome bearing sperm cells are collected as an X-chromosome bearing population.
- 5 40 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 38, further comprising at least one collection container in which droplets containing Y-chromosome bearing sperm cells are collected as a Y-chromosome bearing population.
- 41 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claims 38 or 39, wherein said X-chromosome bearing population and said Y-chromosome bearing population of said sperm cells have a purity selected from the group consisting of between 90% to about 100%, between about 91% to about 100%, between about 92% to about 100%, between about 93% to about 100%, between about 94% to about 100%, between about 95% to about 100%, between about 96% to about 100%, between about 97% to about 100%, between about 98% to about 100%, between about 99% to about 100%
- 10 42 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 41, further comprising a separable event rate wherein said separable event rate is selected from the group consisting of at least 5000 separable events per second, at least 6000 separable events per second, at least 7000 separable events per second, at least 8000 separable events per second, at least 9000 separable events per second, at least 10,000 separable events per second, at least 11,000 separable events per second, at least 12,000 separable events per second, at least 13,000 separable events per second, at least 14,000 separable events per second, at least 15,000 separable events per second, at least 16,000 separable events per second, at least 17,000 separable events per second, at least 18,000 separable events per second, at least 19,000 separable events per
- 15 20 25

67

second, at least 20,000 separable events per second, at least 21,000 separable events per second.

- 43 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 42, further comprising a separation rate
5 selected from the group consisting of at least 500 separations per second, at least 1,000 separations per second, at least 2,000 separations per second, at least 3,000 separations per second, at least 4,000 separations per second, at least 5,000 separations per second, at least 6,000 separations per second, at least 7,000 separations per second, at least 8,000 separations per second, at least 9,000
10 separations per second, at least 10,000 separations per second, 11,000 separations per second.
- 44 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 43, further comprising a droplet formation rate selected from the group consisting of at least 10,000 droplets per
15 second, at least 20,000 droplets per second, at least 30,000 droplets per second, at least 40,000 droplets per second, at least 50,000 droplets per second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80,000 droplets per second, at least 90,000 droplets per second, at least 100,000 droplets per second.
- 20 45 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 44, wherein said detector comprises at least one photomultiplier tube, and wherein said at least one photomultiplier tube converts said electromagnetic radiation into at least one signal, and wherein said photomultiplier tube has a typical operation voltage range, and wherein said
25 photomultiplier tube is operated outside said typical operation voltage range
- 46 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 45, wherein said typical operation

voltage range of said photomultiplier tube is from about 400 volts to about 999 volts

- 47 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 46, wherein said photomultiplier tube is operated in a range from about 0 volts to about 300 volts.
- 5
- 48 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 41, wherein said species of mammal comprises a bovine mammal.
- 49 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 41, wherein said species of mammal comprises an equine mammal.
- 10
- 50 An apparatus to isolate X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 41, wherein said species of mammal comprises an ovine mammal.
- 15 51 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells, comprising the steps of
- a. collecting sperm cells from a male of a species of mammal,
- a. assessing a volume of a capsule containing the nuclear DNA of said sperm cells,
- 20 c. differentiating between said X-chromosome bearing sperm cells and said Y-chromosome bearing sperm cells based upon said volume of said capsule containing said nuclear DNA of said sperm cells,
- 52 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 51, wherein said step of assessing the volume of a capsule containing said DNA comprises determining a phase shift in
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69

said electromagnetic radiation.

- 53 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 52, wherein said step of assessing the volume of a capsule containing said DNA comprises
- 5 a. generating a beam electromagnetic radiation having initial waveform characteristics,
- b. traversing said volume of said capsule containing said DNA with said beam of electromagnetic radiation having initial waveform characteristics,
- c. altering said initial waveform characteristics by traversing said volume of said capsule; and
- 10 d. analyzing altered wave form characteristics of said beam of electromagnetic radiation.
- 54 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 53, wherein said step of altering said initial wave form characteristics by traversing said volume of said capsule
- 15 comprises shifting phase of said initial waveform characteristics of said beam of electromagnetic radiation.
- 55 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 54, further comprising the step of superimposing said initial waveform characteristics and phase shifted waveform characteristics
- 20
- 56 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 55, further comprising the step of comparing an intensity of said initial waveform characteristics with superimposed waveform characteristics
- 25
- 57 A method of isolating X-chromosome bearing sperm cells and Y-chromosome

bearing sperm cells as described in claim 56, further comprising the step of determining said volume of said capsule containing said DNA based upon the difference in said intensity between said superimposed waveform characteristics and said initial waveform characteristics

- 5 58 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 57, further comprising the steps of
- a. introducing said sperm cells into a fluid stream,
 - b. forming droplets a plurality having one of said sperm cells entrained,
 - c. charging each of said droplets differentially based upon the assessed
 - 10 volume of said capsule containing the nuclear DNA of said sperm cells,
 - d. deflecting each of said droplets,
 - e. differentially collecting each of said droplets based upon said volume of said capsule containing said DNA, and
 - f. generating X-chromosome bearing and Y-chromosome bearing
 - 15 populations of said sperm cells
- 59 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 58, further comprising the step of maintaining said amount of DNA within the nucleus of said sperm cells unstained.
- 60 a method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 59, further comprising the step of
- 20 forming droplets having sufficient size to encapsulate said sperm cells.
- 61 a method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 60, further comprises the step of ejecting said fluid stream from a nozzle having an orifice, wherein said orifice has a
- 25 diameter of 100 micrometers
- 62 a method of isolating X-chromosome bearing sperm cells and Y-chromosome


71

bearing sperm cells as described in claim 58, further comprises the steps of

- a. detecting a signal having sperm cell orientation characteristics,
- b. comparing said signal having sperm cell orientation characteristics to a signal having oriented sperm cell characteristics,
- 5 c. determining said sperm cell has unoriented sperm cell characteristics, and
- d. differentially collecting said sperm cell having unoriented sperm cell characteristics.

63 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claims 58 or 62, wherein purity of said X-chromosome bearing and said Y-chromosome bearing populations of said sperm cells is selected from the group consisting of between 90% to about 100%,
10 between about 91% to about 100%, between about 92% to about 100%, between about 93% to about 100%, between about 94% to about 100%, between about 95% to about 100%, between about 96% to about 100%, between about 97% to about 100%,
15 between about 98% to about 100%, between about 99% to about 100%

64 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 63, further comprising the step of establishing a separable event rate wherein said separable event rate is selected
20 from the group consisting of at least 5000 separable events per second, at least 6000 separable events per second, at least 7000 separable events per second, at least 8000 separable events per second, at least 9000 separable events per second, at least 10,000 separable events per second, at least 11,000 separable events per second, at least 12,000 separable events per second, at least 13,000 separable
25 events per second, at least 14,000 separable events per second, at least 15,000 separable events per second, at least 16,000 separable events per second, at least 17,000 separable events per second, at least 18,000 separable events per second, at least 19,000 separable events per second, at least 20,000 separable events per second, at least 21,000 separable events per second

70
72

- 65 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 64, wherein said step of separating said sperm cells comprises a separation rate selected from the group consisting of at least 500 separations per second, at least 1,000 separations per second, at least 2,000 separations per second, at least 3,000 separations per second, at least 4,000 separations per second, at least 5,000 separations per second, at least 6,000 separations per second, at least 7,000 separations per second, at least 8,000 separations per second, at least 9,000 separations per second, at least 10,000 separations per second.
- 10 66 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 65, wherein said step of forming droplets each having one of said sperm cells entrained comprises a droplet formation rate selected from the group consisting of at least 10,000 droplets per second, at least 20,000 droplets per second, at least 30,000 droplets per second, at least 40,000 droplets per second, at least 50,000 droplets per second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80 000 droplets per second, at least 90,000 droplets per second, at least 100,000 droplets per second.
- 15 67 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 51, wherein said species of mammal comprises a bovine mammal
- 20 68 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 51, wherein said species of mammal comprises an equine mammal.
- 25 69 A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 51, wherein said species of mammal comprises an ovine mammal.

77
73

70 A method differentiating particles, comprising the steps of

a. collecting asymmetric particles,

b. establishing said fluid stream with a flow cytometer,

c. introducing said asymmetric particles into said fluid stream,

5 d. orienting said asymmetric particles with respect to a detector,

e. detecting light having characteristics differentially responsive to asymmetric particle orientation to said detector,

g. converting said light having characteristics differentially responsive to asymmetric particle orientation into at least one signal containing

10 asymmetric particle orientation information,

h. analyzing asymmetric particle orientation information, and

i. determining orientation of said asymmetric particles with respect to said detector

71 A method differentiating particles as described in claim 70, wherein said fluid

15 stream comprises a sheath fluid

72 A method differentiating particles as described in claim 70, wherein said detector comprises a photomultiplier tube.

73 A method differentiating particles as described in claim 72, wherein said light comprises florescent light.

20 74 A method differentiating particles as described in claim 73, wherein said florescent light emits from a light emission material bound to said asymmetric particles.

75 A method differentiating particles as described in claim 70, wherein said step of analyzing asymmetric particle orientation information comprises the steps of

25 a. plotting electronic signal over a period of time,

b. integrating the areas of plots corresponding to said electronic signal, and

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74

- c. comparing integrations of said plots to integrations of oriented asymmetric particles

- 76 A method differentiating particles as described in claim 70, further comprising
differentially collecting said asymmetric particles based upon determined
5 orientation of said asymmetric particles with respect to said detector
- 77 A method differentiating particles as described in claim 70, wherein said
asymmetric particles are sperm cells from a male of a species of mammal.
- 78 A method differentiating particles as described in claim 70, further comprising the
steps of:
- 10 a. assessing the volume of a capsule containing the nuclear DNA of said
sperm cells, and
- b. differentiating between said X-chromosome bearing sperm cells and said
Y-chromosome bearing sperm cells based upon the volume of said capsule
containing the nuclear DNA of said sperm cell.
- 15 79 A method differentiating particles as described in claim 78, wherein said capsule
containing said DNA comprises a sperm head.
- 80 A method differentiating particles as described in claim 78, wherein said step of
assessing the volume of a capsule containing said DNA comprises
- 20 a. generating a beam of electromagnetic radiation having initial waveform
characteristics,
- b. traversing said volume of said capsule containing said DNA with said
beam of electromagnetic radiation having initial waveform characteristics,
- c. altering said initial wave form characteristics of said beam of
electromagnetic radiation by traversing said volume of said capsule, and
- 25 d. analyzing altered wave form characteristics

4
75

- 81 A method differentiating particles as described in claim 80, wherein said step of altering said wave form characteristics by traversing said volume of said capsule comprises shifting phase of said initial waveform characteristics
- 82 A method differentiating particles as described in claim 81, further comprising the step of superimposing said initial waveform characteristics and said phase shifted waveform characteristics.
- 83 A method differentiating particles as described in claim 82, further comprising the step of comparing a intensity of said initial waveform characteristics and superimposed waveform characteristics.
- 84 A method differentiating particles as described in claim 83, further comprising the step of determining said volume of said capsule containing said DNA based upon the difference in said intensity
- 85 A method differentiating particles as described in claim 80, further comprising the steps of
- a. forming droplets a plurality having one of said sperm cells entrained,
 - b. charging each of said droplets differentially based upon the determined volume of said capsule containing the nuclear DNA of said sperm cells,
 - c. deflecting each of said droplets, and
 - d. collecting each of said droplets based upon charge of said droplet; and
 - e. generating X-chromosome bearing and Y-chromosome bearing populations of said sperm cells
- 86 A method differentiating particles as described in claim 77, further comprising the steps of
- a. forming droplets a plurality having one of said sperm cells entrained,
 - b. charging each of said droplets differentially based upon an amount of nuclear DNA of said sperm cells,

28
76

- c. deflecting each of said droplets,
- d. collecting each of said droplets based upon charge of said droplet, and
- e. generating X-chromosome bearing and Y-chromosome bearing populations of said sperm cells

5 87 A method differentiating particles as described in claims 85 or 86, wherein said step of forming droplets a plurality having one of said sperm cells entrained comprises forming droplets having sufficient size to encapsulate said sperm cell, wherein said sperm cell comprises an intact live sperm cell having a tail.

88 A method differentiating particles as described in claim 87, further comprising the
10 step of ejecting said fluid stream from a nozzle having an orifice, wherein said orifice has a diameter of 100 micrometers

89 A method differentiating particles as described in claim 88, wherein said X-chromosome bearing and said Y-chromosome bearing populations of sperm cells have a purity selected from the group consisting of between 90% to about 100%,
15 between about 91% to about 100%, between about 92% to about 100%, between about 93% to about 100%, between about 94% to about 100%, between about 95% to about 100%, between about 96% to about 100%, between about 97% to about 100%, between about 98% to about 100%, between about 99% to about 100%.

20 90 A method differentiating particles as described in claim 88, further comprising the step of establishing a separable event rate wherein said separable event rate is selected from the group consisting of at least 5000 separable events per second, at least 6000 separable events per second, at least 7000 separable events per second, at least 8000 separable events per second, at least 9000 separable events per
25 second, at least 10,000 separable events per second, at least 11,000 separable events per second, at least 12,000 separable events per second, at least 13,000 separable events per second, at least 14,000 separable events per second, at least

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15,000 separable events per second, at least 16,000 separable events per second, at least 17,000 separable events per second, at least 18,000 separable events per second, at least 19,000 separable events per second, at least 20,000 separable events per second, at least 21,000 separable events per second.

5 91 A method differentiating particles as described in claim 88, wherein said step of separating said sperm cells comprises a separation rate selected from the group consisting of at least 500 separations per second, at least 1,000 separations per second, at least 2,000 separations per second, at least 3,000 separations per second, at least 4,000 separations per second, at least 5,000 separations per second, at least 6,000 separations per second, at least 7,000 separations per second, at least 8,000 separations per second, at least 9,000 separations per second, at least 10,000 separations per second.

92 A method differentiating particles as described in claim 88, wherein said step of forming droplets each having one of said sperm cells entrained comprises a droplet formation rate selected from the group consisting of at least 10,000 droplets per second, at least 20,000 droplets per second, at least 30,000 droplets per second, at least 40,000 droplets per second, at least 50,000 droplets per second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80,000 droplets per second, at least 90,000 droplets per second, at least 100,000 droplets per second.

93 A method differentiating particles as described in claim 77, wherein said species of mammal comprises a bovine mammal.

94 A method differentiating particles as described in claim 77, wherein said species of mammal comprises an equine mammal.

25 95 A method differentiating particles as described in claim 77, wherein said species of mammal comprises an ovine mammal.

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- 96 A particle differentiation apparatus, comprising
- a. at least one asymmetric particle having orientation characteristics within a fluid stream,
 - b. a irradiation source generating an irradiation beam responsive to said asymmetric particle;
 - c. optics to focus said irradiation beam,
 - d. a light emission material coupled to said asymmetric particle, wherein said light emission material emits light in response to said irradiation beam,
 - e. a detector differentially responsive to said light emitted from said light emission material based upon said particle orientation characteristics, and
 - f. an analyzer coupled to said detector, wherein said analyzer differentiates said asymmetric particles based upon orientation within said fluid stream.
- 97 A particle differentiation apparatus as described in claim 96, wherein said at least one asymmetrical particle comprise at least one sperm cell
- 98 A particle differentiation apparatus as described in claim 96, wherein said optics focus a beam pattern having a height of about equal to the length of said asymmetrical particle along the longitudinal axis to about three times the length of said asymmetrical particle along the longitudinal axis,
- 99 A particle differentiation apparatus as described in claim 98, wherein said at least one sperm cell has a head having a length along the longitudinal axis of about nine micrometers, and wherein said irradiation pattern has a height of about 20 micrometers
- 100 A particle differentiation apparatus as described in claim 96, wherein said at least one asymmetric particle having orientation characteristics further comprises at least one particle differentiation characteristic.
- 101 A particle differentiation apparatus as described in claim 96, wherein said light

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emission material bound to said at least one asymmetric particle comprises a stain bound to nuclear DNA of sperm cells of a male of a species of mammal

- 102 A particle differentiation apparatus as described in claim 101, wherein said
particle differentiation characteristic comprises a difference in amount of said
5 stain bound to said nuclear DNA of X-chromosome bearing sperm cells and said
nuclear DNA of Y-chromosome bearing sperm cells.
- 103 A particle differentiation apparatus as described in claim 96, wherein said detector
comprises at least one photomultiplier tube, and wherein said photomultiplier tube
has a typical operation voltage range, and wherein said photomultiplier tube is
10 operated outside said typical operation voltage range.
- 104 A particle differentiation apparatus as described in claim 103, wherein said typical
operation voltage range of said photomultiplier tube is from about 400 volts to
about 999 volts
- 105 A particle differentiation apparatus as described in claim 104, wherein said
15 photomultiplier tube is operated in a range from about 0 volts to about 300 volts
- 106 A particle differentiation apparatus as described in claim 96, further comprising
droplets breaking off from said fluid stream a plurality having at least one of said
asymmetric particles entrained.
- 20 107 A particle differentiation apparatus as described in claim 106, wherein said
droplets breaking off from said fluid stream have sufficient size to encapsulate one
of said sperm cells, wherein said sperm cells comprise intact live sperm cells
having a tail.
- 108 A particle differentiation apparatus as described in claim 107, further comprising a
25 nozzle having an orifice of about 100 micrometers in diameter

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- 109 A particle differentiation apparatus as described in claim 106, further comprising a droplet charger coupled to said analyzer, wherein said droplets receive a charge differentially based upon said difference in amount of said stain bound the nuclear DNA of X-chromosome sperm cells and the nuclear DNA of Y-chromosome bearing sperm cells
- 5
- 110 A particle differentiation apparatus as described in claim 109, further comprising a droplet charger coupled to said analyzer, wherein said droplets receive a charge differentially based upon said difference in volume of said particles, and wherein said difference in volume of said particles comprises a difference between X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells
- 10
- 111 A particle differentiation apparatus as described in claim 110, further comprising a droplet separator, wherein said droplet separator separates said droplet based upon charge of said droplet.
- 112 A particle differentiation apparatus as described in claim 111, further comprising at least one collection container in which droplets containing said X-chromosome bearing sperm cells are collected as an X-chromosome bearing population.
- 15
- 113 A particle differentiation apparatus as described in claim 111, further comprising at least one collection container in which droplets containing Y-chromosome bearing sperm cells are collected as a Y-chromosome bearing population.
- 20 114 A particle differentiation apparatus as described in claim 112 or 113, wherein said X-chromosome bearing population and said Y-chromosome bearing population of said sperm cells are selected from the group consisting of between 90% to about 100%, between about 91% to about 100%, between about 92% to about 100%, between about 93% to about 100%, between about 94% to about 100%, between about 95% to about 100%, between about 96% to about 100%, between about 97% to about 100%, between about 98% to about 100%, between about 99% to
- 25

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about 100%

- 115 A particle differentiation apparatus as described in claim 114, further comprising the step of establishing a separable event rate wherein said separable event rate is selected from the group consisting of at least 5000 separable events per second, at least 6000 separable events per second, at least 7000 separable events per second, at least 8000 separable events per second, at least 9000 separable events per second, at least 10,000 separable events per second, at least 11,000 separable events per second, at least 12,000 separable events per second, at least 13,000 separable events per second, at least 14,000 separable events per second, at least 15,000 separable events per second, at least 16,000 separable events per second, at least 17,000 separable events per second, at least 18,000 separable events per second, at least 19,000 separable events per second, at least 20,000 separable events per second, at least 21,000 separable events per second.
- 116 A particle differentiation apparatus as described in claim 115, wherein said step of separating said sperm cells comprises a separation rate selected from the group consisting of at least 500 separations per second, at least 1,000 separations per second, at least 2,000 separations per second, at least 3,000 separations per second, at least 4,000 separations per second, at least 5,000 separations per second, at least 6,000 separations per second, at least 7,000 separations per second, at least 8,000 separations per second, at least 9,000 separations per second, at least 10,000 separations per second, at least 11,000 separations per second.
- 117 A particle differentiation apparatus as described in claim 116, wherein said step of forming droplets each having one of said sperm cells entrained comprises a droplet formation rate selected from the group consisting of at least 10,000 droplets per second, at least 20,000 droplets per second, at least 30,000 droplets per second, at least 40,000 droplets per second, at least 50,000 droplets per second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80,000 droplets per second, at least 90,000 droplets per second, at least

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100,000 droplets per second.

- 118 A particle differentiation apparatus as described in claim 102, wherein said species of mammal comprises a bovine mammal.
- 119 A particle differentiation apparatus as described in claim 102, wherein said species
5 of mammal comprises an equine mammal.
- 120 A particle differentiation apparatus as described in claim 102, wherein said species of mammal comprises an ovine mammal.
- 121 A particle differentiation apparatus, comprising
- a. a fluid stream,
 - 10 b. intact live sperm cells introduced into said fluid stream,
 - c. a nozzle having a orifice through which said fluid stream exits,
 - d. an oscillator responsive to said fluid stream, and
 - e. droplets breaking off from said fluid stream, wherein a plurality of said droplets entrain said intact live sperm cells, and wherein said droplets
15 have sufficient size to encapsulate one of said live sperm cells
- 122 A particle differentiation apparatus as described in claim 121, wherein said orifice has a diameter of 100 micrometers
- 123 A particle differentiation apparatus as described in claim 122, wherein said
20 droplets breaking off from said fluid stream break off at a rate selected from the group consisting of at least 10,000 droplets per second, at least 20,000 droplets per second, at least 30,000 droplets per second, at least 40,000 droplets per second, at least 50,000 droplets per second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80,000 droplets per second, at least 90,000 droplets per second, at least 100,000 droplets per second

47
83

- 124 A particle differentiation apparatus as described in claim 123, further comprising a light emission source generating light.
- 125 A particle differentiation apparatus as described in claim 124, further comprising a detector responsive to said light.
- 5 126 A particle differentiation apparatus as described in claim 125, wherein said intact live sperm cells have at least one sex differentiation characteristic
- 127 A particle differentiation apparatus as described in claim 126, wherein said at least one sex differentiation characteristic comprises a volume difference of sperm cell heads of said intact live sperm cells
- 10 128 A particle differentiation apparatus as described in claim 127, wherein said light emission source emits a beam of electromagnetic radiation, and wherein said beam of electromagnetic radiation traverses said volume of said sperm cell heads, and wherein said beam of electromagnetic radiation has ~~an~~ *an* ~~un~~ *un* ~~bal~~ *bal* waveform characteristics differentially responsive to said difference in volume of said sperm cell heads
- 15 129 A particle differentiation apparatus as described in claim 128, further comprising an analyzer responsive to said detector, wherein said analyzer differentiates between said intact live sperm cells based upon said volume difference of said sperm cell heads
- 20 130 A particle differentiation apparatus as described in claim 129, wherein said volume difference of said sperm cell heads in volume comprises a difference between X-chromosome bearing live intact sperm cells and Y-chromosome bearing live intact sperm cells
- 131 A particle differentiation apparatus as described in claim 126, an irradiation source

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generating an irradiation beam responsive to said intact live sperm cells

- 132 A particle differentiation apparatus as described in claim 131, further comprising
optics, wherein said optics focus an irradiation beam pattern having a height equal
5 to about the length of said sperm heads along the longitudinal axis to about three
times the length of said sperm head of said along the longitudinal axis
- 133 A particle differentiation apparatus as described in claim 132, wherein said sperm
heads have a length along the longitudinal axis of about nine micrometers, and
10 wherein said irradiation pattern has a height of about 20 micrometers.
- 134 A particle differentiation apparatus as described in claim 131, wherein said at least
one sex differentiation characteristic comprises a difference in amount of nuclear
DNA of said intact live sperm cells, and wherein said light emission source
comprises a light emission material bound to nuclear DNA, and wherein said light
15 emission material emits light differentially based upon said difference in amount
of said nuclear DNA, and wherein said detector generates at least one
signal in response to said light.
- 135 A particle differentiation apparatus as described in claim 134, further comprising
an analyzer responsive to said detector, wherein said analyzer differentiates
20 between said at least one intact live sperm cell based upon said difference in
amount of nuclear DNA
- 136 A particle differentiation apparatus as described in claim 135, wherein said
difference in said amount of said nuclear DNA comprises a difference between X-
chromosome bearing sperm cells and Y-chromosome bearing sperm cells
- 25 137 A particle differentiation apparatus as described in claims 136, further comprising
a droplet charger coupled to said analyzer, wherein said droplet charger generates
a charge on said droplets differentially based upon said difference in amount of

8/85

said nuclear DNA

- 138 A particle differentiation apparatus as described in claim 129, further comprising a droplet charger coupled to said analyzer, wherein said droplet charger generates a charge on said droplets differentially based upon said volume difference of said sperm cell heads, and wherein said volume difference of said sperm cell heads comprises a difference between X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells
- 5
- 139 A particle differentiation apparatus as described in claims 137 or 138, further comprising a droplet separator, wherein said droplet separator separates said droplets based upon charge of said droplets.
- 10
- 140 A particle differentiation apparatus as described in claim 139, further comprising at least one collection container in which droplets containing said X-chromosome bearing sperm cells are collected as an X-chromosome bearing population.
- 141 A particle differentiation apparatus as described in claim 139, further comprising at least one collection container in which droplets containing Y-chromosome bearing sperm cells are collected as a Y-chromosome bearing population.
- 15
- 142 A particle differentiation apparatus as described in claim 125, wherein said detector comprises at least one photomultiplier tube, and wherein said at least one photomultiplier tube converts light from said light emission source into at least one signal, and wherein said photomultiplier tube has a typical operation voltage range, and wherein said photomultiplier tube is operated outside said typical operation voltage range.
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- 143 A particle differentiation apparatus as described in claim 142, wherein said typical operation voltage range of said photomultiplier tube is from about 400 volts to about 999 volts
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- 144 A particle differentiation apparatus as described in claim 143, wherein said photomultiplier tube is operated in a range from about 0 volts to about 300 volts
- 145 A particle differentiation apparatus as described in claims 140 or 141, wherein said X-chromosome bearing population and said Y-chromosome bearing
5 population of said sperm cells have a purity selected from the group consisting of between 90% to about 100%, between about 91% to about 100%, between about 92% to about 100%, between about 93% to about 100%, between about 94% to about 100%, between about 95% to about 100%, between about 96% to about 100%, between about 97% to about 100%, between about 98% to about 100%,
10 between about 99% to about 100%.
- 146 A particle differentiation apparatus as described in claim 145, further comprising the step of establishing a separable event rate wherein said separable event rate is selected from the group consisting of at least 5000 separable events per second, at least 6000 separable events per second, at least 7000 separable events per second,
15 at least 8000 separable events per second, at least 9000 separable events per second, at least 10,000 separable events per second, at least 11,000 separable events per second, at least 12,000 separable events per second, at least 13,000 separable events per second, at least 14,000 separable events per second, at least 15,000 separable events per second, at least 16,000 separable events per second,
20 at least 17,000 separable events per second, at least 18,000 separable events per second, at least 19,000 separable events per second, at least 20,000 separable events per second, at least 21,000 separable events per second.
- 147 A particle differentiation apparatus as described in claim 146, wherein said step of separating said sperm cells comprises a separation rate selected from the group
25 consisting of at least 500 separations per second, at least 1,000 separations per second, at least 2,000 separations per second, at least 3,000 separations per second, at least 4,000 separations per second, at least 5,000 separations per second, at least 6,000 separations per second, at least 7,000 separations per

87
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second, at least 8,000 separations per second, at least 9,000 separations per second, at least 10,000 separations per second, 11,000 separations per second.

- 148 A particle differentiation apparatus, comprising
- a. particles having at least one particle differentiation characteristic,
 - 5 b. a light emission source differentially responsive to said particle differentiation characteristics,
 - c. at least one photomultiplier tube, wherein said at least one photomultiplier tube converts light from said light emission source into at least one signal, and wherein said photomultiplier tube has a typical operation voltage range, and wherein said photomultiplier tube is operated outside said
10 typical operation voltage range, and
 - d. an analyzer responsive to said at least one signal which differentiates particles based upon said particle differentiation characteristics
- 15 149 A particle differentiation apparatus as described in claim 148, wherein said typical operation voltage range of said photomultiplier tube is from about 400 volts to about 999 volts
- 150 A particle differentiation apparatus as described in claim 149, wherein said photomultiplier tube is operated in a range from about 0 volts to about 300 volts
- 20 151 A particle differentiation apparatus as described in claim 148, further comprising a irradiation source generating an irradiation beam responsive to said particles
- 152 A particle differentiation apparatus as described in claim 151, wherein said light emission source comprises a light emission material bound to said particles which emits said light in response to said irradiation beam.
- 25 153 A particle differentiation apparatus as described in claim 152, wherein said light emission material bound to said particles comprises a stain bound to the nuclear

DNA of sperm cells.

- 154 A particle differentiation apparatus as described in claim 153, wherein said particle differentiation characteristic comprises a difference in amount of said stain bound the nuclear DNA of X-chromosome sperm cells and the nuclear DNA
5 of Y-chromosome bearing sperm cells
- 155 A particle differentiation apparatus as described in claim 152, wherein said particle comprises an asymmetric particle.
- 156 A particle differentiation apparatus as described in claim 155, further comprising optics to focus said irradiation beam responsive to said particle, wherein said
10 optics focus a irradiation pattern having a height of about equal to the length of said asymmetrical particle along the longitudinal axis to about three times the length of said asymmetrical particle along the longitudinal axis
- 157 A particle differentiation apparatus as described in claim 156, further comprising a fluid stream into which said particles are introduced.
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- 158 A particle differentiation apparatus as described in claim 157, wherein said at least one particle differentiation characteristic comprises orientation of said asymmetric particle within said fluid stream, and wherein said photomultiplier tube is differentially responsive to said light emitted from said light emission material
20 based upon said particle orientation characteristics, and wherein said analyzer coupled to said photomultiplier tube differentiates between said asymmetric particles based upon orientation within said fluid stream.
- 159 A particle differentiation apparatus as described in claim 158, wherein said asymmetrical particles comprise sperm cells having sperm cell heads
- 25 160 A particle differentiation apparatus as described in claim 159, wherein said sperm

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cell heads of said sperm cell have a length along the longitudinal axis of about nine micrometers, and wherein said irradiation pattern has a height of about 20 micrometers.

- 161 A particle differentiation apparatus as described in claim 148, wherein said
5 particles have a volume, and wherein said at least one particle differentiation characteristic comprises a volume difference of said particles.
- 162 A particle differentiation apparatus as described in claim 161, wherein said light
emission source emits a beam of electromagnetic radiation, and wherein said beam
of electromagnetic radiation traverses said volume of said particle, and wherein
10 said beam of electromagnetic radiation has initial waveform characteristics altered by said volume of said particles.
- 163 A particle differentiation apparatus as described in claim 162, wherein said
photomultiplier tube is responsive to said waveform characteristics altered by said
volume of said particles.
- 15 164 A particle differentiation apparatus as described in claim 163, wherein said
analyzer responsive to said at least one signal which differentiates particles based
upon said particle differentiation characteristics differentiates between said
particles based upon said difference in volume.
- 165 A particle differentiation apparatus as described in claim 164, wherein said
20 volume difference comprises a difference between X-chromosome bearing and Y-
chromosome bearing sperm cells
- 166 A particle differentiation apparatus as described in claim 160, further comprising
droplets breaking off from said fluid stream a plurality having one of said sperm
25 cells entrained.

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- 167 A particle differentiation apparatus as described in claim 165, further comprising droplets breaking off from said fluid stream a plurality having one of said sperm cells entrained.
- 5 168 A particle differentiation apparatus as described in claim 166, wherein said droplets breaking off from said fluid stream have sufficient size to encapsulate said one of said sperm cells
- 169 A particle differentiation apparatus as described in claim 168, further comprising a nozzle having an orifice of about 100 micrometers in diameter
- 10 170 A particle differentiation apparatus as described in claim 166, further comprising a droplet charger coupled to said analyzer, wherein said droplets receive a charge differentially based upon said difference in amount of said stain bound the nuclear DNA of X-chromosome sperm cells and the nuclear DNA of Y-chromosome bearing sperm cells.
- 15 171 A particle differentiation apparatus as described in claim 167, further comprising a droplet charger coupled to said analyzer, wherein said droplets receive a charge differentially based upon said volume difference of said sperm cell heads, and wherein said difference in volume of said sperm cell heads comprise a difference between X-chromosome bearing sperm cells and Y-chromosome bearing sperm
- 20 cells
- 172 A particle differentiation apparatus as described in claim 170, further comprising a droplet separator, wherein said droplet separator separates said droplet based upon charge of said droplet.
- 173 A particle differentiation apparatus as described in claim 171, further comprising a
- 25 droplet separator, wherein said droplet separator separates said droplet based upon charge of said droplet.

9/91

- 174 A particle differentiation apparatus as described in claim 172, further comprising collection containers responsive to said droplet separator, wherein X-chromosome bearing sperm cell and Y-chromosome bearing sperm cell populations are collected.
- 5 175 A particle differentiation apparatus as described in claim 173, further comprising collection containers responsive to said droplet separator, wherein X-chromosome bearing sperm cell and Y-chromosome bearing sperm cell populations are collected.
- 10 176 An apparatus for differentiating particles as described in claims 174 or 175, wherein said X-chromosome bearing sperm cell population and said Y-chromosome bearing population of said sperm cells are selected from the group consisting of between 90% to about 100%, between about 91% to about 100%, between about 92% to about 100%, between about 93% to about 100%, between about 94% to about 100%, between about 95% to about 100%, between about 96% to about 100%, between about 97% to about 100%, between about 98% to about 100%, between about 99% to about 100%.
- 15 177 A particle differentiation apparatus as described in claim 176, further comprising the step of establishing a separable event rate wherein said separable event rate is selected from the group consisting of at least 5000 separable events per second, at least 6000 separable events per second, at least 7000 separable events per second, at least 8000 separable events per second, at least 9000 separable events per second, at least 10,000 separable events per second, at least 11,000 separable events per second, at least 12,000 separable events per second, at least 13,000 separable events per second, at least 14,000 separable events per second, at least 15,000 separable events per second, at least 16,000 separable events per second, at least 17,000 separable events per second, at least 18,000 separable events per second, at least 19,000 separable events per second, at least 20,000 separable events per second, at least 21,000 separable events per second.
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- 178 A particle differentiation apparatus as described in claim 177, wherein said step of separating said sperm cells comprises a separation rate selected from the group consisting of at least 500 separations per second, at least 1,000 separations per second, at least 2,000 separations per second, at least 3,000 separations per second, at least 4,000 separations per second, at least 5,000 separations per second, at least 6,000 separations per second, at least 7,000 separations per second, at least 8,000 separations per second, at least 9,000 separations per second, at least 10,000 separations per second, 11,000 separations per second.
- 179 A particle differentiation apparatus as described in claim 178, wherein said step of forming droplets each having one of said sperm cells entrained comprises a droplet formation rate selected from the group consisting of at least 10,000 droplets per second, at least 20,000 droplets per second, at least 30,000 droplets per second, at least 40,000 droplets per second, at least 50,000 droplets per second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80,000 droplets per second, at least 90,000 droplets per second, at least 100,000 droplets per second.
- 180 A particle differentiation apparatus as described in claim 179, wherein said sperm cells comprise sperm cells from a bovine mammal.
- 181 A particle differentiation apparatus as described in claim 179, wherein said sperm cells comprise sperm cells from an equine mammal.
- 182 A particle differentiation apparatus as described in claim 179, wherein said sperm cells comprise sperm cells from an ovine mammal.
- 183 A particle differentiation apparatus, comprising
- a. asymmetric particles,
- b. an irradiation source generating an irradiation beam responsive to said asymmetric particles,

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- 5 c. optics to focus said irradiation beam responsive to said asymmetrical particles, wherein said optics focus a beam pattern having a height of about equal to the length of said asymmetrical particles along the longitudinal axis to about three times the length of said asymmetrical particles along the longitudinal axis,
- d. a light emission material coupled to said asymmetric particles, wherein said light emission material emits light in response to said irradiation beam,
- e. a detector responsive to said light.
- 10 184 A particle differentiation apparatus as described in claim 183, wherein said at least one asymmetrical particle comprise at least one sperm cell.
- 15 185 A particle differentiation apparatus as described in claim 184, wherein said at least one sperm cell has a head having a length along the longitudinal axis of about nine micrometers, and wherein said irradiation pattern has a height of about 20 micrometers.
- 186 A particle differentiation apparatus as described in claim 183, wherein said at least one asymmetric particle has at least one particle differentiation characteristic
- 187 A particle differentiation apparatus as described in claim 186, further comprising a fluid stream
- 20 188 A particle differentiation apparatus as described in claim 187, wherein said at least one particle differentiation characteristic comprises orientation of said asymmetric particle within said fluid stream, and wherein said detector is differentially responsive to said light emitted from said light emission material based upon said particle orientation characteristics
- 25 189 A particle differentiation apparatus as described in claim 188, further comprising

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an analyzer coupled to said detector

190 A particle differentiation apparatus as described in claim 189, wherein said analyzer differentiates between said asymmetric particles based upon said orientation of said asymmetric particle within said fluid stream.

5 191 A particle differentiation apparatus as described in claim 190, wherein said light emission material bound to said at least one asymmetric particle comprises a stain bound to the nuclear DNA of sperm cells

192 A particle differentiation apparatus as described in claim 191, wherein said particle differentiation characteristic comprises a difference in amount of said stain bound the nuclear DNA of X-chromosome sperm cells and the nuclear DNA
10 of Y-chromosome bearing sperm cells.

193 A particle differentiation apparatus as described in claim 192, wherein said detector comprises at least one photomultiplier tube, and wherein said photomultiplier tube has a typical operation voltage range, and wherein said
15 photomultiplier tube is operated outside said typical operation voltage range.

194 A particle differentiation apparatus as described in claim 193, wherein said typical operation voltage range of said photomultiplier tube is from about 400 volts to about 999 volts

195 A particle differentiation apparatus as described in claim 194, wherein said
20 photomultiplier tube is operated in a range from about 0 volts to about 300 volts

196 A particle differentiation apparatus as described in claim 193, further comprising droplets breaking off from said fluid stream each having one of said particles entrained.

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- 197 A particle differentiation apparatus as described in claim 196, wherein said droplets breaking off from said fluid stream have sufficient size to encapsulate said one of said sperm cells, wherein said sperm cells comprise intact live sperm cells having at least a head and a neck and a tail.
- 5 198 A particle differentiation apparatus as described in claim 197, further comprising a nozzle having an orifice of about 100 micrometers in diameter
- 199 A particle differentiation apparatus as described in claim 196, further comprising a droplet charger coupled to said analyzer, wherein said droplets receive a charge differentially based upon said difference in amount of said stain bound the nuclear
10 DNA of X-chromosome sperm cells and the nuclear DNA of Y-chromosome bearing sperm cells
- 200 A particle differentiation apparatus as described in claim 199, further comprising a droplet separator, wherein said droplet separator separates said droplet based upon charge of said droplet.
- 15 201 A particle differentiation apparatus as described in claim 200, further comprising at least one collection container in which droplets containing said X-chromosome bearing sperm cells are collected as an X-chromosome bearing population.
202. A particle differentiation apparatus as described in claim 200, further comprising at least one collection container in which droplets containing Y-chromosome
20 bearing sperm cells are collected as a Y-chromosome bearing population.
- 203 A particle differentiation apparatus as described in claims 201 or 202, wherein said X-chromosome bearing population and said Y-chromosome bearing population of said sperm cells are selected from the group consisting of between 90% to about 100%, between about 91% to about 100%, between about 92% to
25 about 100%, between about 93% to about 100%, between about 94% to about

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100%, between about 95% to about 100%, between about 96% to about 100%,
between about 97% to about 100%, between about 98% to about 100%, between
about 99% to about 100%

- 204 A particle differentiation apparatus as described in claim 203, further comprising
5 the step of establishing a separable event rate wherein said separable event rate is
selected from the group consisting of at least 5000 separable events per second, at
least 6000 separable events per second, at least 7000 separable events per second,
at least 8000 separable events per second, at least 9000 separable events per
second, at least 10,000 separable events per second, at least 11,000 separable
10 events per second, at least 12,000 separable events per second, at least 13,000
separable events per second, at least 14,000 separable events per second, at least
15,000 separable events per second, at least 16,000 separable events per second,
at least 17,000 separable events per second, at least 18,000 separable events per
second, at least 19,000 separable events per second, at least 20,000 separable
15 events per second, at least 21,000 separable events per second.
- 205 An apparatus for differentiating particles as described in claim 204, wherein said
step of separating said sperm cells comprises a separation rate selected from the
group consisting of at least 500 separations per second, at least 1,000 separations
per second, at least 2,000 separations per second, at least 3,000 separations per
20 second, at least 4,000 separations per second, at least 5,000 separations per
second, at least 6,000 separations per second, at least 7,000 separations per
second, at least 8,000 separations per second, at least 9,000 separations per
second, at least 10,000 separations per second, 11,000 separations per second.
- 206 An apparatus for differentiating particles as described in claim 205, wherein said
25 step of forming droplets each having one of said sperm cells entrained comprises a
droplet formation rate selected from the group consisting of at least 10,000
droplets per second, at least 20,000 droplets per second, at least 30,000 droplets
per second, at least 40,000 droplets per second, at least 50,000 droplets per

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second, at least 60,000 droplets per second, at least 70,000 droplets per second, at least 80,000 droplets per second, at least 90,000 droplets per second, at least 100,000 droplets per second.

- 207 A particle differentiation apparatus as described in claim 206, wherein said sperm
5 cells comprise a bovine sperm cells
- 208 A particle differentiation apparatus as described in claim 206, wherein said sperm cells comprise sperm cells from an equine mammal.
- 209 A particle differentiation apparatus as described in claim 206, wherein said sperm cells comprise sperm cells from an ovine mammal

98

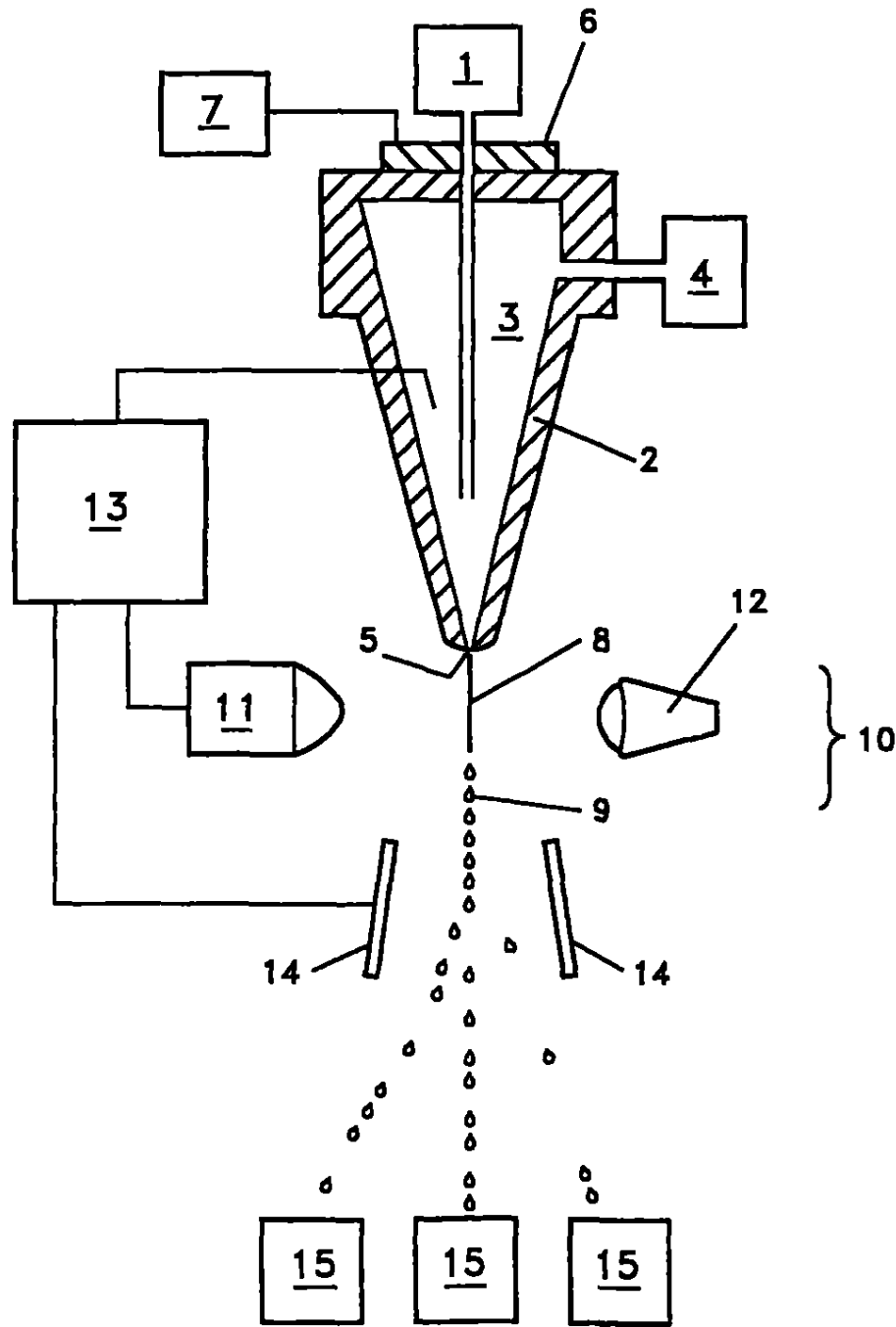


Fig. 1

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24
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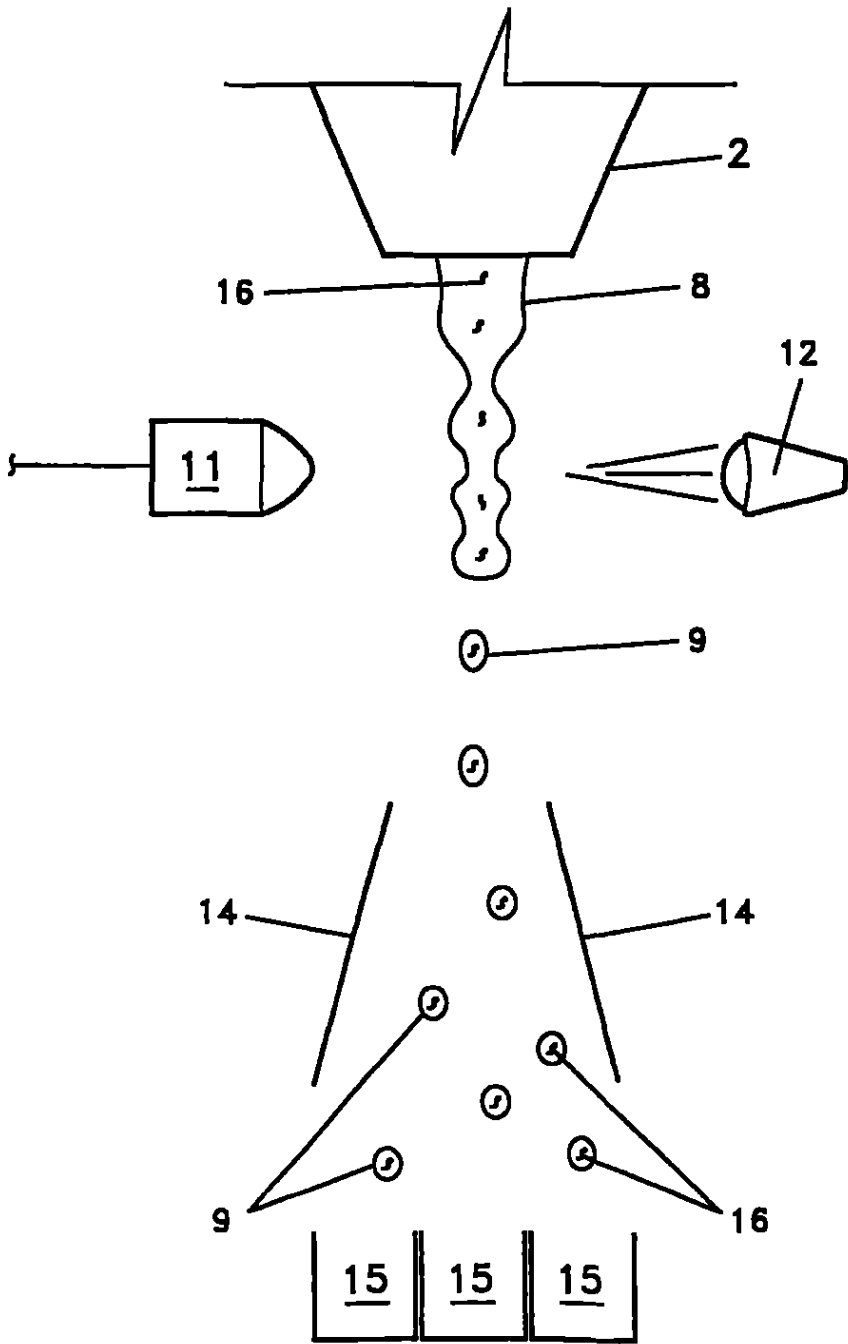


Fig. 2
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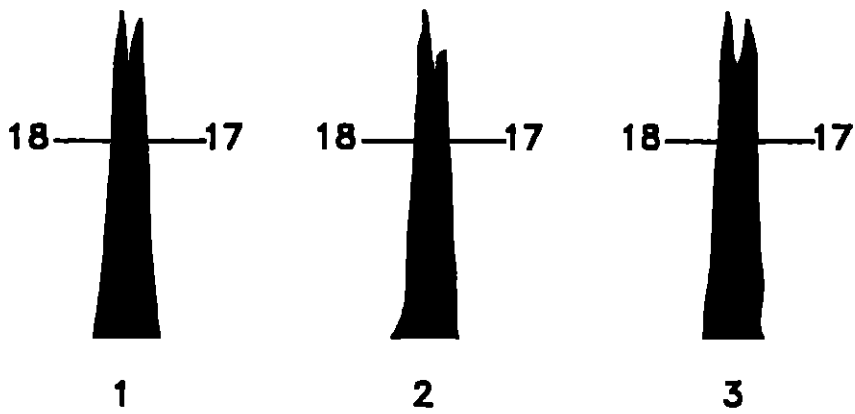


Fig. 3a

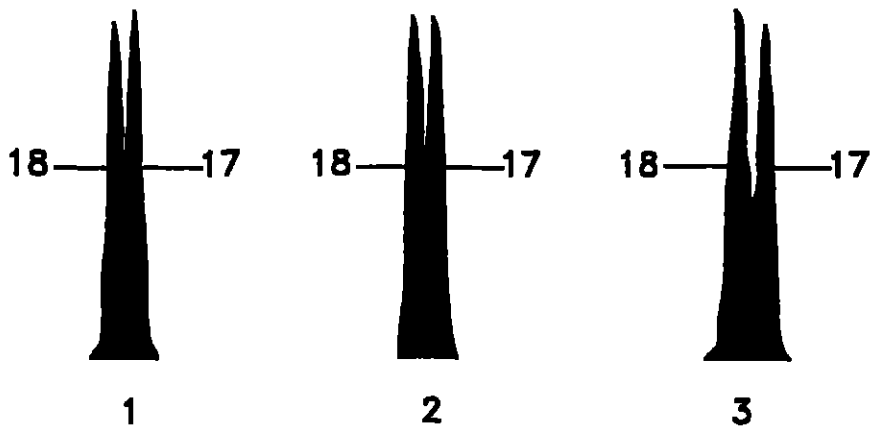


Fig. 3b

Fig. 3

—

10/

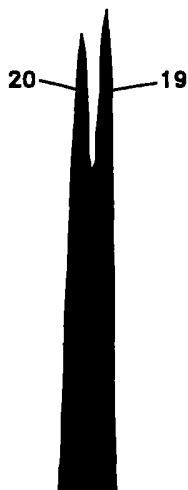


Fig. 4

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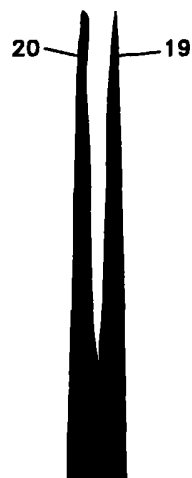


Fig. 5

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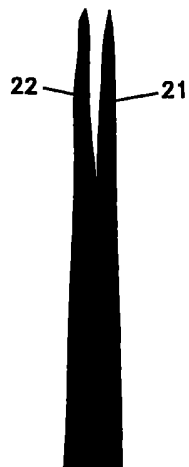


Fig. 6



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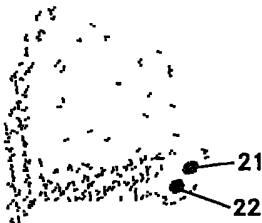
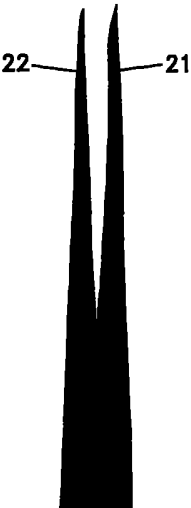


Fig. 7

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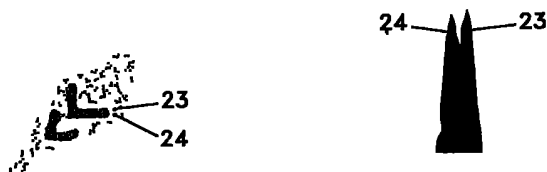


Fig. 8

106

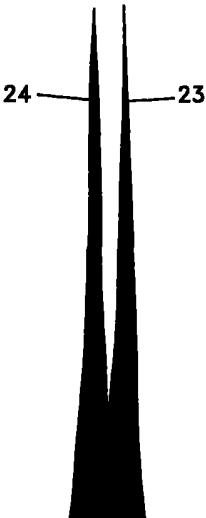


Fig. 9

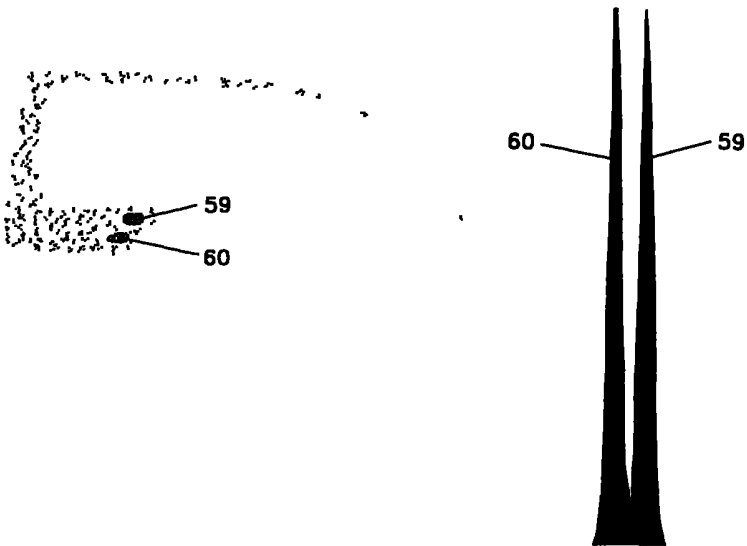


Fig. 10

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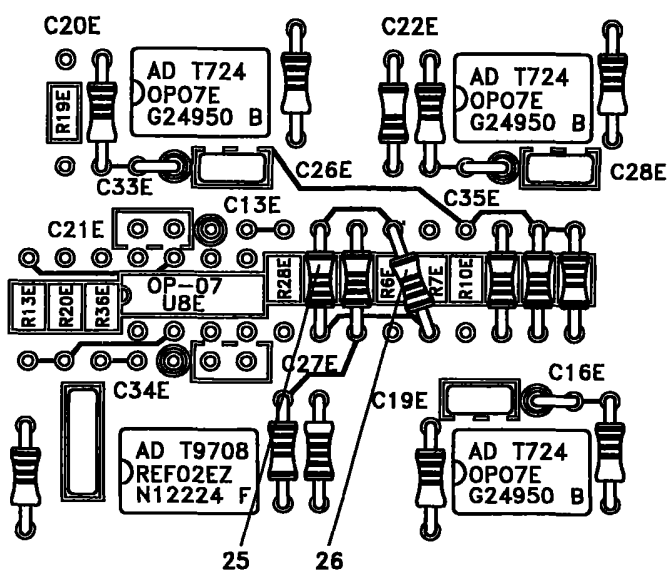


Fig. 11



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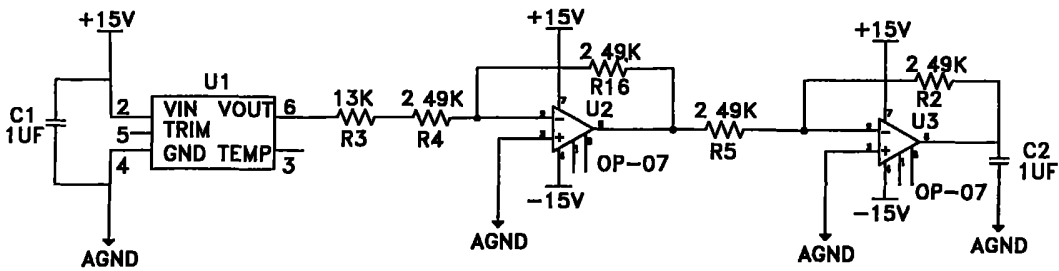


Fig. 12a

110

13/28

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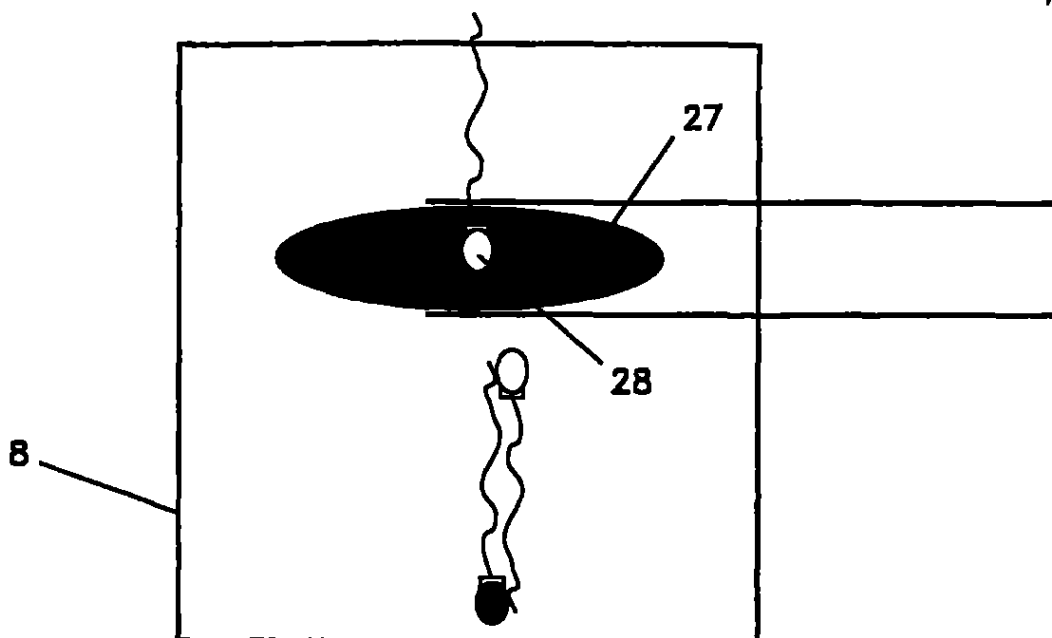


Fig. 13a

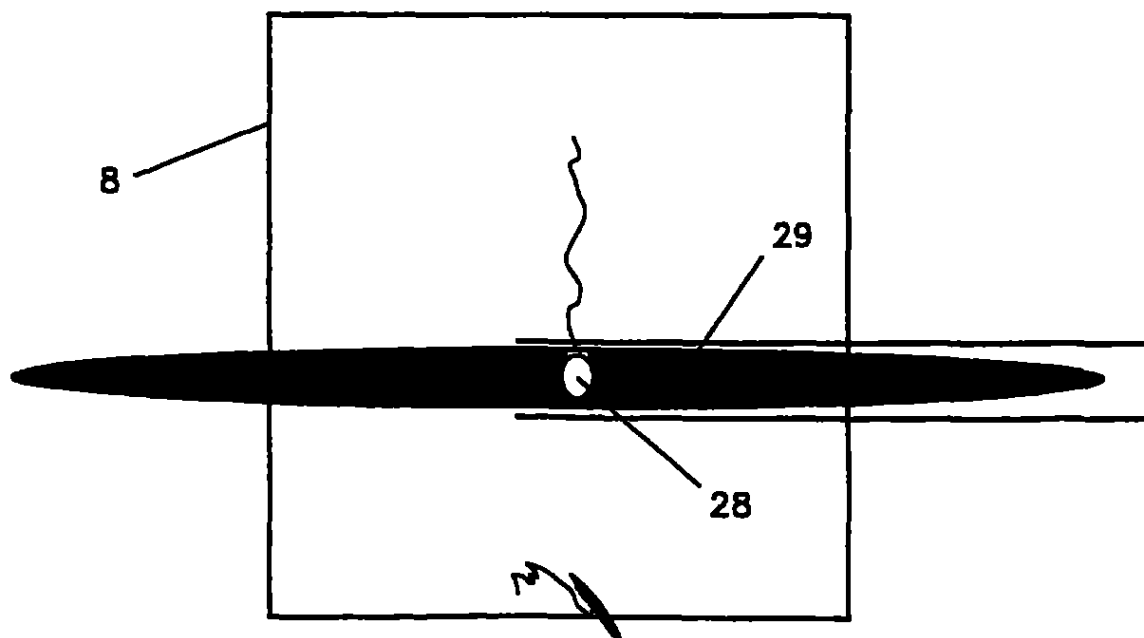


Fig. 13b

Fig. 13

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111

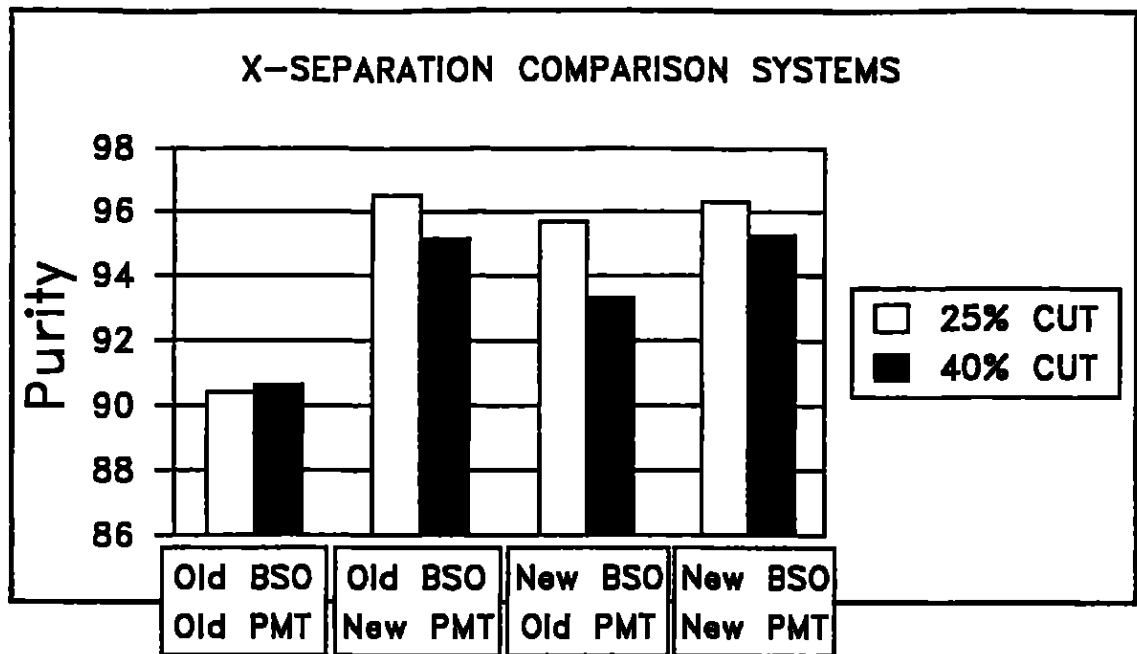


Fig. 14a

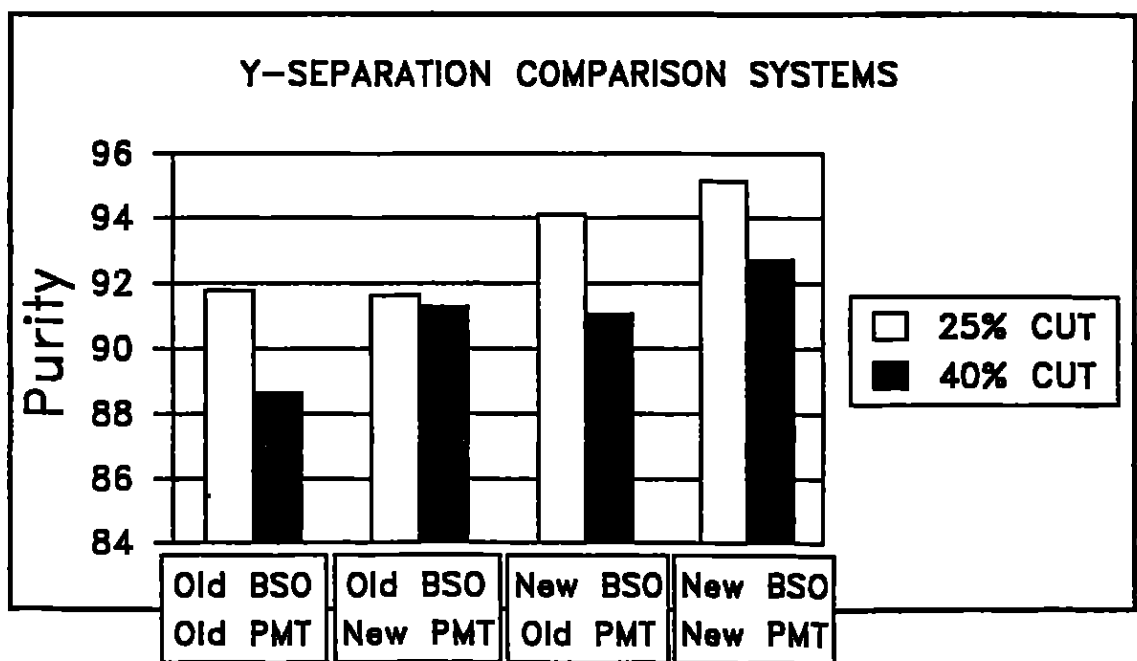


Fig. 14b

Fig. 14

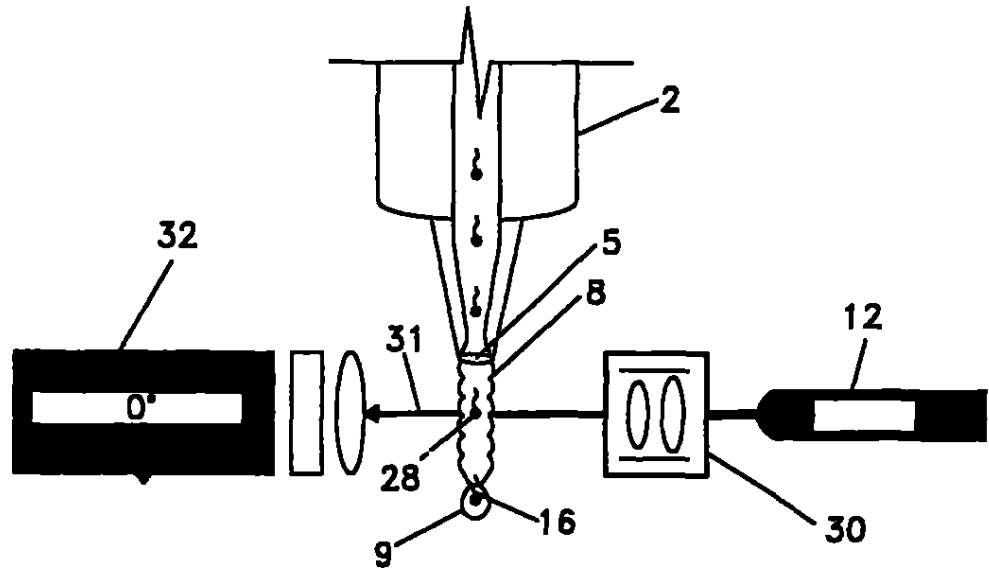


Fig. 15

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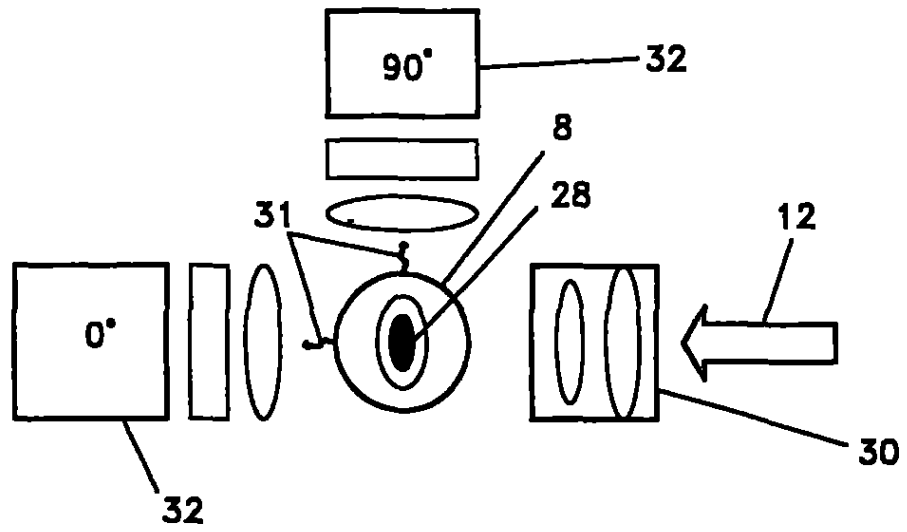


Fig. 16

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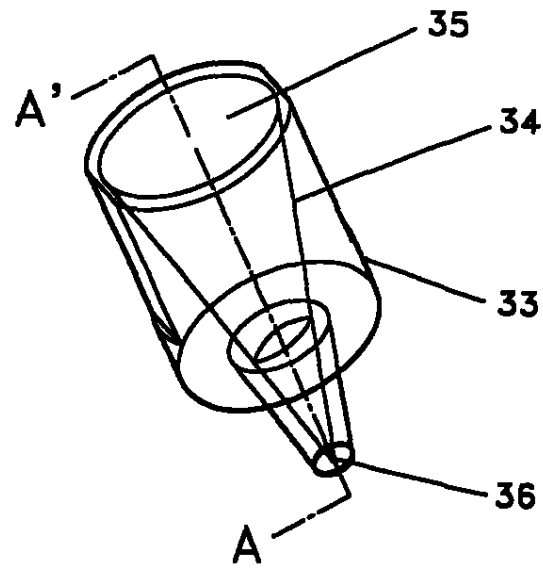
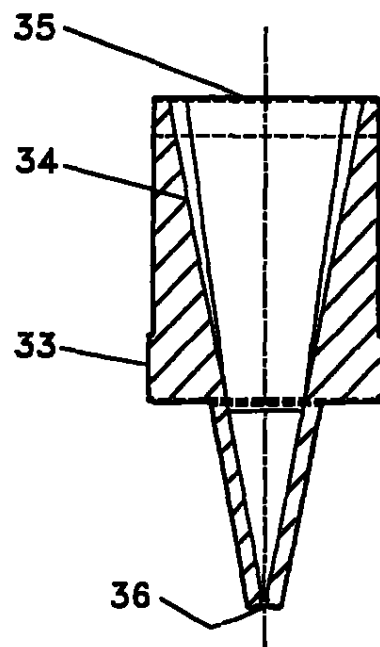


Fig. 17a



Section A-A'

Fig. 17b

Fig. 17

115

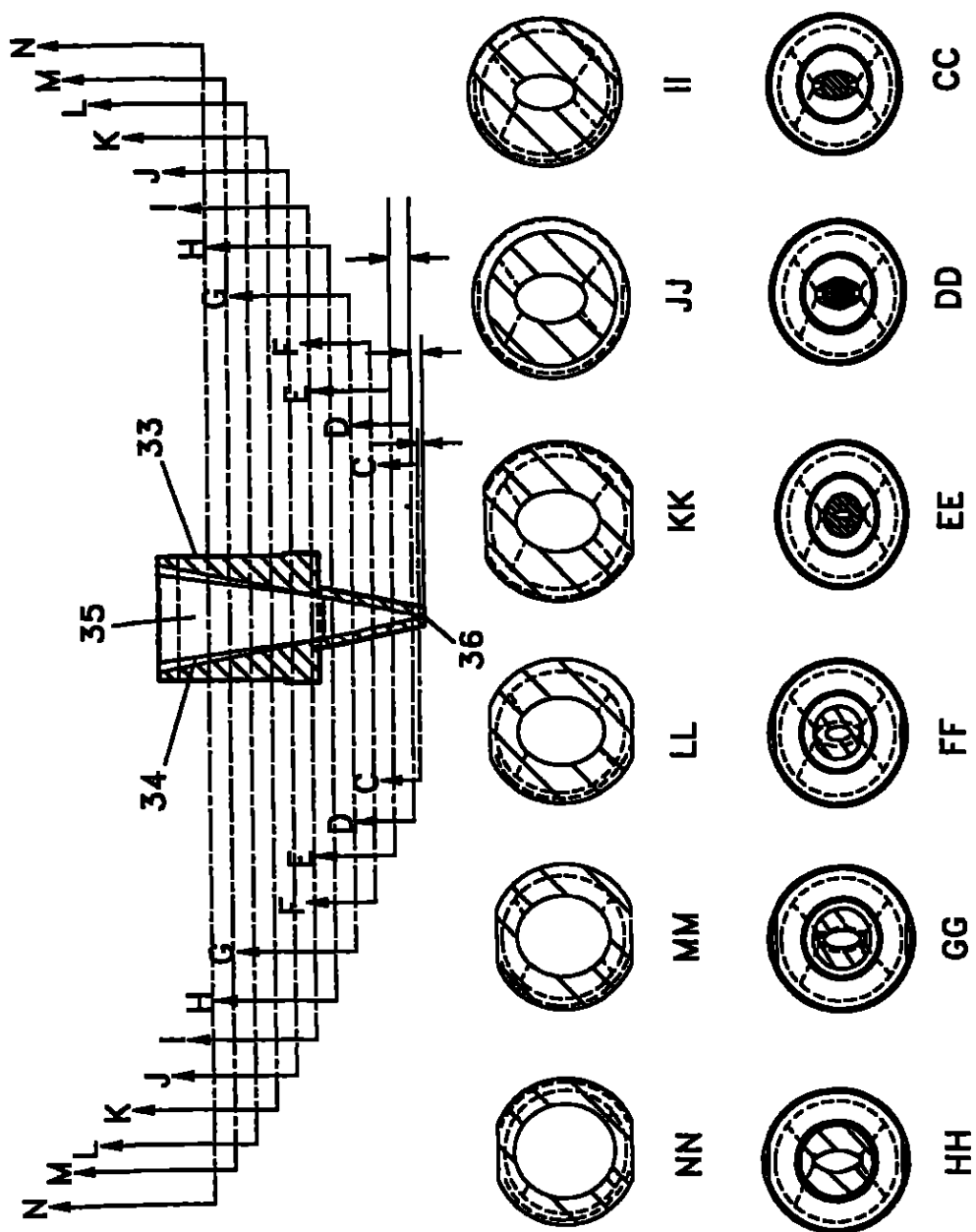


Fig. 18

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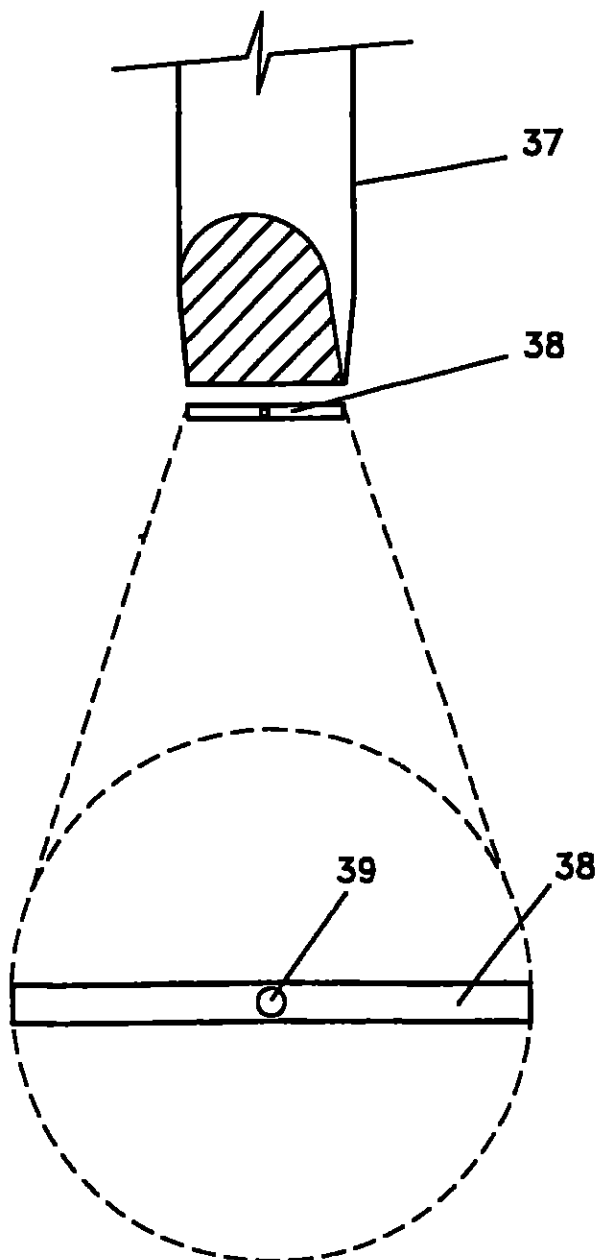


Fig. 19

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117

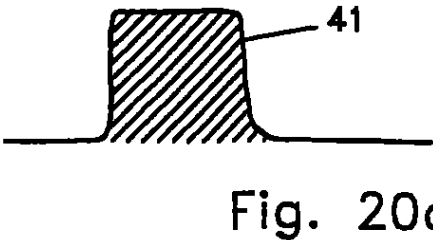
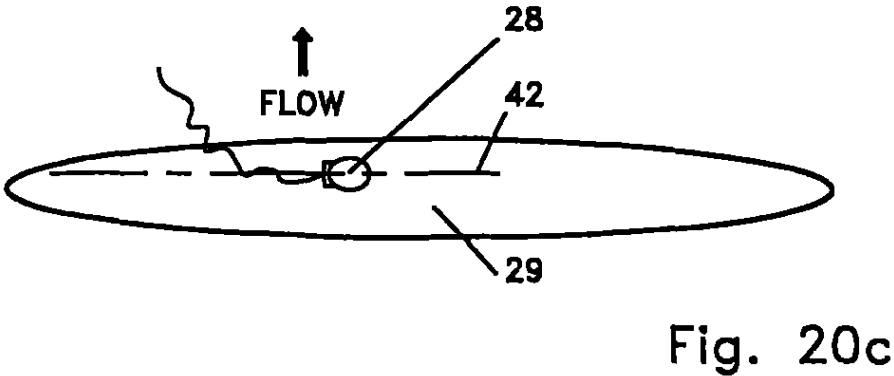
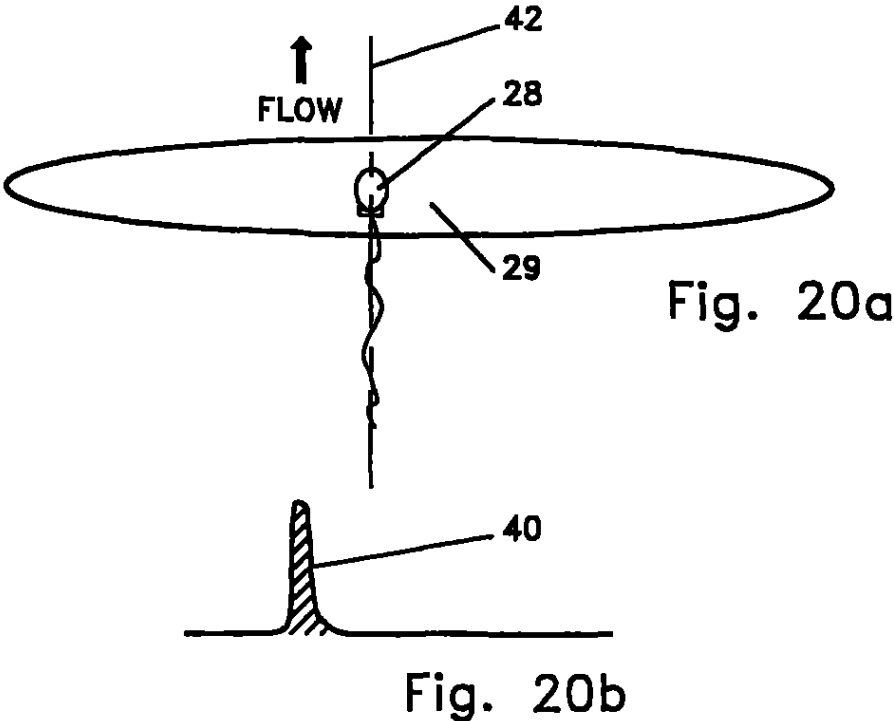
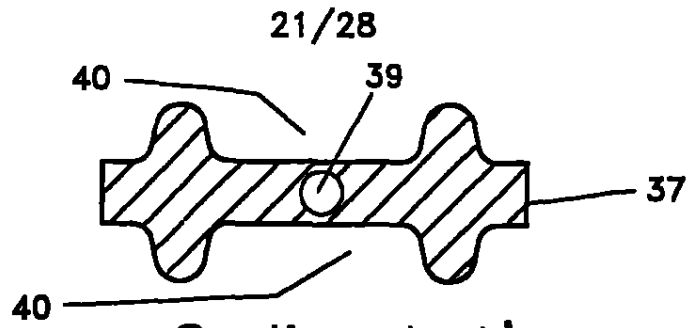


Fig. 20

118



Section A-A'
Fig. 21b

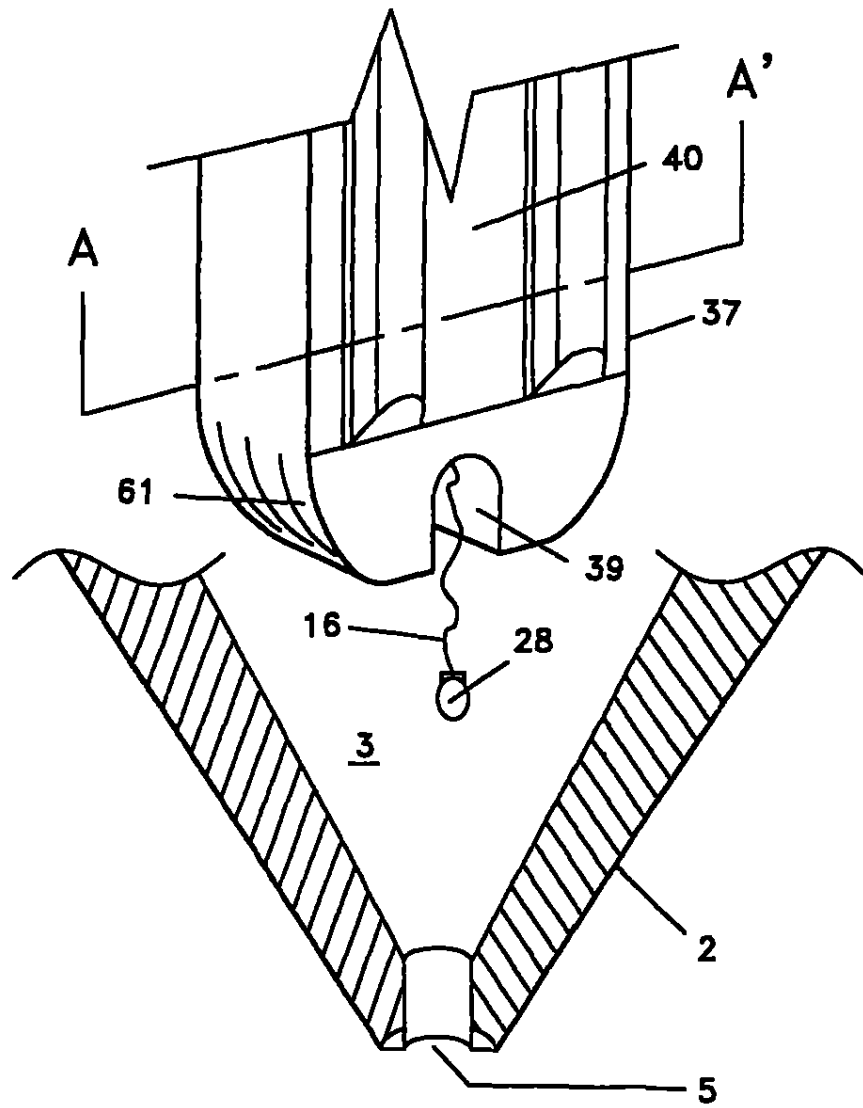
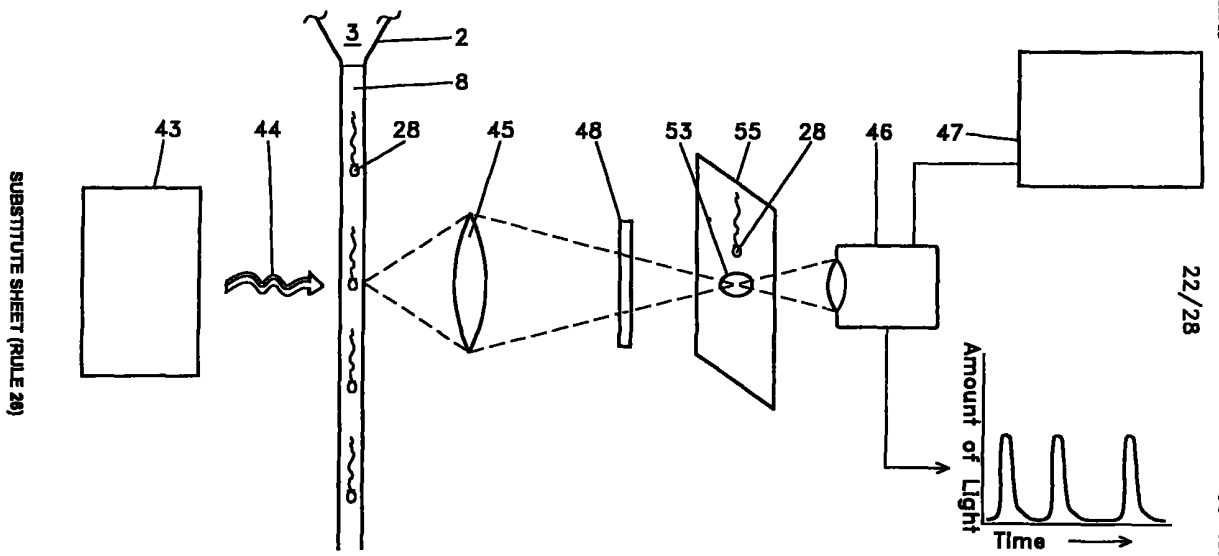


Fig. 21a

Fig. 21

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119



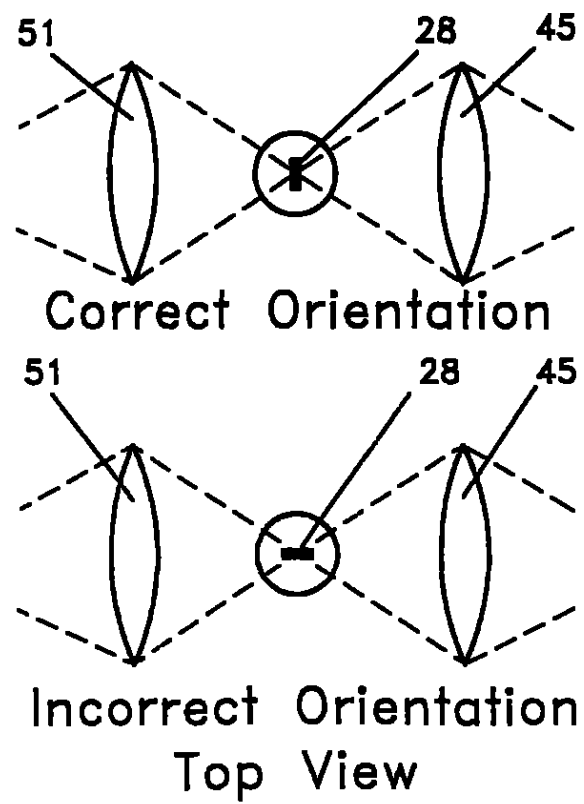
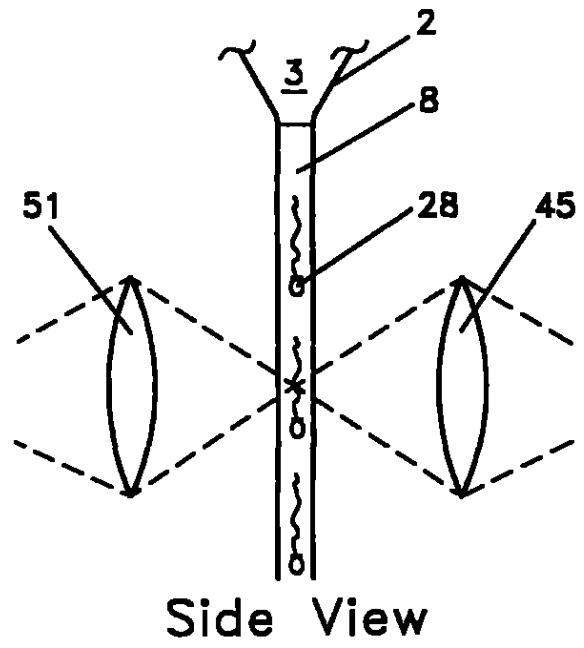
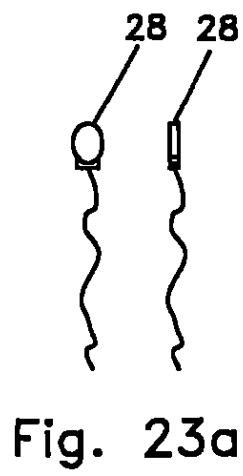


Fig. 23b

Fig 23

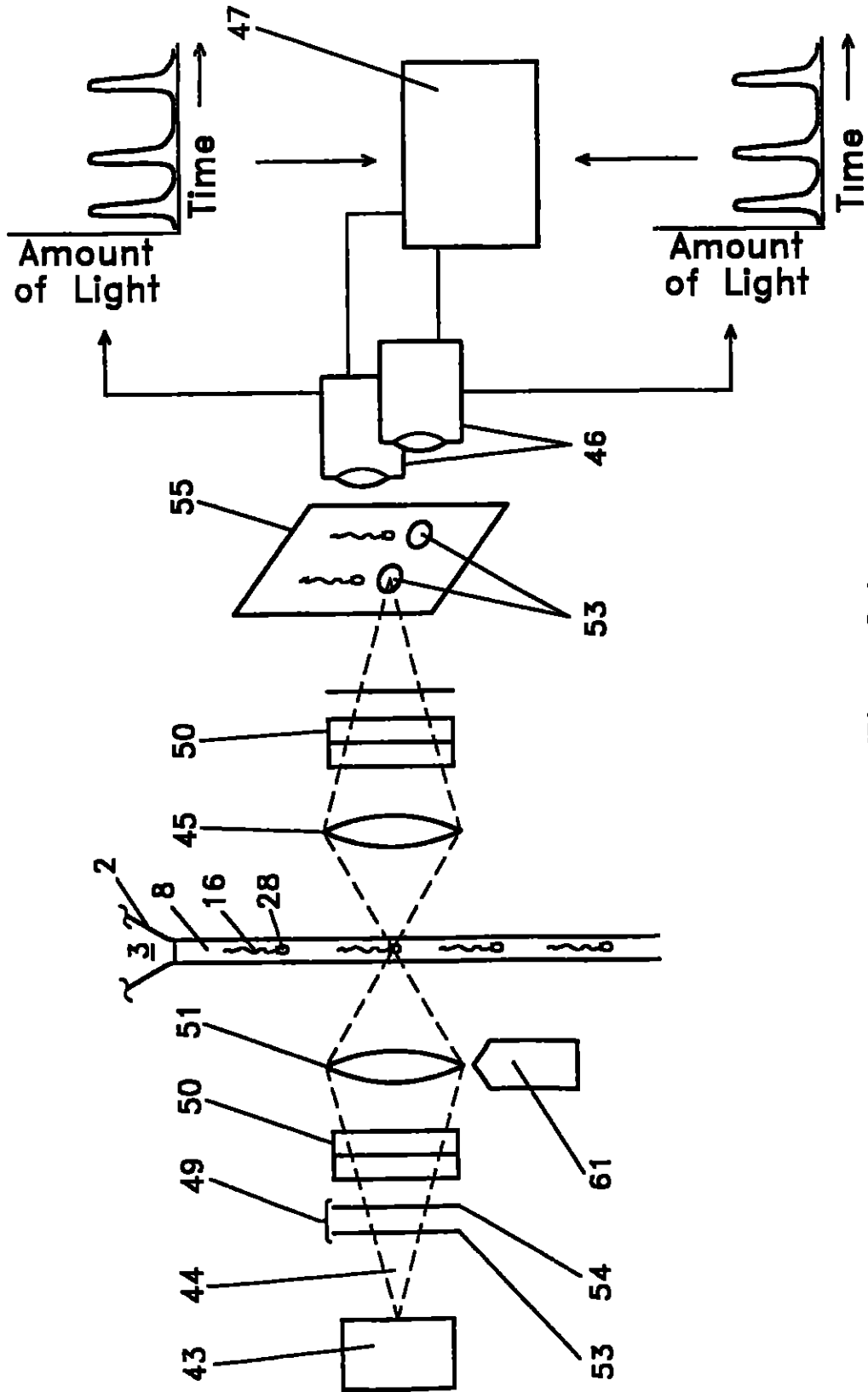


Fig. 24

122

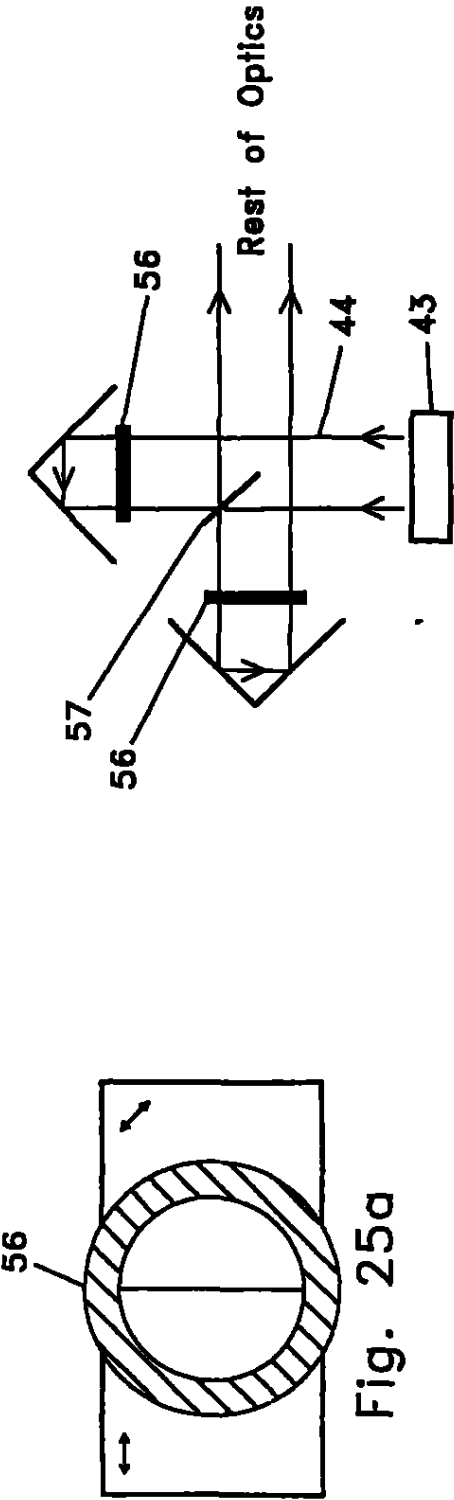


Fig. 25a

Fig. 25d

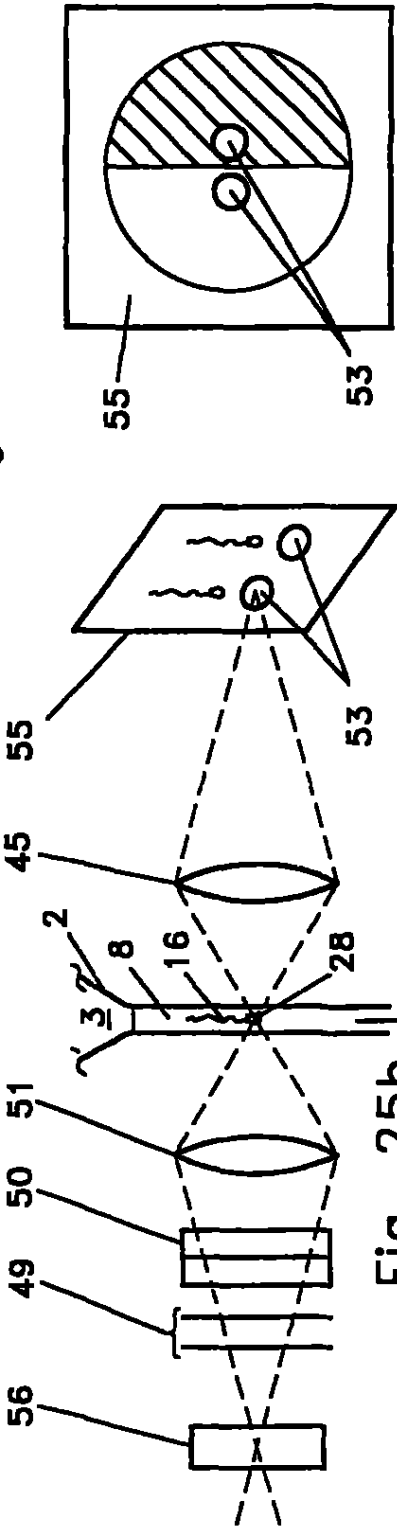


Fig. 25c

Fig. 25

123

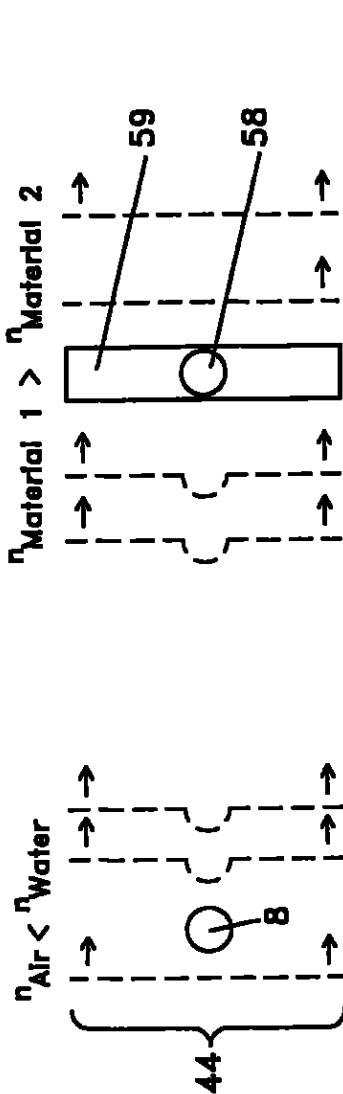


Fig. 26b

Fig. 26a

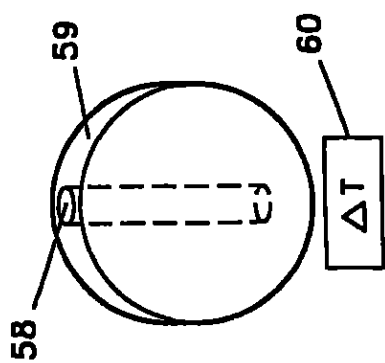
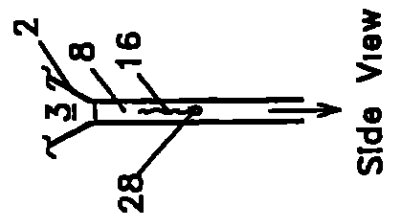


Fig. 26c

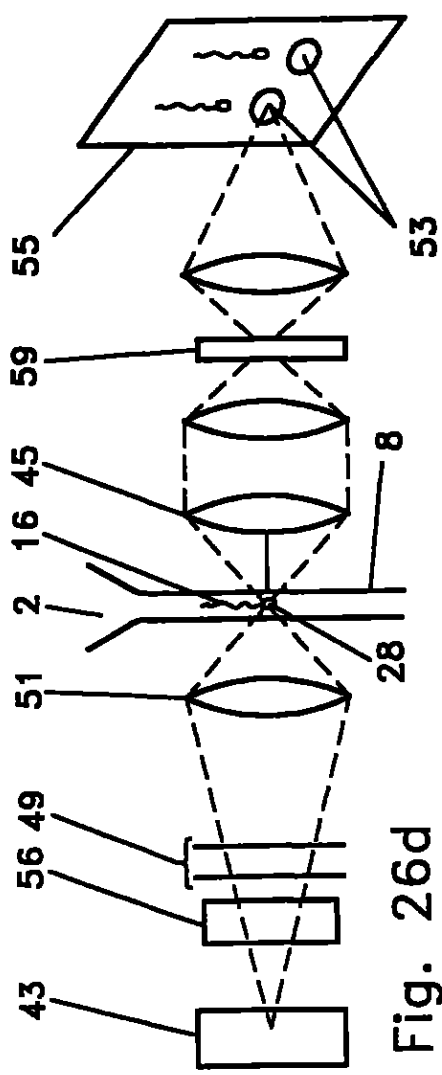


Fig. 26

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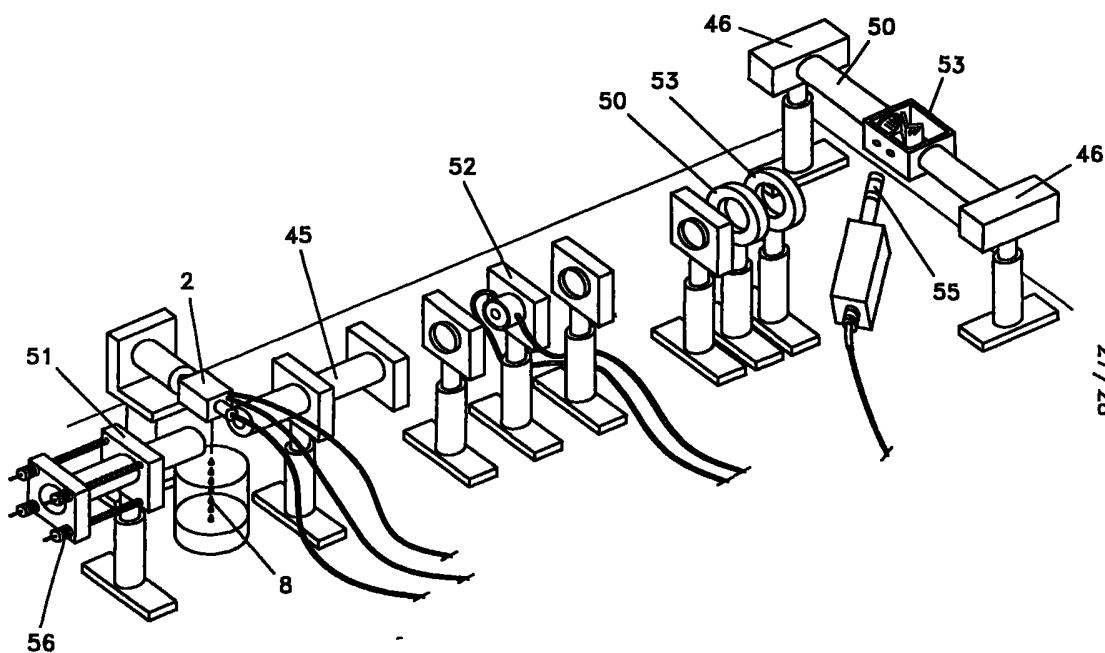


Fig. 27

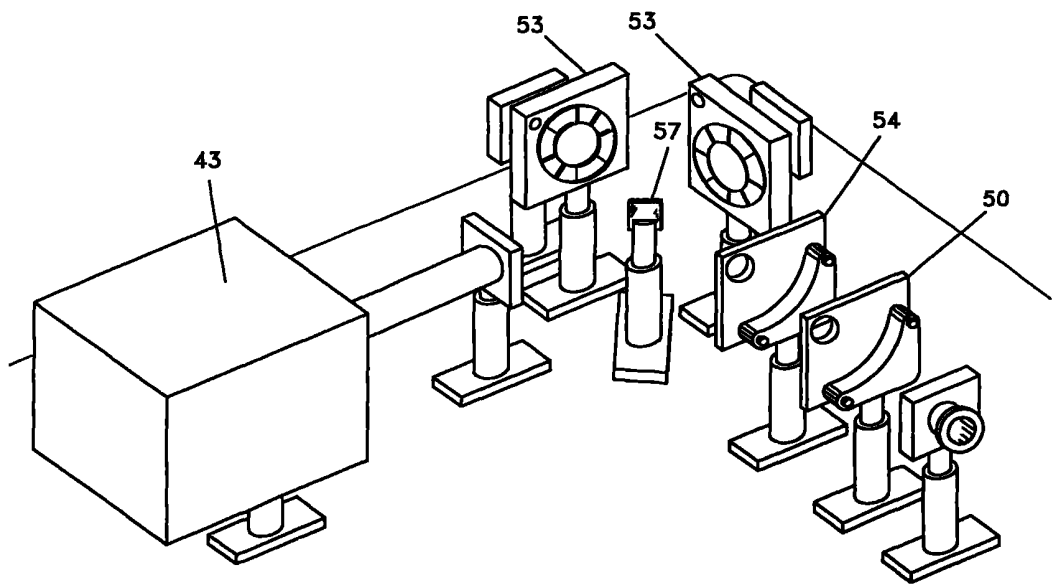


Fig. 28

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AUGUST 09 2001

PTAS



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SANTANGELO LAW OFFICES P C
CRAIG R MILES
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FORT COLLINS, CO 80521

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REEL/FRAME 011836/0015
NUMBER OF PAGES 6

BRIEF ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS)

ASSIGNOR

EVANS, KENNETH M

DOC DATE 05/07/2001

ASSIGNOR

VAN MUNSTER ERIK B

DOC DATE 05/07/2001

ASSIGNEE

XY, INC

1108 NORTH LEMAY AVENUE

FORT COLLINS, COLORADO 80524

SERIAL NUMBER

PATENT NUMBER

PCT NUMBER US0115150

FILING DATE

ISSUE DATE

SEDLEY PYNE EXAMINER
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05-31-2001

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Rev. 8-93

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1. Name of conveying party(ies)

Kenneth M. Evans and Erik B. van Munster

Additional name(s) of conveying party(ies) attached? ☐ Yes
☒ No

2. Name and address of receiving party(ies)

Name: XY Inc.

Internal Address:

Street Address: 1108 North Lemay Avenue

City: Fort Collins State: CO Zip: 80524

Additional name(s) & address attached? ☐ Yes
☒ No

3. Nature of conveyance:

☒ Assignment☐ Merger☐ Security Agreement☐ Change of Name☐ Other

Execution Date(s) of Assignment: May 7 2001

4. Application number(s) or patent number(s)

A. Patent Application No.(s)

PCT/US01/15150, entitled "High Purity X-Chromosome Bearing And Y-Chromosome Bearing Populations of Spermatozoa", filed 09 May 2001

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed.

Name: Craig R. Miles

Internal Address: Santangelo Law Offices, P C

Street Address: 125 South Howes, 3rd Floor

City: Fort Collins State: Colorado Zip: 80521

6. Total number of applications and patents involved. ONE

7. Total fee (37 CFR 3.41) ... \$40.00

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IN THE UNITED STATES PATENT AND
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PCT BRANCH

In Re the Application of XY, Inc

International Application Number PCT/US01/15150

International Filing Date 09 May 2001

US Application No 60/203,089
60/239,752
60/267,571

Priority Date May 9, 2000
October 12, 2000
February 10, 2001

For High Purity X-Chromosome Bearing And Y-Chromosome
Bearing Populations Of Spermatozoa

Receiving Office RO/US

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Assistant Commissioner for Patents
Box Assignment
Washington, D C 20231

Dear Recordation Clerk.

Enclosed for recording are the following documents

- a. an Assignment of Kenneth M Evans and Erik B van Munster along with a
Recordation Cover Sheet,
- b. a firm check in the amount of \$40 00 for the recordation fee,
- c. First Class Mail Certificates for each document, and
- d. this Letter of Transmittal

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Please confirm receipt of the documents by applying your date stamp to the enclosed postcard and returning it to us

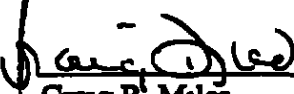
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Please address all future correspondence to Santangelo Law Offices, P C., 125 South Howes, Third Floor, Fort Collins, CO 80521

Dated this 24 day of May, 2001

Respectfully Submitted,

SANTANGELO Law Offices, P C



Craig R. Miles
ATTORNEY FOR APPLICANT
PTO No 45,954
125 South Howes, Third Floor
Fort Collins, Colorado 80522
(970) 224-3100

IN THE UNITED STATES PATENT AND
TRADEMARK OFFICE
PCT BRANCH

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130

In Re the Application of XY, Inc

International Application Number PCT/US01/15150

International Filing Date 09 May 2001

US Application No 60/203,089
60/239,752
60/267,571

Priority Date May 9, 2000
October 12, 2000
February 10, 2001

For High Purity X-Chromosome Bearing And Y-Chromosome
Bearing Populations Of Spermatozoa

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Washington, D C 20231

Dated this 24 day of May, 2001

Shannon M. Nation-Kammerer

131

Atty Docket. XYHighPurPCT

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IN THE UNITED STATES PATENT AND
TRADEMARK OFFICE
PCT BRANCH

In Re the Application of: XY, Inc

International Application Number PCT/US01/15150

International Filing Date 09 May 2001

US Application No 60/203,089
60/239,752
60/267,571

Priority Date May 9, 2000
October 12, 2000
February 10, 2001

For High Purity X-Chromosome Bearing And Y-Chromosome
Bearing Populations Of Spermatozoa

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CERTIFICATE OF MAILING

I, Shannon Nation-Kammerer, hereby certify to the truth of the following items

1 I am an employee of Santangelo Law Offices, P C , 125 South Howes, Third Floor,
Fort Collins, Colorado 80521

2 I have this day deposited the attached Assignment of Patent Rights of Kenneth M
Evans and Erik B van Munster along with a Recordation Cover Sheet with the United States Postal
Service as "First Class Mail", with sufficient postage, for mailing to the Assistant Commissioner for
Patents, Box Assignment, Washington, D C 20231

Dated this 24 day of May, 2001

Shannon Nation-Kammerer

IN THE UNITED STATES PATENT AND
TRADEMARK OFFICE
PCT BRANCH

In Re the Application of XY, Inc

International Application Number PCT/US01/15150

International Filing Date 09 May 2001

US Application No 60/203,089
60/239,752
60/267,571

Priority Date May 9, 2000
October 12, 2000
February 10, 2001

For High Purity X-Chromosome Bearing And Y-Chromosome
Bearing Populations Of Spermatozoa

Receiving Office RO/US

ASSIGNMENT OF PATENT RIGHTS

THIS ASSIGNMENT document is between and among Kenneth M. Evans, whose address is 2125 Primrose Lane, Fort Collins, CO 80526, USA and Erik B van Munster, whose address is Lijnbaansgracht 28C, 1015GP Amsterdam, The Netherlands (individually and collectively the "Assignor" or "Assignors") and XY, Inc, having its principal office at 1108 North Lemay Avenue (the "Assignee")

WHEREAS the Assignors have each contributed to some degree in conceiving or developing technology relating to a "High Purity X-Chromosome Bearing And Y-Chromosome Bearing Populations Of Spermatozoa", including but not limited to the application referred to above, and also including for the purpose of this assignment those improvements which are related to the subject matter of such technology to the extent conceived or developed during the time serving as a consultant or employee of the Assignee, and improvements related to such technology which are identified, developed, or conceived using trade secret or confidential information of the Assignee or which are likely to cause disclosure of such trade secret or confidential information (the "Invention"),

133
WHEREAS each Assignor either by him/herself or in conjunction with other individuals may be an inventor of the Invention

WHEREAS each Assignor acknowledges his/her obligation to assign all right, title and interest in the Invention to Assignee, and

WHEREAS each Assignor desires to formalize his/her obligation for assignment of all right, title and interest to the Invention to Assignee to the extent he/she has any such rights worldwide,

WHEREAS each Assignor acknowledges his/her obligation to assign all right, title and interest in any and all patent rights and any other proprietary rights related to the business of the Assignee, and in particular the Invention, to Assignee,

NOW, THEREFORE, for good and valuable consideration, the parties hereby agree as follows

1 Each Assignor warrants that

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- b. he/she has conveyed no right, title, or interest in the Invention to any party other than the Assignee,
- c. to the extent each individual Assignor conducted any efforts relevant to the ownership of all rights to the Invention under, on behalf of, or through any entity or assumed name, he/she has the authority to act as agent for such an entity and to fully transfer such rights, and
- d. at the time of signing of this Assignment, he/she neither knows nor has reason to know of any outstanding right, title, or interest in the Invention inconsistent with a full assignment of rights to the Assignee other than any right, title, or interest which may exist as a result of any coinventorship

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- b. any applications for United States or foreign patents relating to the Invention, and specifically to the above-entitled application, and to make a claim for any priority to which such applications are entitled, and to any division, substitution, continuation or continuation-in-part, of such applications, all patents which may be granted thereon, and all reissues and extensions thereof,

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c the right to file applications for United States or foreign patents based upon the Invention and to make a claim for any priority to which such applications are entitled, and to any division, substitution, continuation, or continuation-in-part of such applications, all patents which may be granted thereon, and all reissues and extensions thereof

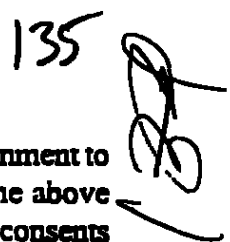
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f all rights to seek protection covering such aspects or claims as the Assignee deems appropriate, specifically including but not limited to claims which seek protection for device, apparatus, method, process, business method, or other coverage, claims which seek protection for as broad a level of coverage as might be desired by the Assignee, claims which address any aspect conceived, developed, or disclosed as separate inventions, claims which address any permutations or combinations of any aspects, claims which address any aspects independent of any initial context considered as preferred embodiments, and claims which seek broad protection in general

3 Each Assignor acknowledges a legal and equitable obligation to assign to the Assignee any and all patent rights and any other proprietary rights related to the business of the Assignee and covenants that he/she has not and will not execute any agreement in conflict with this Assignment and will not take or assist in taking any actions which might tend to diminish the scope of protection available to the Assignee for the Invention. Further, each Assignor warrants that other than rights of the Assignee, he/she maintains the entire right, title, and interest in and to the Invention as may exist by virtue of his/her contribution and has made no conveyance of any rights whatsoever to any other person. Each Assignor also covenants that he/she will promptly and continuously inform the Assignee of any articles, patents, or other references or prior art of which he/she becomes aware which may be material to the assessment of patentability of the Invention.

4 Each Assignor further covenants and agrees that he/she will communicate to the Assignee any facts known to him/her respecting any improvements encompassed by the Invention, and will testify in any legal proceeding, sign all lawful papers, execute all division, continuation, continuation-in-part, substitute, foreign, and reissue applications, make all rightful oaths, and generally do everything possible to aid the Assignee to obtain and enforce full protection and title in and to said Invention and to such improvements in all countries without further consideration but at the expense of the Assignee

135 

5 Each Assignor acknowledges that pursuant to the sale, grant, transfer and assignment to the Assignee of the entire right title and interest in and to the Invention, as provided in the above paragraphs this assignment shall be complete as to all rights owned. As such, each Assignor consents to a waiver of any and all access rights to any applications for United States or foreign patents relating to the Invention, and including but not limited to the above-entitled application, and to any division, substitution, continuation or continuation-in-part, of such applications, all related access rights involving the Invention in any country such as utility-model registrations, inventor's certificates and the like, and all access rights, titles, and interests involving the Invention under any foreign government, to the extent permissible. Access rights may particularly include any and all rights to any files, documents, notes, and evidence regarding any aspect of the prosecution of the Invention in the United States or foreign countries, any right to obtain, view or copy in any form the files, documents, notes and evidence related to prosecution history or status of any application for United States or foreign patents relating to the Invention, any other right to obtain information relating to prosecution history or status of any application for United States or foreign patents relating to the Invention.

6 Each Assignor further covenants and agrees that he/she will sign all lawful papers, consents, and generally do everything possible to aid the Assignee in petitioning waiver of access of each Assignor to the United States or foreign patent offices of any and all applications for United States or foreign patents relating to the invention, as provided above

7 Each Assignor grants the firm of Santangelo Law Offices, P C , the power to insert on this Assignment any further identification which may be necessary or desirable in order to comply with the rules of the United States Patent Office or any foreign patent office for recordation of this document, including but not limited to inserting the application number, the execution date, or the filing date of the above-entitled United States Patent Application based upon the Invention

8 This assignment shall be binding on all parties, their heirs, executors, administrators, successors, and/or assigns, and may be recorded in the United States Patent and Trademark Office or elsewhere. In the event any provision of this Agreement is found to be unenforceable or to be unreasonable in scope, such provision shall be modified to the extent necessary to make it enforceable, and as so modified, this Agreement shall remain in full force and effect.

Date 05-07-01

Kenneth M. Evans 138
Kenneth M. Evans

UNITED STATES OF AMERICA)
STATE OF COLORADO)
COUNTY OF LARIMER)

SUBSCRIBED AND AFFIRMED OR SWORN to before me in the County of Larimer, State of Colorado, United States of America, by Kenneth M. Evans this 7 day of May in the year 2001

WITNESS my hand and official seal pursuant to the authority vested in me as a Notary Public by the State of Colorado

Shannon M. Nation-Kammerer
Notary Public
My Commission Expires 7-15-2003



My Commission Expires 07/15/2003

Date 05-07-01

Erik B. van Munster
Erik B. van Munster

UNITED STATES OF AMERICA)
STATE OF COLORADO)
COUNTY OF LARIMER)

SUBSCRIBED AND AFFIRMED OR SWORN to before me in the County of Larimer, State of Colorado, United States of America, by Erik B. van Munster this 7 day of May in the year 2001

WITNESS my hand and official seal pursuant to the authority vested in me as a Notary Public by the State of Colorado

Shannon M. Nation-Kammerer
Notary Public
My Commission Expires 7-15-2003



My Commission Expires 07/15/2003

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on this line below.

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only		
Identification of IPEA		Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference XYHighPusPCT
International application No. PCT/US01/15150	International filing date (day/month/year) 09 May 2001 (09 05 01)	(Earliest) Priority date (day/month/year) 09 May 2000 (09 05 00)
Title of invention High Purity X-Chromosome Bearing and Y-Chromosome Bearing Populations of Spermatozoa		
Box No. II APPLICANT(S)		
Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country.) XY, INC 1108 North Lemay Avenue Fort Collins, CO 80524 United States of America		Telephone No. (970) 493-3113
		Facsimile No. (970) 493-3114
		Teleprinter No.
		Applicant's registration No. with the Office
State (that is, country) of nationality US		State (that is, country) of residence: US
Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country.) EVANS Kenneth M 2125 Pnmrose Lane Fort Collins, CO 80526 United States of America		
State (that is, country) of nationality US		State (that is, country) of residence: US
Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country.) VAN MUNSTER, Enk B Lijnbaansgracht 280 1015GP Amsterdam The Netherlands		
State (that is, country) of nationality The Netherlands		State (that is, country) of residence: US
☐ Further applicants are indicated on a continuation sheet.		

138
138
138

Box No. III AGENT OR COMMON REPRESENTATIVE, OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative
and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.
☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.
☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier

Name and address: (Family name followed by given names; for a legal entity, full official designation.
The address must include postal code and name of country.)

MILES Craig R
Santangelo Law Offices P C
125 South Howes, Third Floor
Fort Collins, CO 80521
United States of America

Telephone No.

(970) 224-3100

Facsimile No.

(970) 224-3175

Teleprinter No.

Agent's registration No. with the Office
45,954

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments.*

- 1 The applicant wishes the international preliminary examination to start on the basis of:
☒ the international application as originally filed
the description ☒ as originally filed
☐ as amended under Article 34
the claims ☒ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34
the drawings ☒ as originally filed
☐ as amended under Article 34
- 2 ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.
- 3 ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69 1(d)). (This check-box may be marked only where the time limit under Article 19 has not yet expired.)
- * Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination English

- ☒ which is the language in which the international application was filed.
☐ which is the language of a translation furnished for the purposes of international search.
☐ which is the language of publication of the international application.
☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States (that is all States which have been designated and which are bound by Chapter II of the PCT)

excluding the following States which the applicant wishes not to elect:

None

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV for the purposes of international preliminary examination.

- | | | |
|---|--|--------|
| 1 | translation of international application | sheets |
| 2 | amendments under Article 34 | sheets |
| 3 | copy (or where required, translation) of amendments under Article 19 | sheets |
| 4 | copy (or where required, translation) of statement under Article 19 | sheets |
| 5 | letter | sheets |
| 6 | other (specify) | sheets |

For International Preliminary Examining Authority use only

received	not received
☐	☐
☐	☐
☐	☐
☐	☐
☐	☐
☐	☐

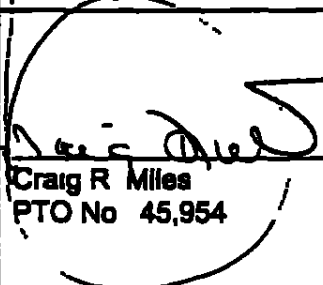
The demand is also accompanied by the item(s) marked below:

- | | |
|--|---|
| 1 ☒ fee calculation sheet | 5 ☐ statement explaining lack of signature |
| 2 ☐ original separate power of attorney | 6 ☐ sequence listing in computer readable form |
| 3 ☐ original general power of attorney | 7 ☐ other (specify) |
| 4 ☐ copy of general power of attorney reference number if any | |

Letter of Transmittal,
Certificates of Exp. Mail

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

	<u>12 10 01</u>
Craig R Miles	Date
PTO No 45,954	

For International Preliminary Examining Authority use only

1 Date of actual receipt of DEMAND:

2 Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3 ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5 below does not apply

☐ The applicant has been informed accordingly

4 ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5

5 ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date the delay in arrival is EXCUSED pursuant to Rule 82

For International Bureau use only

Demand received from IPEA on:

From the INTERNATIONAL SEARCHING AUTHORITY

To
CRAIG R. MILES
SANTANGELO LAW OFFICES P C
125 SOUTH HOWES THIRD FLOOR
FORT COLLINS CO 80521

140
PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

Date of Mailing
(day/month/year)

15 MAR 2002

Applicant's or agent's file reference
XYHIGHFURPCT

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No
PCT/US01/15150

International filing date
(day/month/year)

09 May 2001 (09 05 2001)

Applicant
XY INC

- 1 ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith
Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46r)

When* The time limit for filing such amendments is normally 3 months from the date of transmittal of the international search report; however for more details, see the notes on the accompanying sheet.

Where* Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No. (41 22) 740.14.33

For more detailed instructions see the notes on the accompanying sheet

- 2 ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

- 3 ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Office;

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made

- 4 Further action(s) The applicant is reminded of the following:

Shortly after 18 months from the priority date the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application or of the priority claim, must reach the International Bureau as provided in rules 90 bis 1 and 90 bis 3 respectively before the completion of the technical preparations for international publication.

Within 19 months from the priority date a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 30 months from the priority date the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington D C 20231
Facsimile No (703) 305 3230

Authorized officer

Dorthea Lawrence for
Sandra Salmer

Telephone No (703) 308-0196

Form PCT/ISA/220 (July 1998)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To
CRAIG R. MILES
SANTANGELO LAW OFFICES P C
125 SOUTH HOWES THIRD FLOOR
FORT COLLINS CO 80521

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

Date of Mailing
(day/month/year)

15 MAR 2002

Applicant's or agent's file reference
XYHIGHIPURPCT

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No
PCT/US01/15150

International filing date
(day/month/year)

09 May 2001 (09 05 2001)

Applicant
XY INC

1 ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith
Filing of amendments and statement under Article 19
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When* The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where* Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No. (41 22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2 ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3 ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Further action(s) The applicant is reminded of the following.

Shortly after 18 months from the priority date the international application will be published by the International Bureau.
If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application or of the priority claim, must reach the International Bureau as provided in rules 90 bis 1 and 90 bis 3 respectively before the completion of the technical preparations for international publication.

Within 19 months from the priority date a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 30 months from the priority date the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington D C 20231
Facsimile No. (703) 305 3230

Authorized officer

Dorthea Lawrence for
Sandra Smucker

Telephone No. (703) 308-0196

Form PCT/ISA/220 (July 1998)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference XYHIGHPURPCT	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as where applicable item 5 below
International application No PCT/US01/15150	International filing date (day/month/year) 09 May 2001 (09 05 2001)	(Earliest) Priority Date (day/month/year) 09 May 2000 (09 05 2000)
Applicant XY INC		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau

This international search report consists of a total of 4 sheets



It is also accompanied by a copy of each prior art document cited in this report.

1 Basis of the Report

a. With regard to the language the international search was carried out on the basis of the international application in the language in which it was filed unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b))

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application the international search was carried out on the basis of the sequence listing



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2 ☐ Certain claims were found unsearchable (See Box I)

3 ☒ Unity of invention is lacking (See Box II)

4. With regard to the title



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows

5 With regard to the abstract



the text is approved as submitted by the applicant.



the text has been established according to Rule 38.2(b) by this Authority as it appears in Box III. The applicant may within one month from the date of mailing of this international search report submit comments to this Authority

6 The figure of the drawings to be published with the abstract is Figure No. _____



as suggested by the applicant



because the applicant failed to suggest a figure



because this figure better characterizes the invention



None of the figures

INTERNATIONAL SEARCH REPORT

International application No

PCT US01/15150

143

142

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

- 1 ☐ Claim Nos
because they relate to subject matter not required to be searched by this Authority namely
- 2 ☐ Claim Nos
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out specifically
- 3 ☐ Claim Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6 4(a)

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application as follows
Please See Continuation Sheet

- 1 ☐ As all required additional search fees were timely paid by the applicant this international search report covers all searchable claims.
- 2 ☐ As all searchable claims could be searched without effort justifying an additional fee this Authority did not invite payment of any additional fee
- 3 ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos. 1-50 96-209
- 4 ☐ No required additional search fees were timely paid by the applicant. Consequently this international search report is restricted to the invention first mentioned in the claims it is covered by claims Nos

Remark on Protest ☒ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees

INTERNATIONAL SEARCH REPORT

International application No.

PCT US01/15130

A CLASSIFICATION OF SUBJECT MATTER IPC(7) 401N 1/02 C12M 3/00 US CL 435/2 288 7 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U S 435/2 288 7 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched NONE Electronic data base consulted during the international search (name of data base and, where practicable search terms used) USPATFULL on West, WPIDS AGRICOLA on STN		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate of the relevant passages	Relevance to Claim No
X	BEYHAN et al Sexual dimorphism in IVF-IVF bovine embryos produced from X and Z chromosomes-bearing spermatozoa sorted by high speed flow cytometry 01 July 1999	1 11 13 22 25 29
Y	Vol 52 No 1 pages 35-48 especially abstract.	30 31
A P	US 6 154,276 A (MARIELLA Jr) 28 November 2000.	1 50 96-209
A	US 5 985 216 A (RENS et al) 16 November 1999	1 50 96-209
A P	WO 01/51612 A1 (GALLI et al) 19 July 2001	1 50 96-209
☐ Further documents are listed in the continuation of Box C ☐ See patent family annex.		
Special categories of cited documents.		
A	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use exhibition or other means	"Z" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 14 January 2002 (14 01 2002)		Date of mailing of the international search report 15 MAR 2002
Name and mailing address of the ISA: US Commissioner of Patents and Trademarks Box PCT Washington D C 20531 Facsimile No (703)305-3230		Authorized officer Doretha Lawrence For Signature: SAUSA 107 Telephone No (703) 308-0196

172/1

BOX II OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I claims 1-31 drawn to a method of isolating X and Y sperm cells by determining a sex differentiation characteristic and separating on the basis of that characteristic

Group II claims 32-50 drawn to an apparatus to isolate X and Y sperm cells by determining a difference in volume of the sperm heads comprising a beam of electromagnetic radiation detector analyzer and sperm cells

Group III claims 51-69 drawn to a method of separating X and Y sperm cells by determining the volume of the capsule containing the DNA

Group IV claims 70-95 drawn to a method of differentiating particles

Group V claims 96-120 drawn to an apparatus to differentiate particles comprising an asymmetric particle in a fluid stream, an irradiation source optics light emission material detector and analyzer

Group VI claims 121-147 drawn to an apparatus to differentiate particles comprising a nozzle an oscillator droplets and sperm cells

Group VII claims 148-182 drawn to an apparatus comprising particles light emission source a photo-multiplier tube and an analyzer

Group VIII claims 183-209 drawn to an apparatus comprising asymmetric particles an irradiation source optics light emission material detector

The inventions listed as Groups I-VIII do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons

PCT Rules 13.1 or 13.2 do not provide for multiple methods or multiple apparatuses

The methods of Group I-III and IV all require distinct materials for example the method of Group II requires assessment of the volume of the capsule containing DNA while the methods of Groups I or IV do not require such as determination

The methods of Groups I-III or IV do not require the apparatuses of Groups II-V VI VII or VIII

The apparatuses of Groups II-V VI VIII all have distinct components and are therefore distinct apparatuses

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) XYHighPurPCT

Box No. I TITLE OF INVENTION	
High Purity Y-chromosome Bearing and Y-chromosome Bearing Populations of Spermatozoa	
Box No. II APPLICANT ☐ This person is also inventor	
Name and address. (Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	Telephone No. (970) 493-3113
XY, INC. 1108 North Lemay Avenue Fort Collins, CO 80524 United States of America	Facsimile No. (970) 493-3114
	Teleprinter No.
	Applicant's registration No. with the Office
State (that is, country) of nationality US	State (that is, country) of residence US
This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
Name and address. (Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (if this check-box is marked, do not fill in below.)
EVANS, Kenneth M 2125 Pimrose Lane Fort Collins, CO 80526 United States of America	Applicant's registration No. with the Office
	State (that is, country) of nationality US
	State (that is, country) of residence US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE, OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative	
Name and address. (Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country.)	Telephone No. (970) 224-3100
MILES, Craig R Santangelo Law Offices, P C 125 South Howes Third Floor Fort Collins, CO 80521 United States of America	Facsimile No. (970) 224-3114
	Teleprinter No.
	Agent's registration No. with the Office 45,954
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

149

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) <i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
Name and address: <i>(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> VAN MONSTER, Erik B Lijnbaansgracht 28C 1015GP Amsterdam The Netherlands	This person is ☐ applicant only ☐ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality The Netherlands	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> 	This person is. ☐ applicant only ☐ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality 	State (that is, country) of residence:
This person is applicant for the purposes of ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> 	This person is ☐ applicant only ☐ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality 	State (that is, country) of residence:
This person is applicant for the purposes of ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> 	This person is ☐ applicant only ☐ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality 	State (that is, country) of residence:
This person is applicant for the purposes of ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

Box No. 1 DESIGNATION OF STATES

Mark the applicable check-boxes below: at least one must be marked.

The following designations are hereby made under Rule 4.9(a)

Regional Patent

- ☒ AP ARIPO Patent GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- (7) ☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- (2) ☒ EP European Patent: AT Austria, BE Belgium, CH & LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, TR Turkey and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line)

- | | | |
|---|--|--|
| ☒ AE United Arab Emirates | ☒ GE Georgia | ☒ MW Malawi |
| ☒ AG Antigua and Barbuda | ☒ GH Ghana | (4) ☒ MX Mexico |
| ☒ AL Albania | ☒ GM Gambia | ☒ MZ Mozambique |
| ☒ AM Armenia | ☒ HR Croatia | ☒ NO Norway |
| ☒ AT Austria and utility model | ☒ HU Hungary | ☒ NZ New Zealand |
| (5) ☒ AU Australia | ☒ ID Indonesia | ☒ PL Poland |
| ☒ AZ Azerbaijan | ☒ IL Israel | ☒ PT Portugal |
| ☒ BA Bosnia and Herzegovina | ☒ IN India | ☒ RO Romania |
| | ☒ IS Iceland | ☒ RU Russian Federation |
| ☒ BB Barbados | (6) ☒ JP Japan | ☒ SD Sudan |
| ☒ BG Bulgaria | ☒ KE Kenya | ☒ SE Sweden |
| ☒ BR Brazil | ☒ KG Kyrgyzstan | ☒ SG Singapore |
| ☒ BY Belarus | ☒ KP Democratic People's Republic of Korea | ☒ SI Slovenia |
| ☒ BZ Belize | ☒ KR Republic of Korea | ☒ SK Slovakia and utility model |
| (3) ☒ CA Canada | ☒ KZ Kazakhstan | ☒ SL Sierra Leone |
| ☒ CH & LI Switzerland and Liechtenstein | ☒ LC Saint Lucia | ☒ TJ Tajikistan |
| (8) ☒ CN China | ☒ LK Sri Lanka | ☒ TM Turkmenistan |
| ☒ CO Colombia | ☒ LR Liberia | ☒ TR Turkey |
| ☒ CR Costa Rica | ☒ LS Lesotho | ☒ TT Trinidad and Tobago |
| ☒ CU Cuba | ☒ LT Lithuania | |
| ☒ CZ Czech Republic and utility model | ☒ LU Luxembourg | ☒ TZ United Republic of Tanzania |
| ☒ DE Germany and utility model | ☒ LV Latvia | ☒ UA Ukraine |
| ☒ DK Denmark and utility model | ☒ MA Morocco | ☒ UG Uganda |
| ☒ DM Dominica | ☒ MD Republic of Moldova | (1) ☒ US United States of America continuation in part |
| ☒ DZ Algeria | | ☒ UZ Uzbekistan |
| ☒ EE Estonia and utility model | ☒ MG Madagascar | ☒ VN Viet Nam |
| ☒ ES Spain | ☒ MK The former Yugoslav Republic of Macedonia | ☒ YU Yugoslavia |
| ☒ FI Finland and utility model | ☒ MN Mongolia | ☒ ZA South Africa |
| ☒ GB United Kingdom | | ☒ ZW Zimbabwe |
| ☒ GD Grenada | | |

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet

- ☒ All possible states and all ☒
- ☒ countries that have become ☒
- members of the PCT since issuance of this form ☒

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

Supplemental Box

if the Supplemental Box is not used, this sheet should not be included in the request.

- 1 If in any of the Boxes, except Boxes Nos. VII(1) to (4) for which a special continuation box is provided, the space is insufficient to furnish all the information, in such case, write "Continuation of Box No. ..." (indicate the number of the Box) and furnish the information in the same manner as required according to the contents of the Box in which the space was insufficient, in particular:
- (i) If more than two persons are to be indicated as applicants and/or inventors and no "continuation sheet" is available, in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.
- (ii) If, in Box No. II or in any of the sub-boxes of Box No. III the indication "the States indicated in the Supplemental Box" is checked, in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant.
- (iii) If, in Box No. II or in any of the sub-boxes of Box No. III the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America, in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor.
- (iv) If, in addition to the agent(s) indicated in Box No. IV there are further agents, in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV.
- (v) If, in Box No. V the name of any State (or OAPI) is accompanied by the indication "parent of addition," or "certificate of addition," or if, in Box No. V the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part" in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application.
- (vi) If in Box No. VI there are more than five earlier applications whose priority is claimed, in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.
- 2 If, with regard to the precautionary designation statement contained in Box No. V the applicant wishes to exclude any State(s) from the scope of that statement in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

CONTINUATION OF BOX VI

- 1 This application claims benefit of United States Patent Application Number 60/203,089 filed on (09 05 00) 09 May 2000, and
- 2 This application claims benefit of United States Patent Application Number 60/239,752 filed on (12 10 00) 12 October 2000, and
- 3 This application claims benefit of United States Patent Application Number 60/267,571 filed on (10 02 01) 10 February 2001

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application, country	regional application ^a , regional Office	international application, receiving Office
item (1) (09.05 00) 09 May 2000	60/203,089	US		
item (2) (12 10.00) 12 October 2000	60/239,752	US		
item (3) (10.02 01) 10 February 2001	60/267,571	US		
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office) identified above as:

☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other see Supplemental Box^a Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii))**Box No. VII INTERNATIONAL SEARCHING AUTHORITY**

Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen, the two-letter code may be used)

ISA / US

Request to use results of earlier search, reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority).

Date (day/month/year)

Number

Country (or regional Office)

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos VIII (i) to (v) (mark the applicable check-boxes below and indicate in the right column the number of each type of declaration):

Number of declarations

- ☐ Box No. VIII (i) Declaration as to the identity of the inventor
- ☐ Box No. VIII (ii) Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent
- ☐ Box No. VIII (iii) Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application
- ☒ Box No. VIII (iv) Declaration of inventorship (only for the purposes of the designation of the United States of America)
- ☐ Box No. VIII (v) Declaration as to non-prejudicial disclosures or exceptions to lack of novelty

1

157

Box No. VIII (iv) **DECLARATION ON INVENTORSHIP** (only for the purposes of the designation of the United States of America)
The declaration must conform to the following prescribed wording provided for in Section 214; see Notes to Section 214, PCT (I) in (a) (in general) and the specific Notes to Box No. VIII (iv). If this Box is not used, its then should not be included in the request.

Declaration of Inventorship (Rules 4.170v) and 516a.1(a)(iv))
(for the purposes of the designation of the United States of America).

I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought.

This declaration is directed to the international application of which is form a part (if filing declaration with application).

This declaration is directed to international application No. PCT/

(if furnishing declaration pursuant to Rule 26ter).

I hereby declare that my residence, mailing address, and citizenship are as stated next to my name.

I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.16, my claim to foreign priority, and I have identified below under the heading "Prior Applications," by application number, country or Member of the World Trade Organization, day, month and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.

Prior Applications US s/n 60/203,099, IN s/n 60/239,752 and US s/n 60/267,571

I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name: Kenneth M. Evans

Residence: 2125 Primrose Lane, Fort Collins, CO 80526, USA

(city and either US state, if applicable, or country)

Mailing Address: 2125 Primrose Lane, Fort Collins, CO 80526, USA

Citizenship: US

Inventor's Signature:

(If not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor not that of the agent)

Date: May 9, 2001

(if signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)

Name: Erik A. van Marster

Residence: IJzermansgracht 28C, 1015GP Amsterdam, The Netherlands

(city and either US state, if applicable, or country)

Mailing Address: IJzermansgracht 28C, 1015GP Amsterdam, The Netherlands

1619 Stover Street, Fort Collins, CO 80525, USA

Citizenship: The Netherlands

Inventor's Signature:

(If not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor not that of the agent)

Date: May 9, 2001

(if signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (iv)"

Box No. IX CHECK LIST LANGUAGE OF FILING

This international application contains: (a) the following number of sheets in paper form request (including declaration sheets) 7 description (excluding sequence listing part) 51 claims 40 abstract 1 drawings 28 Sub-total number of sheets 127 sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form, see (b) below) Total number of sheets 127 (b) sequence listing part of description filed in computer readable form (i) ☐ only (under Section 801(a)(i)) (ii) ☐ in addition to being filed in paper form (under Section 801(a)(ii)) Type and number of carriers (diskette, CD-ROM, CD-R or other) on which the sequence listing part is contained (additional copies to be indicated under item 9(ii) in right column)		This international application is accompanied by the following items (mark the applicable check-boxes below and indicate in right column the number of each item). 1 ☒ fee calculation sheet 1 2 ☒ original separate power of attorney 1 3 ☐ original general power of attorney 4 ☐ copy of general power of attorney "reference number if any" 5 ☐ statement explaining lack of signature 6 ☐ priority document(s) identified in Box No. VI as item(s): 7 ☐ translation of international application into (language): 8 ☐ separate indications concerning deposited microorganism or other biological material 9 ☐ sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other)) (i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application) (ii) ☐ (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter (iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column 10 ☒ other (specify): Letter of Transmittal, USPO Letter of Transmittal
Figure of the drawings which should accompany the abstract: Fig. 10	Language of filing of the international application: Certificates of Express mailing English	

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from result of the request).

 5901
Craig R Miles

For receiving Office use only	
1 Date of actual receipt of the purported international application.	2 Drawings. ☐ received. ☐ not received.
3 Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application.	
4 Date of timely receipt of the required corrections under PCT Article 11(2):	
5 International Searching Authority (if two or more are competent) ISA /	6 ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only	
Date of receipt of the record copy by the International Bureau.	

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's
file reference

XYHighPurPCT

Applicant

XY, Inc.

CALCULATION OF PRESCRIBED FEES

1 TRANSMITTAL FEE

240.00

T

2 SEARCH FEE

450.00

S

International search to be carried out by ITS
(If two or more International Searching Authorities are requested to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3 INTERNATIONAL FEE

Basic Fee

Where item (b) of Box No. IX applies, enter Sub-total number of sheets

Where item (b) of Box No. IX does not apply enter Total number of sheets

b1 first 30 sheets

382.00

b1

b2 97

number of sheets
in excess of 30

x 9.00

fee per sheet

= 873.00

b2

b3 additional component (only if sequence listing part of description
is filed in computer readable form under Section 801(a)(i), or
both in that form and on paper under Section 801(a)(ii))

400 x

fee per sheet

=

b3

Add amounts entered at b1, b2 and b3 and enter total at B

1255.00

B

Designation Fees

The international application contains all designations.

6

number of designation fees
payable (maximum 6)

x 82.00

amount of designation fee

= 492.00

D

Add amounts entered at B and D and enter total at I

1747.00

I

(Applicants from certain States are entitled to a reduction of 75% of the
international fee. Where the applicant is (or all applicants are) so entitled, the total
to be entered at I is 25% of the sum of the amounts entered at B and D.)

45.00

P

4 FEE FOR PRIORITY DOCUMENT (if applicable)

5 TOTAL FEES PAYABLE

Add amounts entered at T, S, I and P and enter total in the TOTAL box

2482.00

TOTAL

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☐ authorization to charge
deposit account (see below)

☐ postal money order

☐ cash

☐ coupons

☒ cheque

☐ bank draft

☐ revenue stamps

☐ other (specify)

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

(This mode of payment may not be available at all receiving Offices)

☐ Authorization to charge the total fees indicated above

☐ (This check-box may be marked only if the conditions for deposit accounts
of the receiving Office so permit) Authorization to charge any deficiency
or credit any overpayment in the total fees indicated above.

☐ Authorization to charge the fee for priority document.

Receiving Office RO/

Deposit Account No.

Date:

Name

Signature

PATENT COOPERATION TREATY

154
154

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

WRITTEN OPINION

(PCT Rule 66)

To
CRAIG R. MILES
SANTANGELO LAW OFFICES, P.C.
188 SOUTH HOWES THIRD FLOOR
FORT COLLINS, CO 80501

Date of Mailing
(day/month/year) 01 OCT 2002

Applicant's or agent's file reference
XYHIOHPUSPCT

REPLY DUE within ONE month
from the above date of mailing

International application No.
PCT/US01/16150

International filing date (day/month/year)
09 MAY 2001

Priority date (day/month/year)
09 MAY 2000

International Patent Classification (IPC) or both national classification and IPC
IPC(7): A01N 1/08; C12M 3/00 and US Cl. 435/2, 338 7

Applicant
XY INC.

1 This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority

2. This opinion contains indications relating to the following items

I ☒ Basis of the opinion

II ☐ Priority

III ☒ Non-establishment of opinion with regard to novelty inventive step or industrial applicability

IV ☒ Lack of unity of invention

V ☒ Reasoned statement under Rule 66.2(a)(i) with regard to novelty inventive step or industrial applicability
reasons and explanations supporting such statement

VI ☐ Certain documents cited

VII ☐ Certain defects in the international application

VIII ☐ Certain observations on the international application

3 The applicant is hereby invited to reply to this opinion

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(4).

How? By submitting a written reply accompanied, where appropriate by amendments according to Rule 66.3 for the form and the language of the amendments see Rules 66.5 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments see Rule 66.4 bis.
For an informal communication with the examiner see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 66.2 is: 09 SEPTEMBER 2002

Name and mailing address of the IPEA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20531

Authorized officer
Christopher Tate
CHRISTOPHER TATE

Facsimile No. (703) 305-5230

Telephone No. (703) 305-0166

WRITTEN OPINION

International application No

PCT/US01/15150

155

I Basis of the opinion

1 With regard to the elements of the international application:

- ☒ the international application as originally filed
- ☒ the description.
 pages 1-31 as originally filed
 pages NONE filed with the demand
 pages NONE filed with the letter of
- ☒ the claims
 pages 52-91 as originally filed
 pages NONE as amended (together with any statement) under Article 19
 pages NONE filed with the demand
 pages NONE filed with the letter of
- ☒ the drawings
 pages 1-28 as originally filed
 pages NONE filed with the demand
 pages NONE filed with the letter of
- ☒ the sequence listing part of the description
 pages NONE as originally filed
 pages NONE filed with the demand
 pages NONE filed with the letter of

2 With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
 These elements were available or furnished to this Authority in the following language which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b))
- ☐ the language of publication of the international application (under Rule 48.3(b))
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3 With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the written opinion was drawn on the basis of the sequence listing.

- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4 ☒ The amendments have resulted in the cancellation of

- ☒ the description, pages NONE
- ☒ the claims Nos. NONE
- ☒ the drawings sheets/fig NONE

5 ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as originally filed

WRITTEN OPINION

International application No.
PCT/US01/15150

156

III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of

☐ the entire international application.

☒ claims Nos. 51-85

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination (*specify*).

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. _ are so unclear that no meaningful opinion could be formed (*specify*)

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 51-85.

2 A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions.

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard

WRITTEN OPINION

International application No. 157
PCT/US01/15150

IV Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees the applicant has:

- ☒ restricted the claims.
☐ paid additional fees.
☐ paid additional fees under protest.
☐ neither restricted nor paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 83.1 not to invite the applicant to restrict or pay additional fees:

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

- ☐ all parts.
☒ the parts relating to claims Nos. 1-31.

V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability, citations and explanations supporting such statement**1 statement****Novelty (N)**

Claims 12 24, 25, 30 31

YES

Claims 1-11 13-23 26-29

NO

Inventive Step (IS)

Claims 25 26

YES

Claims 1-23, 26-31

NO

Industrial Applicability (IA)

Claims 1-31

YES

Claims NONE

NO

2 citations and explanations

Claims 1-11 13-23, and 26-29 lack novelty under PCT Article 33(3) as being anticipated by Beyhan et al.

Beyhan et al. reasonably teach a method of isolating X- and Y-chromosome bearing sperm cells from bovine using the claimed steps (see entire document).

Claims 1-23 and 26-31 lack an inventive step under PCT Article 33(3) as being obvious over Beyhan et al.

Beyhan et al. reasonably teach a method of isolating X- and Y-chromosome bearing sperm cells from bovine using the claimed steps (see entire document). However if not expressly taught, the adjustment of conventional working parameters (such as maintaining unstained DNA within the nucleus of the spermatozoa and/or substituting other livestock mammals such as ovine or equine from which to isolate X- and Y-chromosome bearing sperm cells) would have been merely a matter of judicious selection and routine optimization which is within the purview of the skilled artisan having the cited reference as a guide.

Claims 25 and 26 meet the criteria set out in PCT Article 33(3)-(4), because the prior art does not teach or fairly suggest an overall method of isolating X- and Y-chromosome bearing sperm cells which include the steps recited in claims 25 and 26.

Claims 1-31 meet the criteria set out in PCT Article 33(4) under industrial applicability because the method can be used to efficiently isolate X- and Y-chromosome bearing sperm cells from various mammalian species.

NEW CITATIONS

NONE

WRITTEN OPINION

159
international application No.

PCT/US01/15150

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Boxes I VIII

Sheet 10

TIME LIMIT

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d) Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

160 1581

POBLACIONES DE ESPERMA DE ALTA PUREZA QUE CONTIENEN
CROMOSOMA X E Y.

CAMPO TECNICO

Las poblaciones de espermatozoides de alta pureza que contienen cromosoma X e Y aislados y tecnologías para aislar espermatozoides, partículas, o eventos basados en las características de diferenciación tales como masa, volumen, contenido de ADN, o lo similar

ANTECEDENTES

Las poblaciones de espermatozoides de alta pureza que contienen cromosoma X e Y aislados pueden ser utilizadas para realizar inseminación artificial en vivo o in vitro o la fertilización de ovarios u óvulos de varios mamíferos tales como bovinos, equinos, ovinos, cabras, porcinos, perros, gatos, camellos, elefantes, bueyes, búfalos, o lo similar Ver además también la patente norteamericana U S No 5 135 759, incorporada a la presente a modo de referencia

Sin embargo, las tecnologías convencionales para la separación de espermatozoides en poblaciones que contienen cromosoma X y cromosoma Y dan como resultado en una

población de espermatozoides que presentan una baja puridad. Independientemente del método de separación, los espermatozoides no han sido separados rutinariamente en muestras de esperma que contienen cromosoma Y y cromosoma X que presentan una alta puridad tal como 90%, 95% o más del 95%.

Un número de técnicas, directamente o indirectamente basadas en las diferencias de tamaño o masa de la densidad se han divulgado con respecto a la separación del espermatozoide que contiene cromosoma X del espermatozoide que contiene cromosoma Y. Tal como se divulga por la patente norteamericana U S No 4 474 875, una fuerza flotante es aplicada a todas las células de esperma simultáneamente y el espermatozoide que contiene el cromosoma X y el que contiene el cromosoma Y pueden ser aislados en diferentes ubicaciones en el medio de la separación. La patente norteamericana U S No 4 605 558, divulga que el esperma puede ser hecho diferencialmente sensible a una densidad gradual mientras que la patente norteamericana U S No 4 009 260 explota las diferencias en la velocidad de migración, o la velocidad de inmersión entre el espermatozoide que contiene el cromosoma Y y el espermatozoide que contiene el cromosoma X a través de una columna de medio retardado.

Un problema común a cada una de las técnicas mencionadas anteriormente puede ser que cada una actúa en todos los espermatozoides en una manera de bulto, significando que todos los espermatozoides se someten al mismo tratamiento en el mismo momento, y las células de esperma que contiene el cromosoma Y salen más rápido, más fácil o a una diferente posición que las células de esperma que contiene el cromosoma X. Como tal, las células de espermatozoides individuales no pueden ser evaluadas y puede no haber una medición actual de volumen, masa, densidad u otras características de células de esperma. La evaluación de uno por uno de células de esperma puede proveer ventajas en que el proceso de separación actual puede ser monitoreado, los datos cuantitativos objetivos pueden ser generados incluso durante el proceso de separación, y los parámetros de separación pueden ser alterados tanto como se desee. Además, estas tecnologías pueden no ser acopladas con los dispositivos de clasificación de célula de flujo.

Las técnicas de citómetro de flujo para la separación de espermatozoides también ha sido divulgada. Utilizando estas técnicas el espermatozoide puede ser manchado con un fluorocromo y forzado a que fluya en una corriente angosta o banda pasando por una fuente de excitación o irradiación tal como un rayo láser. Mientras que las partículas

manchadas o células pasan a través de la excitación o la fuente de irradiación, el fluorocromo emite la luz fluorescente. La luz fluorescente puede ser recolectada por un conjunto de lentes ópticas, enfocadas en un detector, tal como un tubo fotomultiplicador el cual genera y multiplica una señal electrónica, la cual puede ser luego analizada por medio de un analizador. Los datos pueden ser luego visualizados como histogramas o cromatogramas de parámetros individuales o múltiples. El número de células y células por fluorescencia pueden ser utilizadas como coordenadas. Ver también la patente norteamericana U S No 5 135 759, incorporada a la presente a modo de referencia. Sin embargo, con respecto a este tipo de tecnología una variedad de problemas pueden mantenerse sin resolver y puede ser difícil el aislamiento de las células de espermatozoos que contienen cromosoma X y cromosoma Y de las poblaciones altamente purificadas.

Un problema significativo con las tecnologías de citómetro de flujo convencionales puede ser la orientación de objetos, partículas o células en la vaina de la corriente de fluido. Esto puede ser particularmente problemático cuando el objeto o célula es irregular en su forma con respecto a más de un eje, tal como por ejemplo el espermatozoide. Un aspecto de este problema puede ser

establecido en la orientación inicial del objeto dentro de la vaina de corriente de fluido. Un segundo aspecto de este problema puede ser mantener la orientación del objeto con respecto al detector (tubo fotomultiplicador o lo similar) durante el período en que es medida la luz emitida desde el objeto.

Otro problema significativo con las tecnologías de citómetro de flujo convencionales pueden ser las fallas para encapsular los objetos o células en una gota de líquido. Especialmente, cuando las gotas son formadas alrededor de objetos de formas irregulares, las gotas pueden no ser del suficiente tamaño para rodear en su totalidad todas las características de los objetos o células. Por ejemplo, durante la operación de citometría de flujo tal como fue mencionado anteriormente las gotas pueden ser formadas a una alta velocidad, incluso desde 10 000 a 90 000 gotas por segundo y en algunas aplicaciones tantas como 80 000 gotas por segundo. Cuando los espermatozoides son encapsulados en gotas, especialmente a estas altas velocidades, una porción de la cola o cuello puede no ser encapsulada en la gota. Aquella porción de la cola o cuello no encapsulada en la gota puede ser sensible con la boquilla o puede ser sensible al medio ambiente que rodea la gota en una manera que interfiere con la formación

subsecuente de gotas o con la desviación propia de la gota. Como resultado algunos de los espermatozoides pueden no ser analizados en su totalidad reduciendo la eficacia del procedimiento, o no pueden ser resueltos suficientemente para ser asignados a una población, o pueden ser desviados en trayectorias errantes o una combinación de la totalidad de lo mencionado puede suceder.

Otro problema significativo con las tecnologías de citómetro de flujo convencionales, así también como otras tecnologías, puede ser una coincidencia de los eventos medidos. Un aspecto de este problema puede ser que el flujo de luz incidente de un primer evento continúa produciendo señales luego de que el flujo de luz incidente de un segundo evento comienza a generar una señal. Como tal, los dos eventos se mantienen al menos parcialmente sin resolver uno con respecto del otro. Otro aspecto de este problema puede ser que dos o más eventos son iniciados simultáneamente y el flujo de luz incidente comprende la contribución de todos los eventos. Como tal, la multiplicidad de eventos puede no ser resuelta en su totalidad y los objetos correspondientes a la multiplicidad de eventos pueden ser incorrectamente asignados a una población o no ser asignados a una población, o ambos casos. Específicamente, con respecto a la citometría de

flujo, las partículas individuales, objetos, células, o espermatozoides en suspensión de flujo a través de un rayo de luz con los cuales éstos interactúan proveyendo una respuesta medible, tal como la emisión fluorescente. En la citometría de flujo convencional, los espermatozoides manchados con Hoechst atraviesan un rayo de láser que resulta en una emisión de luz fluorescente. La emisión de luz fluorescente del fluorocromo excitado que rodea al ADN puede ser lo suficientemente brillante para producir un flujo de electrones en los tubos fotomultiplicadores convencionales por un período de tiempo luego de que el evento de emisión actual haya terminado. Mas aun, en un citómetro de flujo convencional, el rayo láser puede producir un patrón que presenta una altura de 30 μm mientras que el ancho puede ser aproximadamente de 80 μm . El núcleo de un espermatozoide bovino, el cual contiene fluorocromo unido al ADN puede ser de alrededor de 9 μm en longitud haciendo la altura del rayo láser tres (3) veces más grande que el núcleo. Esta diferencia puede permitir la excitación del láser de la unión del fluorocromo en más de un espermatozoide dentro del patrón de rayo de láser en una sola vez. Cada uno de estos problemas de citometría de flujos convencionales reducen la habilidad de resolver los eventos individuales uno con respecto del otro.

Otro problema significativo con las tecnologías de citómetro de flujo convencional, y otras tecnologías, puede ser que los objetos de forma irregular, tal como los espermatozoides, generan señales diferentes (forma, duración o cantidad) dependiendo de su orientación dentro del paso de detección/excitación. Como tal, los individuales dentro de la población homogénea puede generar un amplio espectro de características de emisión que pueden superponerse con las características de emisión de los individuales de otras poblaciones homogéneas obviando o reduciendo la disponibilidad para resolver los individuales de las dos poblaciones.

Otro problema significativo con las tecnologías de citómetro de flujo convencionales, y otras tecnologías, puede ser que los objetos no son expuestos uniformemente a la fuente de excitación. Las ópticas de forma de rayo convencional pueden no proveer una exposición uniforme a la luz láser cuando los objetos están cerca de la periferia del rayo.

Otro problema significativo con las tecnologías de citómetro de flujo convencionales puede ser que los objetos, tales como los espermatozoides, pueden ser expuestos a la fuente de excitación por períodos de tiempo innecesariamente largo. La irradiación de las células,

tales como espermatozoides con luz láser puede dañar las células o el ADN contenido dentro de las mismas

Otro problema significativo con las tecnologías de citómetro de flujo convencional puede ser que pueda existir una interrupción del flujo laminar dentro de la boquilla por medio del tubo de inyección. La interrupción del flujo laminar puede cambiar la orientación de los objetos de forma irregulares dentro del flujo o reducir la velocidad de clasificación y la pureza de el espermatozoide que contiene cromosoma X o el espermatozoide que contiene cromosoma Y de las poblaciones clasificadas

Pueden existir problemas adicionales con las tecnologías que utilizan manchas que contienen el ADN nuclear de las células de espermatozoide. Primero, debido a que el ADN en el núcleo es altamente condensado y es de forma plana, manchando esteoquímicamente el ADN puede ser difícil o imposible. Segundo, el núcleo manchado puede tener un alto índice de refracción. Tercero, la mancha que contiene el ADN para formar un complejo de mancha ADN puede reducir las probabilidades de fertilización o la viabilidad de los embriones subsecuentes. Cuarto, el complejo mancha ADN es típicamente irradiado con luz ultravioleta para hacer que la mancha florezca. Esta irradiación puede

169 ~~169~~
1

afectar la viabilidad del espermatozoide Debido a estos varios problemas, puede ser preferible utilizar un método que requiera menos o ninguna mancha, o menos o la no radiación de ultravioleta, o menos o nada de ambos

Con respecto a la generación de las muestras de alta puridad de poblaciones de células de esperma que contienen cromosoma X y cromosoma Y (ya sea vivo, fijo, viable, no viable, intacto, sin cola o como núcleo), o generalmente con respecto a la detección de pequeñas diferencias en las señales fotogeneradas entre los eventos seriales que presentan un flujo de luz incidente relativamente alto, o con respecto a la orientación de los objetos de forma irregular en una corriente de fluido, o en la eliminación de eventos coincidentes dentro de un paso óptico, o la remoción no deseada de los objetos orientados para el análisis, la presente invención se dirige a cada uno de los problemas mencionados anteriormente en una manera práctica

III DIVULGACIÓN DE LA INVENCION

Un objeto amplio de la invención puede ser proveer poblaciones que contienen cromosoma x y cromosoma Y de alta puridad de espermatozoides El aislamiento de las poblaciones que suceden no naturalmente de espermatozoides

que tienen una alta puridad tienen numerosas aplicaciones incluyendo la selección de sexo de la descendencia de mamíferos, varios protocolos in vitro para la fertilización de óvulos, varios protocolos en vivo tales como inseminación artificial, métodos de negocio que incluyen la producción de animales preciados o carne de animales, o la preservación de animales en extinción o raros, para mencionar unas pocas solicitudes para las poblaciones de alta puridad de espermatozoides

Otro amplio aspecto de la invención incorpora ambos dispositivos y métodos para la producción de muestras de esperma de alta puridad que contienen cromosomas X y cromosomas Y

Las realizaciones particulares de la invención son descritas, las cuales pueden utilizarse en varias aplicaciones tales como fueron mencionadas anteriormente, que pueden ser utilizadas para realizar los objetos específicos para la diferenciación entre eventos fotoemisivos brillantes que presentan pequeñas diferencias medibles en el flujo de luz total, la orientación de objetos de forma irregular en una corriente de fluido, la minimización de los eventos coincidentes dentro de un paso óptico, la remoción de la señal contribuida por los objetos

171 

no orientados no deseados dentro de un paso óptico (incluyendo la remoción del objeto mismo), y la encapsulación de los objetos de forma irregular dentro de una gota. Como tal, los objetos específicos de la invención pueden ser algo variados.

Otro aspecto amplio de la invención puede ser el proveer muestras de espermatozoides que contienen cromosoma X y cromosoma Y (vivo, fijo, viable, no viable, intacto, sin cola o núcleo de espermatozoide) que presenta un nivel graduado de alta pureza en el rango de 80%, 85%, 90%, 95%, o incluso mayor que 95%.

Otro objeto significativo de las realizaciones particulares de la invención puede ser la clasificación de espermatozoides en poblaciones que contienen cromosoma X y cromosoma Y que presenta una alta pureza incluso en altas velocidades de separación. Las altas velocidades de separación pueden producir espermatozoide vivo de cada sexo a velocidades de 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 o incluso 10 000 por segundo o mayor.

Otro objeto significativo de las realizaciones particulares de la invención puede ser el de eliminar sustancialmente o remover el espermatozoide (vivo, fijo,

viable, no viable, intacto, sin cola o núcleo de esperma) que presenta la orientación indeseada en la porción de excitación/detección en el paso de flujo de un citómetro de flujo

Otro objeto significativo de las realizaciones particulares de la invención puede ser proveer muestras de inseminación artificial de espermatozoides que contienen cromosoma X y cromosoma Y que tienen un alto nivel de pureza

Otro objeto significativo de las realizaciones particulares de la invención puede ser proveer muestras de inseminación in vitro de espermatozoides que contienen cromosoma X y cromosoma Y que presentan un alto nivel de pureza

Otro objeto significativo de una realización particular de la invención puede ser preseleccionar el sexo de los descendientes femeninos inseminados con una muestra de inseminación artificial de alta pureza, el sexo de descendencia del óvulo fertilizado con muestras de inseminación artificial de alta pureza, con un promedio de éxito de selección de 80%, 85%, 90%, 95%, o mayor que 95%

Otro objeto significativo de las realizaciones particulares de la invención puede ser diferenciar los eventos fotoemisivos que presentan pequeñas diferencias en un total de flujo de luz emitido

Otro objeto significativo de las realizaciones particulares de la invención puede ser el de eliminar sustancialmente o reducir la cantidad de ruido de fondo generado por un tubo fotomultiplicador, incluso en la ausencia de luz, durante el período posterior a la exposición de un flujo de luz incidente alto

Otro objeto significativo de las realizaciones particulares de la invención pueden ser el de eliminar sustancialmente la saturación del fotocátodo de los tubos fotomultiplicadores utilizados en conjunto con el citómetro de flujo o de otra manera

Otro objeto significativo de las realizaciones particulares de la invención puede ser el reducir el número de electrones que migran desde el fotocátodo de un tubo fotomultiplicador hacia el primer dínodo

Otro objeto significativo de las realizaciones particulares de la invención puede ser el reducir el flujo

174 ~~171~~

total de electrones hacia el electrón N de un tubo fotomultiplicador

Otro objeto significativo de las realizaciones particulares de la invención puede ser permitir la incrementación del flujo de luz hacia el fotocátodo del tubo fotomultiplicador sin aumentar proporcionalmente la cantidad de señal de fondo generada por el tubo fotomultiplicador

Otro objeto significativo de las realizaciones particulares de la invención puede ser incrementar el promedio de la señal de fondo de los eventos fotoemisivos medidos

Otro objeto significativo de las realizaciones particulares de la invención puede ser permitir la ampliación incrementada de la señal generada del tubo fotomultiplicador durante los eventos de flujo de luz incidentes altos o la serie de eventos de luz incidentes altos sin saturar el fotocátodo del tubo fotomultiplicador

Otro objeto significativo de las realizaciones particulares de la invención puede ser incrementar la resolución aparente de cromatogramas o histogramas que

resultan de la clasificación de esperma manchada con fluorocromo, u otras células, u otros objetos, presentando pequeñas diferencias en el flujo de luz emitida luego de la excitación de los fluorocromos unidos

Otro objeto significativo de las realizaciones particulares de la invención puede ser el de mejorar la calibración de los instrumentos de citómetro de flujo de clasificación cuando son utilizados para clasificar espermatozoides

Otro objeto significativo de las realizaciones particulares de la invención puede ser incrementar la velocidad de clasificación de esperma de los sistemas de citómetro de flujo

Otro objeto significativo de las realizaciones particulares de la invención puede ser incrementar la pureza de las muestras de esperma clasificadas por el citómetro de flujo

Otro objeto significativo de las realizaciones particulares de la invención puede ser proveer técnicas de clasificación de esperma que contiene cromosoma X y cromosoma Y en donde las pequeñas diferencias en la

cantidad de ADN cromosoma Y sobre la cantidad de ADN cromosoma X con respecto a la cantidad total del ADN nuclear

Otro objeto significativo de las realizaciones particulares de la invención puede ser proveer técnicas las cuales mejoran la resolución aparente de los histogramas generados durante el proceso de clasificación de esperma que contiene cromosoma X y de esperma que contiene cromosoma Y con un citómetro de flujo

Otro objeto significativo de las realizaciones particulares de la invención puede ser proveer ópticas de formación de rayo, las cuales minimizan la coincidencia de objetos dentro del paso de detección/excitación

Otro objeto significativo de las realizaciones particulares de la invención puede ser proveer ópticas de formación de rayo que minimizan los lúmenes totales a los cuales un objeto es expuesto para atravesar el rayo de excitación. Un aspecto de este objeto puede ser para reducir los lúmenes totales a los cuales un objeto está expuesto. Un segundo aspecto de este objeto puede ser el incrementar la energía de la fuente de luz sin reducir los lúmenes totales a los cuales el objeto está expuesto.

Otro objeto significativo de las realizaciones particulares de la invención puede ser la provisión de ópticas formadoras de rayo que permitan la exposición uniforme de objetos que pasan a través del paso óptico

Otro objeto significativo de las realizaciones particulares de la invención puede ser proveer una boquilla que oriente los objetos de forma irregulares en una corriente de fluido. Un aspecto de este objeto puede ser orientar los objetos alargados en la misma dirección. Un segundo aspecto de este objeto puede ser la orientación de los objetos dorso-lateralmente aplanados en la misma dirección.

Otro objeto significativo de las realizaciones particulares de la invención puede ser el encapsular en la totalidad los objetos de forma irregular dentro de una gota de fluido.

Otro objeto significativo de las realizaciones particulares de la invención puede ser el diferenciar los objetos orientados indeseablemente de los objetos orientados deseablemente en una corriente de fluido.

Otro objeto de una realización de la invención puede ser proveer la tecnología de contraste de interferencia diferencial, en donde el plano objeto consiste de una corriente de fluido que transporta los objetos de interés, y de esta manera el plano imagen puede ser utilizado para medir la señal desde los objetos que pasan

Otro objeto de una realización de la invención puede ser el proveer ópticas que forman dos imágenes separadas lateralmente una con respecto a la otra de tal manera que una puede ser utilizada para medir el volumen actual, y otra para determinar la orientación. De esta manera, los objetos que no fueron orientados propiamente para permitir una medición precisa de su volumen pueden ser descartados. Esto puede ser realizado por modificaciones para que los pulsos de luz, resultantes de estas dos imágenes puedan ser detectados independientemente utilizando dos orificios de perno en el plano de imagen. Las ópticas son sintonizadas de tal manera que una primera imagen puede dar un ascenso a un pulso de luz proporcional al volumen del objeto, y que la segunda imagen puede dar un ascenso a un pulso de luz dependiente de la orientación que haya tenido el objeto cuando éste fue medido.

Otro objeto de una realización de la invención puede proveer una manera de compensar el hecho de que los objetos son contenidos dentro de la corriente de fluido. La corriente de fluido puede ser un cilindro de agua, por ejemplo, el cual actúa como una lente cilíndrica, de esta manera distorsionando la imagen del objeto. Ópticamente, esto corresponde al cilindro de mayor índice refractivo (agua) que sus alrededores (aire). La compensación divulgada en esta invención puede consistir de, por ejemplo, un cilindro que presenta un índice inferior refractivo que sus alrededores, aunque otros elementos compensadores de varias formas e índices refractivos pueden además ser diseñados tanto como lo requiera la situación. Asegurándose de que la luz pase a través de este elemento de compensación, el efecto óptico de la corriente de fluido puede ser compensado por el comportamiento opuesto exacto del elemento de compensación.

IV BREVE DESCRIPCIÓN DE LOS DIBUJOS

La figura 1 muestra un citómetro de flujo generalizado.

La figura 2 muestra una segunda vista de un citómetro de flujo generalizado.

La figura 3 muestra una comparación de un histograma de una sola variable de un citómetro de flujo (#1, #2 y #3) sin la invención amplificada (figura 3A) con histogramas de una sola variable para el mismo citómetro de flujo utilizando una realización particular de la invención de ampliación (figura 3B) ilustrando la resolución mejorada entre las poblaciones de esperma bovina que contienen cromosoma X y cromosoma Y

La figura 4 ilustra histogramas de una sola variable y de dos variables que ilustran la resolución convencional entre las poblaciones de esperma bovina que contienen cromosoma X y cromosoma Y

La figura 5 muestra histogramas de dos variables y de una sola variable que ilustran la resolución mejorada entre la población de esperma bovina que contiene cromosoma X y cromosoma Y utilizando una realización particular de la invención de ampliación

La figura 6 muestra un segundo ejemplo de histogramas de dos variables y de una sola variable que ilustran la resolución convencional entre las poblaciones de esperma bovina que contienen cromosoma X y que contienen cromosoma Y

La figura 7 muestra un segundo ejemplo de un histograma univariable y bivariable que ilustra la resolución mejorada entre las poblaciones de esperma bovina que contiene cromosoma X y cromosoma Y utilizando una realización particular de la invención de amplificación

La figura 8 muestra histogramas de dos variables y de una sola variable que ilustran la resolución convencional entre las poblaciones de espermatozoides equinos que contiene cromosoma X y cromosoma Y

La figura 9 muestra histogramas de una sola variable y de dos variables que ilustran la resolución mejorada entre las poblaciones que contienen cromosoma X y cromosoma Y de espermatozoides equinos utilizando una realización particular de la invención de amplificación

La figura 10 muestra histogramas de una sola variable y de dos variables que ilustran la resolución mejorada entre las poblaciones de espermatozoides equinos que contienen cromosoma X y cromosoma Y utilizando una realización particular de la invención de amplificación

La figura 11 muestra una realización particular del tablero de circuito modificado para realizar la invención de amplificación con respecto a un citómetro de flujo MoFlo

La figura 12 muestra el diagrama esquemático eléctrico de una realización particular de la invención de amplificación con respecto a un citómetro de flujo MoFlo

La figura 13 muestra un patrón de rayo láser que utiliza las ópticas de forma de rayo convencional (figura 13A) y el patrón de rayo láser que utiliza ópticas de forma de rayo alto reducido (figura 13B)

La figura 14 muestra un gráfico de barra que compara la puridad del espermatozoide que contiene cromosoma X separado (figura 14A) y el espermatozoide que contiene cromosoma Y (figura 14B) que utiliza la tecnología convencional o utiliza la invención de amplificación independientemente o en conjunto con las ópticas de forma de rayo de altura reducida

La figura 15 muestra una vista frontal de las ópticas de forma de rayo de altura reducida

La figura 16 muestra una vista superior de las ópticas de forma de rayo de altura reducida

La figura 17 muestra una perspectiva y dos vistas en corte del objeto que orienta la boquilla de la invención

La figura 18 muestra una serie graduada de secciones transversales del objeto que orienta la boquilla de la invención

La figura 19 muestra una vista frontal y una posterior de una realización del tubo de inyección biselado de la invención

La figura 20 ilustra la remoción de los espermatozoides sin orientar no deseados (RUUS) por medio de las señales de comparación de los espermatozoides orientados (figuras 20A y 20B) y las señales del espermatozoide sin orientar (figuras 20C y 20D)

La figura 21 muestra una perspectiva de otra realización del tubo de inyección biselado de la invención que presenta una cuchilla biselada en forma de paleta

La figura 22 muestra una tecnología de ópticas convencionales acoplada a un citómetro de fluido

La figura 23A muestra la forma y tamaño de un espermatozoide típico y la figura 23B muestra la diferencia entre los espermatozoides correctamente y no correctamente orientados

La figura 24 muestra una realización de la invención que presenta una construcción que permite la medición de dos señales, por ejemplo volumen y orientación

Las figuras 25A Y 25B muestran una realización de la invención que presenta dos mitades con una perforación de perno que corresponde a cada mitad, la figura 25C muestra un plano de imagen de una realización de la invención La figura 25D muestra una realización de la invención presentando dos polarizadores rotables independientemente

Las figuras 26A y 26B ilustran el método de compensación para la corriente de fluido para una realización de la invención, la figura 26C muestra una realización de un elemento de compensación, la figura 26D muestra otra realización de un elemento de compensación en

185 ~~2~~
1

donde las imágenes de una corriente de fluido y del elemento de compensación caen en la parte superior de uno con respecto al otro en el plano de imagen

La figura 27 muestra una realización de las ópticas de interferencia de la invención

La figura 28 muestra una segunda vista de las ópticas de interferencia de la invención

V MODOS PARA LLEVAR A CABO LA INVENCION

La invención incluye aislar poblaciones de esperma o células de esperma de alta puridad que contienen cromosoma X y cromosoma Y. Las poblaciones de esperma que contienen cromosoma X y cromosoma Y pueden comprender poblaciones de espermatozoides vivos intactos, y pueden además comprender poblaciones de espermatozoides sin cola (núcleo de esperma), o las poblaciones de otras formas de esperma viable o no viable tal como sea deseado. Mientras que los ejemplos particulares son provistos los cuales describen la invención en el contexto de la separación de células de esperma vivas intactas cada célula de esperma tiene una cabeza, cuello y cola y puede entenderse que las tecnologías descritas pueden tener varias aplicaciones con

respecto al núcleo de esperma también Las poblaciones de esperma que contienen cromosoma X y cromosoma Y pueden además ser entendidas para que abarquen los espermatozoides de cualquier macho de una especie mamífera incluyendo pero no limitado a espermatozoides para humanos y espermatozoides para animales comúnmente conocidos tales como bovinos, equinos, ovinos, cánidos, felinos, cabras o porcinos, así como también animales menos conocidos tales como elefantes, cebras, camellos o kudu Esta lista de animales es sólo ejemplar de la gran variedad de animales de la cual el espermatozoide puede ser clasificado rutinariamente a un 90% de puridad o mayor, y no es su intención el limitar la descripción de la invención al espermatozoide de cualquier especie particular de mamíferos

Los espermatozoides separados de alta puridad de varias especies de mamíferos pueden ser incorporados dentro de productos que pueden ser utilizados con protocolos de inseminación artificial o como parte de métodos de negocios comerciales tales como aquellos descritos en la patente norteamericana No De Solicitud 60/211 093 60/224 050, o la Solicitud de Tratado de Cooperación de Patente PCT No US99/17165, o pueden ser utilizados con los protocolos de inseminación de dosis bajas tal como se describe en la

187 ⁴/₅

Solicitud de Tratado de Cooperación de Patente PCT No US98/27909, o utilizado en la fertilización in vitro de ovarios desde animales, incluyendo humanos tal como es descrito en la patente americana U S Solicitud No 60/253 785, cada una de las mencionadas anteriormente están incorporadas como referencia a la presente solicitud

El uso del término "puridad" o "alta puridad" debe entenderse como el porcentaje de población de espermatozoide aislado que contiene una característica de diferenciación particular o combinación deseada de características. Por ejemplo, donde una población de espermatozoides son separados basado sobre el contenido de cromosoma X opuesto a un cromosoma Y, una población que contiene cromosoma X que presenta 90% de puridad comprende una población de espermatozoides del cual 90% del espermatozoide individual contiene cromosoma X mientras que el 10% de esta población de espermatozoides contiene cromosoma Y. Como tal, la alta puridad con respecto a las poblaciones que contienen cromosoma X o poblaciones que contienen cromosoma Y pueden comprender una puridad seleccionada del grupo que consiste de entre 90% a alrededor de 100%, entre 91% a 100%, entre alrededor de 92% a alrededor de 100%, entre alrededor de 93% a alrededor de 100%, entre alrededor de 94% a alrededor de 100%, entre alrededor de 95% a alrededor

de 100%, entre alrededor de 96% a alrededor de 100%, entre alrededor de 97% a alrededor de 100%, entre alrededor de 98% a alrededor de 100%, entre alrededor de 99% a alrededor de 100%

Es importante, mientras que realizaciones numerosas de la invención describen la alta puridad de poblaciones de esperma que contienen cromosoma X y cromosoma Y aislados, y mientras que la descripción además divulga los dispositivos de separación de espermatozoides de alta puridad y métodos de cómo aislar y cómo utilizar las poblaciones de esperma de alta puridad aislado, el concepto básico de la invención debe entenderse para ser aplicado a otros tipos de partículas o eventos que presentan características de diferenciación de partículas o eventos de diferenciación de características. Debe entenderse que la invención puede ser aplicable a una variedad de circunstancias en las cuales la resolución de pequeñas diferencias en señales fotogeneradas puede ser necesaria, tales como productos para detección de defectos, fraccionado de flujo de campo, cromatografía líquida, electroforesis, tomografía computada, cámaras de rayo gamma, instrumentos de vuelo de tiempo, o lo similar tal como puede ser fácilmente entendido por aquellos entendidos en el arte.

Más aun, mientras que esta divulgación provee descripciones de realizaciones de aparatos y métodos para la separación de flujo de espermatozoides que contienen cromosoma X de espermatozoides que contienen cromosoma Y, la descripción de estas realizaciones de la invención no está dirigida a reducir el alcance de la invención a sólo la separación de flujo de espermatozoide o sólo a las disposiciones de separación de espermatozoide por citómetro de flujo de alta puridad pero en cambio estos ejemplos están dirigidos a ejemplificar los conceptos básicos de la invención en una manera práctica para que éstos puedan ser aplicados a una amplia variedad de aplicaciones

Haciendo referencia ahora a las figuras 1 y 2, una realización de citómetro de flujo de la invención es ilustrada la cual incluye una fuente de célula o partícula (1) la cual actúa para establecer o suministrar partículas o células manchadas con al menos un fluorocromo para su análisis. Las partículas o células son depositadas dentro de una boquilla (2) en una manera tal que las partículas o células son introducidas dentro de una corriente de fluido o vaina de fluido (3). La vaina de fluido (3) es generalmente suministrada por medio de alguna fuente de fluido de vaina (4) para que mientras que la fuente de célula o partícula suministre las células o partículas

190 1/2

dentro de la vaina de fluido éstas son concurrentemente alimentadas a través de la boquilla (2)

De esta manera puede ser fácilmente entendido cómo el fluido de vaina (3) forma un medio ambiente de fluido de vaina para las partículas o células. Dado que varios fluidos son provistos hacia el citómetro de flujo con algo de presión, éstos fluyen fuera de la boquilla (2) y salen por el orificio de la boquilla (5). Por medio de la provisión de algún tipo de oscilador (6) el cual puede variar precisamente controlado a través de un control de oscilador (7), las ondas de presión pueden ser estabilizadas dentro de la boquilla (2) y transmitidas a los fluidos que salen de la boquilla (2) en el orificio de boquilla (5). Dado que el oscilador (6) actúa sobre el fluido de vaina (3), la corriente (8) sale del orificio de boquilla (5) eventualmente y forma regularmente gotas (9). Debido a que las partículas o células son rodeadas por la corriente de fluido o la vaina del medioambiente de fluido, las gotas (9) pueden unirse dentro de las mismas células o partículas aisladas individualmente, o pueden ser células de esperma con respecto a algunas realizaciones de la invención.

Dado que las gotas (9) pueden unirse a las partículas o células, el citómetro de flujo puede ser

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191

utilizado para separar las partículas, células, células de esperma o lo similar basado sobre las características de la partícula o célula. Esto es realizado a través de una disposición de detección de partícula o célula (10). La disposición de detección de célula o partícula incluye al menos algún tipo de detector o sensor (11) el cual responde a las partículas o células contenidas dentro de la corriente de fluido (8).

La disposición de detección de célula o partícula (10) puede causar una acción dependiendo sobre la presencia relativa o ausencia relativa de una unión fluorocromo a la partícula o célula que puede ser excitado por medio de una fuente de irradiación tal como un excitador láser (12) que genera un rayo de irradiación sensible a la partícula. Mientras que cada tipo de partícula, célula, o ADN nuclear de células de esperma puede ser manchado con al menos un tipo de cantidades diferentes de fluorocromo unidos a cada partícula individual o célula basada en el número de sitios de aglutinamiento disponibles al tipo particular de fluorocromo utilizado. Con respecto a los espermatozoides, la disponibilidad de los sitios de aglutinamiento para la mancha Hoechst 33342 es dependiente sobre la cantidad de ADN contenida dentro de cada esperma. Debido a que el espermatozoide que contiene cromosoma X contiene más ADN que el espermatozoide que contiene cromosoma Y, el

espermatozoide que contiene cromosoma X puede unir una gran cantidad de fluorocromo que el espermatozoide que contiene cromosoma Y. De esta manera, por medio de la medición de la fluorescencia emitida por el fluorocromo unido luego de la excitación, es posible diferenciar entre el espermatozoide que contiene cromosoma X y el espermatozoide que contiene cromosoma Y.

Para realizar la separación y el aislamiento basados sobre las características de células o partículas, la luz emitida puede ser recibida por el censor (11) y alimentada a algún tipo de disposición de discriminación de separación o analizador (13) acoplado a un cargador de gota el cual carga diferencialmente cada gota (9) basada sobre las características de la célula o partícula contenida dentro de la gota (9). De esta manera, la disposición de discriminación de separación o analizador (13) actúa para permitir que las placas de deflexión electrostáticas (14) desvíen las gotas (9) basadas en ya sea o no que contienen la célula o partícula apropiada.

Como resultado, el citómetro de flujo actúa para separar las partículas o células (16) haciendo que las mismas sean dirigidas a uno o más contenedores de recolección (15). Por ejemplo, cuando el analizador

193

diferencia las células de esperma basado sobre una característica de células de esperma, las gotas que contienen espermatozoides que presentan cromosoma X pueden ser cargadas positivamente y de esta manera desviadas en una dirección, mientras que las gotas que transporta el espermatozoide que contiene cromosoma Y pueden ser cargadas negativamente y de esta manera desviadas hacia el otro lado, y la corriente malgastada (la corriente en que las gotas no transportan una partícula o célula o transportan células no deseadas o sin clasificar) puede ser dejada sin cargar y de esta manera recolectada en un corriente no desviada dentro de un tubo de succión o lo similar tal como se divulga en la solicitud de patente norteamericana U S No 09/001 394, incorporada a la presente a modo de referencia Naturalmente, numerosas trayectorias de desviación pueden ser establecidas y recolectadas simultáneamente

Para separar las células, partículas, células de esperma o espermatozoide rutinariamente (nucleido, no viable, viable, fijo, vivo, intacto) dentro de poblaciones de alta pureza que contiene cromosoma X y cromosoma Y, el aparato de diferenciación de partícula o métodos utilizados deben proveer una alta resolución de las características de

diferenciación que son utilizadas en base al análisis y separación

Con respecto al espermatozoide, la diferenciación entre la luz emitida por el fluorocromo unido al ADN nuclear de las células de esperma que contienen cromosoma X y la luz emitida por el fluorocromo unido al ADN nuclear de las células de esperma que contienen cromosoma Y puede ser difícil tal como fue discutido anteriormente

En varias aplicaciones, tal como fue descrito anteriormente, el total de la luz emitida de los eventos fotoemisores incidentes hacia al detector, el cual puede ser un tubo fotomultiplicador, puede ser alto mientras que la diferencia entre la luz emitida de cada evento fotoemisor a ser diferenciado puede ser pequeña. El problema puede ser exacerbado cuando los eventos fotoemisores suceden serialmente a un alto promedio de velocidad y el período de tiempo entre los eventos fotoemisores es corto, tales como con una clasificación de célula de alta velocidad utilizando citómetros de flujo. Cuando se separa partículas, células o células de esperma basadas sobre la diferencia en el contenido de fluorocromo, el flujo de células pasa una fuente de excitación y un alto número de eventos emisores por segundo puede ser establecido. Como

195-~~82~~

resultado, la cantidad de luz emitida generada en la corriente de partículas, células o células de esperma puede ser enorme. Mientras que la velocidad de la corriente es incrementada, el punto de intersección con la fuente de excitación se vuelve muy brillante. Este alto nivel de luz incidente sobre el fotocátodo del tubo fotomultiplicador puede causar una señal muy baja con respecto al promedio de ruido. La cantidad de ruido puede además ser exacerbada cuando tal fluorocromo tal como el Hoechst 33342 es utilizado para etiquetar el ADN nuclear de células de esperma.

La mayoría de las soluciones a los problemas han enfocado en reducir la cantidad total de flujo de luz sobre el tubo fotocátodo por medio de la ubicación de filtros ópticos enfrente del tubo fotomultiplicador. Esta solución no cambia la proporción de señal a ruido y subsecuentemente atenta a incrementar la sensibilidad del tubo generador que genera ruido adicional mientras que el tubo fotomultiplicador se satura de la cantidad de señal de fondo.

Típicamente, los tubos fotomultiplicadores son accionados en un rango de voltaje de operación de alrededor de 400 voltios a alrededor de 900 voltios. El límite

196

5/1

inferior de la operación lineal de los tubos fotomultiplicadores estándar tales como los tubos fotomultiplicadores R928 y R1477 disponibles de Hamamatsu Corporation, pueden ser de alrededor de 300 voltios. Como tal, los equipos o instrumentos los cuales utilizan tubos fotomultiplicadores son configurados para operar tales tubos fotomultiplicadores a 400 voltios o más. Incluso cuando la reducción del número de electrones en el ánodo es deseado, tal como se divulga en la patente norteamericana U S No 4 501 366 y 5 880 457, el voltaje entre el fotocátodo y primer dinodo es mantenido a un alto voltaje y la reducción de electrones en el ánodo es realizada por ya sea la reducción de voltaje a los dinodos remanentes, o al ruido oscuro inherente o ruido de tiro que es filtrado electrónicamente.

Inesperadamente, reduciendo la cantidad de voltaje del tubo fotomultiplicador por debajo de los 400 voltios a alrededor de 280 voltios o a alrededor de 250 voltios, o incluso a 0 voltios puede permitir pequeñas diferencias en la luz fotoemisiva que puede ser diferenciada incluso cuando la luz total emitida de cada evento fotoemisor es alta, o incluso cuando existe un alto número de eventos seriales brillosos por segundo. Con respecto al promedio de eventos fotoemisores generados a partir de la irradiación de

197-101

fluorocromos unidos al ADN nuclear de espermatozoide, la invención permite la velocidad de eventos fotoemisores que pueden ser realizados durante la separación de espermatozoides en poblaciones que contienen cromosoma X y cromosoma Y a ser incrementado a una velocidad de evento separable de al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos separables por segundo, al menos 13 000 eventos separables por segundo, al menos 14 000 eventos separables por segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000 eventos separables por segundo, al menos 19 000 eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 25 000 eventos separables por segundo, al menos 30 000 eventos separables por segundo, al menos 35 000 eventos separables por segundo, o mayor

Un ejemplo específico, existiendo los citómetros de flujo de clasificación Cytomation SX MoFlo son configurados

198 *EP*

para operar el tubo fotomultiplicador a un mínimo de 400 voltios. La ganancia puede ser ajustada para operar el tubo fotomultiplicador a mayores voltajes pero no a menores voltajes. El citómetro de flujo SX MoFlo puede ser convertido por medio de la reconfiguración de los controles del fotomultiplicador. El resistor R16C (2 49 kilohms) en el canal tres puede ser reemplazado por un resistor de 2 0K para alterar la ganancia del amplificador que controla el tubo fotomultiplicador. Esta conversión permite al tubo fotomultiplicador operar a alrededor de 280 voltios. La conversión similar del citómetro de flujo SX MoFlo con dos resistores de 3 75 kilohm en paralelo, o un resistor de 1 3 kilohm puede permitir que los tubos fotomultiplicadores sean operados a voltajes de alrededor de 200 voltios o justo por encima de los cero voltios, respectivamente. Además con respecto a esta conversión, el filtro de densidad neutral en frente al fotocátodo puede además ser removido como resultado de la operación del tubo fotomultiplicador fuera del rango de voltaje de operación típico.

Esta conversión inesperadamente incrementa la relación señal a ruido del evento fotoemisor que es traducido a un señal electrónica por medio del tubo fotomultiplicador. La señal del limpiador puede ser de esta

manera amplificada por medio del incremento del amplificador de ganancia al convertidor análogo a digital del analizador (13) al nivel apropiado y la salida puede ser generada como histogramas de una sola variable o de dos variables

Haciendo referencia ahora a la figura 3, una comparación de histogramas de una sola variable generados en tres diferentes citómetros de flujo SX MoFlo (#1, #2, #3) previa al uso de la invención (figura 1A), utilizando la invención (figura 1B) con respecto a la separación de esperma bovina eyaculada en vivo intacto es ilustrado Tal como puede entenderse de los histogramas de una sola variable, en la resolución (la diferenciación aparente de la población que contiene cromosoma X de la población que contiene cromosoma Y representada por el valle entre picos) de el espermatozoide que contiene cromosoma X vivo intacto (17) del espermatozoide que contiene cromosoma Y vivo (18) puede ser sustancialmente mejorado por el uso de la invención

La velocidad de separación promedio o los promedios de clasificación de espermatozoide vivo intacto previo al uso de esta realización de la invención con los citómetros de flujo SX MoFlo fue de alrededor de $17.9 \times 10^6/4.5$ horas

200
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de tanto los espermatozoides que contienen cromosoma X y los espermatozoides que contienen cromosoma Y (por ejemplo alrededor de 1100 separaciones o clasificaciones por segundo en cada una de las dos corrientes, la primer corriente es el espermatozoide que contiene cromosoma X y la segunda corriente es el espermatozoide que contiene cromosoma Y) a alrededor de 87% de puridad con un rango de 84% a 93% de puridad El promedio de eventos separables fue de 22 000, 23 000 y 20 000 respectivamente para estas tres clasificaciones

Las velocidades de clasificación promedio del espermatozoide vivo luego de la conversión mencionada anteriormente fue de alrededor de $4.3 \times 10^6 / 4.5$ horas de clasificación (por ejemplo, alrededor de 2500 clasificaciones por segundo por corriente) a alrededor de 90.8% de puridad con un rango de 89% a alrededor de 92% Los eventos por segundo fueron de 13 000, 15 000 y 19 500 respectivamente para estas tres clasificaciones

Tal como puede entenderse de los datos, no sólo esta realización de la invención resultó en el incremento de puridad de la población de espermatozoides separados pero además permitió que la velocidad de separación o velocidad de clasificación sea más que el doble mientras

201/20

que la velocidad de evento separable fue actualmente reducida

Similarmente, haciendo referencia ahora a las figuras 4 y 5, las cuales muestran histogramas de dos variables de la clasificación de espermatozoides de toro vivo intacto con el citómetro de flujo #1 SX MoFlo previo al uso de la invención (figura 4) y luego de la conversión mencionada anteriormente (figura 5) Previo al uso de la invención, el citómetro de flujo SX MoFlo fue inicialmente operado a 440 voltios en el fotocátodo con el láser ajustado a 135 MW, de ganancia de X y con un filtro de densidad neutral de 1 0 (1/10amplitud) a alrededor de 10 000 eventos por segundo Luego del uso de la invención, el citómetro de flujo SX MoFlo fue operado a alrededor de 262 voltios en el fotocátodo, con el láser ajustado a alrededor de 100 MW, una ganancia de 4X, sin el filtro de densidad neutral, a alrededor de 10 000 eventos separables por segundo Tal como puede entenderse de estos datos, existe un gran incremento en la resolución como evidencia por medio del incremento de la profundidad del valle entre la población que contiene cromosoma X (19) y la población que contiene cromosoma Y (20)

202 ~~7~~ 2

Similarmente, haciendo referencia ahora a las figura 6 y 7, las cuales muestran histogramas de dos variables a partir de la clasificación de espermatozoides de toro vivo intacto, el citómetro de flujo #2 SX MoFlo previo a su uso de esta realización de la invención (figura 6) y luego de utilizar esta realización de la invención (figura 7) operado en los mismos parámetros tal como se ilustra en las figuras 3 y 4 respectivamente. Nuevamente, puede existir una gran cantidad de incremento en la resolución tal como se evidencia por la profundidad del valle entre la población que contiene cromosoma X (21) y la población que contiene cromosoma Y (22).

Haciendo referencia ahora a las figuras 8 y 9, las cuales muestran histogramas de dos variables a partir de la separación o clasificación de espermatozoide equino vivo intacto con el citómetro de flujo SX MoFlo⁷ antes de utilizar esta realización de la invención (figura 8) y luego de la utilización de esta realización de la invención (figura 9). Cuando se usa esta realización de la invención, el espermatozoide equino vivo fue separado o clasificado con una energía de láser a 100 mW con el voltaje del tubo fotomultiplicador por debajo de los 300 voltios. Las velocidades de separación o velocidades de clasificación excedieron las 4800 clasificaciones por segundo promedio a

203 2/2

12 000 eventos por segundo La resolución incrementada de la población que contiene cromosoma X (23) y la población que contiene cromosoma Y (24) es dramática Los datos muestran que alrededor de 8 canales de separación pueden ser realizados con esta realización de la invención tal como es comparado con los 5 canales de separación entre los picos sin el uso de esta realización de la invención La pureza de la población que contiene cromosoma X y la población que contiene cromosoma Y fue de alrededor de 93%

Haciendo referencia ahora a la figura 10, la cual ilustra un histograma de una sola variable y un ploteo por punto de dos variables de la clasificación del nucleido de esperma de un semental manchado Hoechst 33342 (S-05400) separado utilizando esta realización de la invención El nucleido fue separado del esperma del semental eyaculado frescamente El esperma fue lavado por centrifugado, sonificado y las cabezas resultantes y colas separadas utilizando el centrifugado gradual de densidad Percoll Las cabezas aisladas fueron lavadas, fijadas con 2% de formalina y luego manchadas con Hoechst 33342 El nucleido manchado fue estabilizado utilizando sodio de ácido (0.5%) La muestra fue ejecutada a 5000 eventos por segundo para producir los histogramas El nucleido manchado fue de esta manera utilizado para calibrar un citómetro de flujo SX

204 2/2

MoFlo⁷ convertido tal como fue mencionado anteriormente para incorporar el tubo fotomultiplicador de la realización de la invención. La compensación fue utilizada para nivelar las dos poblaciones (nucleido manchado X y nucleido manchado Y) en el ploteo de dos variables. Nótese que las dos poblaciones de nucleido de esperma equina son casi completamente resueltas a la línea de base tal como se ilustra en el ploteo de una sola variable.

Haciendo referencia ahora a la figura 11, una modificación específica para el citómetro de flujo SX MoFlo⁷ incluye el uso de dos resistores en paralelo para proveer el valor correcto de 1.8K. Dos resistores de 3.57K (25) y (26) son iguales a alrededor de 1.785K los cuales pueden ser suficientemente cercanos al valor para ser efectivos. Con esta modificación el tubo fotomultiplicador en este instrumento particular puede ser ejecutado a alrededor de 200 voltios. Naturalmente, una modificación similar puede ser realizada para otros instrumentos citómetros de flujo u otros instrumentos los cuales utilizan un tubo fotomultiplicador para medir la cantidad de luz emitida de eventos particulares. La figura 12 provee un diagrama esquemático eléctrico para esta realización particular de la invención.

102-502

Otra realización importante de la invención puede ser ópticas de patrón de rayo de irradiación de altura reducida Tal como se ilustra en la figura 13A, las ópticas de forma de rayo de irradiación convencional generan un patrón de rayo (27) que puede tener una altura que puede ser mayor que la altura de la cabeza de célula de esperma (28) que pasa a través del mismo Como resultado, más de una cabeza de célula de esperma individual que contiene fluorocromo unido al ADN puede entrar al patrón de rayo de irradiación al mismo tiempo En ese caso, el fluorocromo unido al ADN contenido dentro de las cabezas de esperma múltiple puede ser excitado simultáneamente y la fluorescencia dentro de un evento emisor singular Como tal, los eventos emisores subsecuentes o previos pueden incluir flujo de luz coincidente contribuido de otras cabezas de esperma en el patrón de rayo (27) Esto resulta en una diferencia reducida en el promedio de flujo de luz entre los eventos de luz emisores los cuales distinguen entre el esperma que contiene cromosoma X y el que contiene cromosoma Y También puede reducirse la diferencia en el promedio de flujo de luz entre los eventos que comparan las emisiones de luz del espermatozoide que contiene cromosoma X o el espermatozoide que contiene cromosoma Y Es importante que la coincidencia de excitación de fluorocromo unido a múltiples ADN incrementa el promedio de brillo de

los eventos realizando la diferencia de medición en el flujo de luz entre los eventos generando un menor porcentaje del flujo de luz emitido en su totalidad. Esto hace más difícil la cuantificación de las diferencias entre los eventos.

Por medio de la reducción de la altura de la forma de rayo tal como se ilustra en la figura 13B, la coincidencia de las cabezas de esperma múltiples estando dentro del patrón de rayo de altura reducida (29) durante el mismo evento medido es reducido. Este resultado es un promedio incrementado de la diferencia entre los eventos emisores de luz los cuales distinguen entre los espermatozoides que contienen cromosoma X y cromosoma Y. También puede reducirse el promedio total de flujo de luz para cada evento emisor medido. Para las realizaciones particulares de la invención utilizadas para clasificar esperma bovina los cuales tienen un núcleo de alrededor de $9\mu\text{m}$, se ha encontrado que la altura del rayo debe ser de alrededor de $20\mu\text{m}$. En esta aplicación, se ha encontrado que la altura del rayo vertical menor que $20\mu\text{m}$ no provee una ganancia adicional en la resolución.

Haciendo referencia a la figura 14, puede entenderse que el uso de óptica de patrón de rayo de

irradiación de altura reducida puede mejorar la puridad del espermatozoide bovino que contiene cromosoma X (figura 14A) y la clasificación de poblaciones de esperma bovina que contiene cromosoma Y (figura 14B) que han sido manchados con una mancha Hoechst 33342. Esto es verdad tanto para las compuertas de clasificación de 25% y 40% de los picos de una sola variable. Tal como puede entenderse posteriormente a partir de la figura 14, las ópticas de forma de rayo de altura reducida pueden mejorar la puridad del espermatozoide separado independiente de cualquier otro aspecto de la invención, tal como la modificación de la realización del circuito fotomultiplicador de la invención (nuevo PMT) tal como fue descrito anteriormente, o puede ser utilizado en conjunto con la realización del fotomultiplicador modificado de la invención para incrementar la puridad de las muestras de espermatozoides separados incluso más.

Otra ventaja de las ópticas de rayo de altura reducida puede ser que el tiempo de tránsito del espermatozoide en el rayo láser de excitación o el rayo de excitación puede ser reducido. Una cantidad reducida de tiempo de irradiación dentro del rayo láser de excitación puede dar como resultado menor tensión o daño al espermatozoide.

Haciendo referencia nuevamente a la figura 14B como puede entenderse que el patrón de rayo de altura reducida puede ser utilizado en conjunto con el patrón de rayo de irradiación que tiene una mayor área que el utilizado convencionalmente. Por ejemplo, los patrones de rayo convencionales (27), tales como los ilustrados en la figura 14A, tienen un patrón elíptico de alrededor de $30\mu\text{m}$ a $80\mu\text{m}$ mientras que la invención fue utilizada para clasificar esperma bovina que genera una resolución óptima entre las poblaciones que contienen cromosoma X y cromosoma Y cuando el rayo tiene un patrón de rayo de $20\mu\text{m}$ a $160\mu\text{m}$ (29). El patrón de rayo $20\mu\text{m}$ x $160\mu\text{m}$ tiene aproximadamente 13 veces el área de $30\mu\text{m}$ x $80\mu\text{m}$ de patrón de rayo. Como tal, puede existir una proporción inversa en la pérdida de energía en el punto incidente. Esto hace posible incrementar la energía de excitación del láser sin importar la incrementación del daño de irradiación al espermatozoide. Por ejemplo, si un instrumento tiene ópticas de forma de rayo convencional que producen un patrón de rayo de irradiación de $30\mu\text{m}$ x $80\mu\text{m}$ y el láser de excitación es energizado convencionalmente a 150mW , entonces las realizaciones particulares de la invención con un patrón de rayo de $20\mu\text{m}$ x $160\mu\text{m}$ puede tener un láser de excitación energizado a 300mW sin

209
202

incrementar la cantidad total de energía en el punto incidente Alternativamente, el láser de excitación puede ser ejecutado a 150 mW para tomar ventaja de la energía de irradiación inferior por unidad de área, reduciendo el daño de irradiación, alargando la vida del láser y lo similar

En comparación a las ópticas de forma de rayo convencional y los dispositivos de ampliación de tubo fotomultiplicador, las ópticas de forma de rayo de altura reducida de la invención y la amplificación del tubo fotomultiplicador de la invención puede incrementar la puridad de las poblaciones de espermatozoides que contiene cromosoma X y cromosoma Y por alrededor de 4%, o más

Las ópticas de forma de rayo de la invención (30) pueden ser instaladas a un citómetro de flujo tal como se ilustra en las figuras 15 y 16 Tal como puede entenderse, la luz emitida (31) por la excitación de láser del fluorocromo unido al ADN contenido con el espermatozoide puede ser detectado por medio del tubo fotomultiplicador (32) situado a 0 y 90° con respecto a la superficie plana de la cabeza del esperma (28) mientras fluye a través del patrón de rayo de láser de excitación

10/2
210

Tal como puede entenderse, el espermatozoide manchado debe ser bombeado a través del rayo de excitación o rayo de irradiación de una manera precisa para que cada cabeza de esperma sea orientada con la superficie plana de la cabeza de esperma dirigida hacia el tubo fotomultiplicador que es el detector de grado 0

La medición precisa del contenido ADN del espermatozoide puede sólo ser medido de la superficie plana de la cabeza de esperma en forma de paleta (28) De esta manera, únicamente esa proporción del espermatozoide que ingresa en el rayo de excitación en la orientación apropiada puede ser medido precisamente y seleccionado en base al contenido de ADN

Haciendo ahora referencia a las figuras 17, 18 y 19, las realizaciones particulares de la invención pueden además tener un objeto de orientación de boquilla (33) que fuerza hidrodinámicamente la cabeza de esperma achatada en la orientación apropiada mientras que éstos pasan enfrente del fotomultiplicador Tal como se ilustra en la figura 17, la boquilla de orientación tiene superficies interiores (34) que forman una forma tipo cono El cono interno cambia gradualmente desde una forma circular en el extremo de entrada (35) a una forma altamente elíptica cerca del

orificio (36) en donde la corriente sale por la punta El orificio (36) puede ser circular en vez de elíptico De esta manera, el aspecto interno de la boquilla de orientación (34) va desde una entrada redonda hacia una elipse angosta a una salida redonda corta previa al orificio (36) Esta forma interna es además clarificada por las secciones transversales de la boquilla de orientación ilustrada por la figura 18

Tal como es ilustrado por las figuras 19 y 21, el tubo de inyección (37) (el cual puede ser de alrededor de 0 061 pulgadas en diámetro) puede ser utilizado con la boquilla de orientación (o con una boquilla convencional) (33) la cual puede ser biselada cercana a la punta para formar una boquilla (38) La cuchilla achatada (38) puede ser orientada en un ángulo de 90° desde la dimensión mayor de la elipse en la orientación de la boquilla (33) El diámetro interno de la boquilla de inyección puede ser de alrededor de 0 010 pulgadas en diámetro que forma un orificio redondo (39) en el centro de la cuchilla de tubo de aguja achatada (38)

En realizaciones particulares del tubo de inyección biselado la cuchilla biselada puede ser configurada en una forma de paleta ilustrada por la figura 21 La cuchilla

biselada en forma de paleta puede asistir en mantener el flujo laminar de la vaina de fluido dentro de la boquilla (ya sea la boquilla convencional o la boquilla de orientación) Como tal, el flujo laminar de líquido mantenido por la cuchilla biseladas en forma de paleta presenta menos interrupción de los objetos inyectados dentro del mismo El espermatozoide introducido dentro del flujo laminar de la vaina de fluido mantenida por una realización del tubo inyector de la invención que tiene una cuchilla biselada en forma de paleta permite un incremento de 20%, 30%, 40%, 50% o más en los promedios de clasificación de espermatozoides sobre la inyección convencional de tecnología de tubo La clasificación de alta velocidad de los espermatozoides a velocidades de 4000 a 10000 clasificaciones para cada sexo por segundo puede ser realizada La alta pureza (90% o mayor) de las poblaciones que contienen cromosoma X y cromosoma Y puede ser establecida incluso en estas altas velocidades de clasificación El tubo inyector de la invención con la punta en forma de paleta biselada puede ser utilizada independientemente para una combinación con otras invenciones descritas aquí u otras tecnologías tales como las descritas en la patente norteamericana U S Solicitud No 09/454488 o la Solicitud de Patente Internacional No

PCT/US00/42350, cada una de las cuales incorporadas a la presente a modo de referencia

Tal como se muestra en la Figura 21, ciertas realizaciones del tubo inyector de cuchilla biselada de la invención o la forma de paleta de cuchilla biselada de la invención pueden además incluir surcos de mejoramiento de flujo laminar (40) tal como se ilustra en la figura 21. Los surcos de mejoramiento de flujo laminar (40) asisten en mantener un flujo laminar hacia el orificio del tubo inyector. Nuevamente, el flujo laminar mejorado permite que más espermatozoides se mantengan en la correcta orientación en la vaina de fluido laminar de flujo dando como resultado mayores números de promedios de eventos clasificables los cuales a cambio otorgan mayores promedios de clasificación para cada sexo o espermatozoide.

En otra realización de la invención, el orificio de boquilla de orientación (39) u otra boquilla convencional puede ser dimensionada para formar gotas las cuales encapsulan esperma vivo intacto mientras que éstos salen del orificio (39). El encapsulado de las células de esperma no sucede en la tecnología de transporte de célula de esperma convencional. En cambio una porción de la cola de célula de esperma reside fuera de la gota. Por ejemplo, la

célula de esperma bovina tiene una longitud de alrededor de 13.5 microsegundos cuando la corriente de fluido tiene una presión de alrededor de 50 libras por pulgada cuadrada (por ejemplo, la longitud del tiempo para la total longitud de la célula de esperma para que pase a través del rayo de irradiación a alrededor de 50 libras por pulgada cuadrada de presión de corriente de fluido) Las técnicas de formación de gotas convencionales para el transporte de células de esperma bovinas establecen una gota de alrededor de 14 microsegundos (por ejemplo, la longitud del tiempo del diámetro de la gota que toma para pasar a través del rayo de irradiación) de una boquilla que presenta un orificio con un diámetro de alrededor de 70 micrómetros el cual puede ser sensible a un oscilador operado a alrededor de 35 kilohertz Como tal, una porción de la cola de célula de esperma fácilmente protubera de la gota Para prevenir que la cola de célula de esperma protubere de la gota, una realización de la encapsulación de gota de la invención provee un orificio de alrededor de 100 micrómetros que puede producir una gota de alrededor de 28 microsegundos alrededor de 50 libras por pulgada cuadrada a alrededor de 30 kilohertz Encapsulando enteramente la célula de esperma viva intacta, incluyendo la porción de cola, la célula de esperma interactúa con la boquilla en menor cantidad luego de la carga de la gota y la

descripción de la gota puede ser más precisa. Esto conduce a una menor contaminación cruzada de espermatozoides que contiene cromosoma X con espermatozoides que contiene cromosoma Y y además permite desviar el espermatozoide para que sea recolectado más uniformemente. El esperma que es uniformemente desviado puede ser dirigido a las superficies de recolección que son amortiguadas por varios líquidos. La amortiguación del espermatozoide separado puede ser importante en la reducción de las tensiones tal como se describe en la solicitud de patente norteamericana U S No 09/001 394, incorporada a la presente a modo de referencia. Con respecto a los espermatozoides de otras especies de mamíferos, la invención puede ser variada para producir tamaños de gota para encapsular las longitudes variantes de las células de esperma. Dependiendo de la longitud del espermatozoide y de la presión de la corriente de fluido la encapsulación de la gota de la invención puede todavía alcanzar las velocidades de formación de gota de al menos 10 000 gotas por segundo, al menos 20 000 gotas por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo hasta

216
2/12

alrededor de 200 000 gotas por segundo en algunas realizaciones de la encapsulación de gota de la invención

Incluso con la orientación de boquilla de la invención todavía puede existir un cierto número de partículas o espermatozoides, los cuales no están propiamente orientados en el patrón de rayo Tal como fue descrito anteriormente, si la orientación de la cabeza de esperma no es la apropiada entonces el contenido de ADN no puede ser medido con precisión basado sobre la luz emitida Las realizaciones particulares de la presente invención proveen la remoción de espermatozoides sin orientar y no deseados (RUSS) o partículas dentro de una corriente de fluido

Haciendo referencia ahora a las figuras 16 y 20A, puede entenderse que la medición precisa del contenido ADN del espermatozoide depende sobre la superficie plana de la cabeza de esperma en forma de paleta (28) estando orientada propiamente con el detector De esta manera, sólo aquella porción del espermatozoide que ingresa al rayo de excitación en la orientación apropiada tal como se ilustra por medio de las figuras 16 y 20^a puede ser medida precisamente y clasificada en poblaciones que contienen cromosoma X y cromosoma Y basado sobre el contenido de ADN

217 $\frac{2}{12}$

Tal como se ilustra por las figuras 20A y 20B, el espermatozoide que transita a través del rayo de excitación en la orientación apropiada genera un ploteo de señal de emisión orientada (40) que puede ser formada diferentemente que el ploteo de señal de emisión no orientado (41) que es generado por el espermatozoide sin orientar ilustrado por la figura 20D. Naturalmente, la forma del ploteo de señal de emisión sin orientar (41) generado por el espermatozoide desorientado puede variar dependiendo del grado de orientación inapropiado del rayo de excitación. Estas orientaciones inapropiadas pueden incluir la orientación ilustrada en la figura 20C pero además puede incluir todas las maneras de orientaciones que rota la cabeza de esperma en cualquier porción de una rotación que orienta la superficie de la cabeza en forma de paleta fuera de la alineación con el detector (alineación apropiada ilustrada por la figura 16), o cualquier porción de una rotación que orienta el eje de la cabeza de esperma (42) fuera de la alineación con la dirección de flujo. Naturalmente, la orientación apropiada puede ser definida diferentemente de especie a especie. Para algunas especies, en las cuales la cabeza de esperma no tiene forma de paleta, la orientación apropiada dentro del rayo de excitación, o relativo a los detectores o de otra manera puede ser definida por otras características anatómicas o características de señal. No

218 $\frac{F}{2}$

obstante, una señal optimizada para la orientación apropiada de los espermatozoides de varias especies dentro de la ventana de excitación puede ser generada como es estándar de los ploteos de señal de emisión para la comparación subsecuente con los eventos de emisión seriales

Por medio de la comparación de la forma (o del área integrada o de ambas) de cada ploteo de señal de emisión con el ploteo de señal de emisión estándar (o área integrada estándar o ambas) establecido para un espermatozoide orientado de una especie de mamífero, un espermatozoide sin orientar puede ser identificado, la señal substraída de los histogramas de una sola variable o de dos variables, y el espermatozoide sin orientar puede ser afirmativamente removido, si se lo desea, para que el espermatozoide sin orientar no sea recolectado dentro de las poblaciones que contienen cromosoma X y cromosoma Y

Es importante destacar mientras que la invención mejora la resolución entre las dos poblaciones de espermatozoides siendo separadas las cuales incrementan la velocidad a la cual la población puede ser separada una de otra, y mejora la puridad de las poblaciones a ser separadas. Como tal, es ahora posible clasificar

219 ^{p/2}

espermatozoides a velocidades sorprendentemente altas La clasificación o las velocidades de eventos separables pueden ser tan altas como 35 000 por segundo (no incluyendo los eventos coincidentes, múltiples espermatozoides dentro de la ventana de detección/excitación al mismo tiempo) La clasificación o velocidades de evento separable correlativa con la alta separación o velocidades de clasificación las cuales pueden ser de 5000 a 8000 espermatozoides vivos intacto de cada sexo por segundo con una pureza de 90%, 92%, 93%, 95% o mayor Las invenciones descritas anteriormente además permiten que las poblaciones de alta pureza que contienen cromosoma X y cromosoma Y sean obtenidas alrededor de 97% hasta 98% o incluso mayores por medio de la reducción de las velocidades de clasificación o separación a alrededor de 200 espermatozoides vivos para cada sexo por segundo

Tal como puede entenderse, las invenciones descritas anteriormente son particularmente importantes en realizar las mayores velocidades de evento separable o clasificable posibles y las mayores velocidades de separación resultantes posibles las cuales pueden ser al menos de 1000 separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones

220²

por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo para cada sexo por segundo

La invención permite clasificación a alta velocidad tal como fue mencionado anteriormente, de espermatozoides incluso cuando estos son difíciles de manchar, o tienen otras características químicas o anatómicas, que hacen la diferenciación entre las poblaciones que contienen cromosoma X y cromosoma Y más difícil. Incluso en estos casos difíciles, las poblaciones de alta pureza que contienen cromosoma Y y cromosoma X de espermatozoide bovino pueden ser aisladas a una alta pureza de 92% a 93% alcanzando las velocidades de eventos clasificables de alrededor de 15 000 a 20 000 eventos clasificables por segundo o mayores tal como se describe anteriormente, o la clasificación o velocidades de separación de espermatozoide vivo intacto para cada sexo (conteniendo cromosoma X y conteniendo cromosoma Y) de 2000 espermatozoides vivos intactos para cada sexo por segundo, o mayor.

Haciendo ahora referencia a las figuras 22 y 23, una realización de la invención utiliza la tecnología de contraste de interferencia diferencial para medir el

volumen de una partícula o cápsula Una realización básica de la invención puede comprender partículas que tienen un volumen diferente, tales como cabezas de células de esperma 28 que tienen una diferencia en el volumen entre las células de esperma que contienen cromosoma X y que contienen cromosoma Y Una fuente de radiación electromagnética (43) genera una radiación electromagnética (44) que tiene características de forma de onda diferenciales sensibles a la diferencia en el volumen entre las partículas o cabezas de células de esperma (28) La radiación electromagnética la cual puede ser una luz de láser, pero además pueden ser numerosos tipos de radiación electromagnética que incluye, pero no estando limitado a radiación de microondas, radiación ultravioleta o lo similar Luego de atravesar la partícula o cápsula o el volumen de cabeza de esperma, la radiación electromagnética puede ser enfocada a través de una lente objetiva (45) sobre un detector (46) sensible a las características de forma de onda de la radiación electromagnética (44) El detector puede ser acoplado a un analizador (47) El analizador puede diferenciar entre las partículas basadas en el cambio de las características de forma de onda previo a atravesar el volumen de la partícula y luego de atravesar el volumen de la partícula y puede analizar la señal basada en las áreas integradas o las formas de señales o ambas En

algunas realizaciones de la invención las características de forma de onda analizadas pueden comprender la superposición de las características de la forma de onda iniciales con las características de la forma de onda alteradas luego de atravesar el volumen de la partícula, cápsula o cabeza de células de esperma La superposición de las características de forma de onda iniciales y la característica de forma de onda alterada puede modular diferencialmente la intensidad de la radiación electromagnética basada en la cantidad de medio que atraviesa la radiación electromagnética La invención puede además incluir filtros adicionales (48), tales como filtros de colores

Haciendo ahora referencia a la figura 24, una realización de las ópticas de invención incluye utilizar ópticas de contraste de interferencia diferencial, las cuales incrementan la distancia actual sobre la cual la luz es dividida comparada a la microscopía DIC convencional la cual corresponde al límite de resolución del microscopio En esta realización de la invención, el divisor inducido es mayor que el tamaño de los objetos, de esta manera dando una elevación a las dos imágenes individuales, lateralmente separadas, que se originan desde un objeto La segunda modificación incluye el uso de placas de material

birrefringente, tales como las placas Savart, en una ubicación alejada del lente objetivo. Esta realización de la invención es fácil de construir dado que los materiales birrefringentes no deben ser ubicados dentro del alojamiento del objetivo. En los microscopios DIC convencionales el material birrefringente es utilizado para formar un prisma llamado Wollaston, que debe ser ubicado dentro del alojamiento del objetivo, siendo necesario el uso de lentes de objetivo costosos que han sido fabricados especialmente para este propósito.

Los componentes de una realización de la invención pueden ser dispuestos en línea uno con respecto al otro y consiste de una fuente de luz (43), por ejemplo, una lámpara de arco Mercury, un elemento de ajuste espectral por ejemplo un filtro de paso de banda, un elemento de ajuste de polarización (49), por ejemplo, una lámina polarizadora (53) y una placa de onda (54) sensible a un montaje rotativo, un condensador de luz (51) que permite que la luz sea condensada sobre la partícula o la célula de esperma, por ejemplo, una lente condensadora o juegos de lentes u objetivo de microscopio, una corriente de fluido (8) que pueden contener partículas o células de esperma (28), por ejemplo, un chorro de fluido eyectado bajo presión, un recolector de luz (45) para recolectar la luz

224 224

de la partícula o célula, por ejemplo un objetivo de microscopio de distancia de trabajo mayor 50X y una pieza de material birrefringente en la forma de una placa Savart, montada de esta manera que su orientación y ubicación puede ser controlada precisamente, un selector de luz de imagen (55) para seleccionar sólo la luz correspondiente a la partícula o célula de esperma, por ejemplo, un juego de orificios de perno un orificio de perno (53) para cada una de las imágenes formadas

En una realización de la invención, los componentes pueden ser dispuestos de tal manera que la fuente de luz (43) o su imagen están ubicadas en el plano focal posterior del condensador de luz (45) generalmente referido como la iluminación tipo Köhler. La imagen del plano objeto puede coincidir de mejor manera con el selector de luz de objetivo (55) o los orificios de perno (53) para capturar la luz desde las partículas individuales o células de esperma. Tal como se ilustra en las figuras 27 y 28 los componentes pueden ser montados en una tabla óptica firme, o banco utilizando montajes, postes o soportes. Los componentes pueden ser montados de tal manera que el enfoque del plano objeto puede ser hecho con precisión. Esto puede ser realizado por medio del equipado de la corriente de fluido con un controlador de posición de

225-242

corriente, tales como micrómetros, para poner la corriente dentro y fuera de foco. Además puede ser necesario equipar el condensador de luz (45) con un controlador de posición condensador de luz (56) que permite que sea enfocado sobre el plano objeto. Puede ser necesario tomar un cuidado especial sobre el montaje de los elementos birrefringentes o los divisores de rayo (50) para lo cual un elemento de rotación de tres ejes es preferible.

Ahora haciendo referencia a la figura 25, las realizaciones de la presente invención pueden además incluir el uso de ambas imágenes generadas para determinar la orientación de una partícula asimétrica de corriente de fluido, incluyendo pero no estando limitado a espermatozoides tales como células de espermatozoos de toro. Una evaluación de orientación de las realizaciones de la invención puede incluir una disposición óptica que permite el control del estado de polarización de la luz que ingresa al sistema para tanto las imágenes generadas independientemente. Las ópticas de interferencia de la invención pueden además proveer el elemento de ajuste de polarización (56) que controla el estado de polarización de la luz que ingresa al sistema. Para la detección de orientación de la invención el elemento de ajuste de polarización (56) puede ser seleccionado de tal manera que

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226

consiste de dos partes, que están dispuestas sobre el selector de luz de imagen (55) que en una realización de la invención contiene los orificios de esperma (53). Esto puede ser realizado por medio de la ubicación del elemento de ajuste de polarización (56) en el plano focal posterior del condensador de luz (51), o por medio del uso de otras ópticas para realizar la misma tarea. Un ejemplo simple de este componente puede ser una pieza de media sombra, por ejemplo, que consiste de dos partes semicirculares de material polarizante, tal como una lámina polarizadora, el ángulo de orientación del cual puede ser elegido independientemente. Cada orificio de perno en el plano de imagen puede caer en una de las mitades de dicho hemisferio. Los ángulos de polarización pueden ser elegidos de tal manera que la señal de los orificios de perno (53) corresponden al volumen, y relativamente independiente del ángulo de orientación del objeto que pasa, y del otro orificio de perno (53) como una señal que depende a un gran grado, sobre este ángulo de orientación. Las dos señales pueden ser procesadas por medio del analizador (47) de una manera similar a un citómetro de flujo de multicanal convencional, a modo de ejemplos. Con respecto a este ejemplo, los ploteos de punto de dos variables pueden ser realizados y además permite al usuario seleccionar las ventanas en este ploteo.

Una mejora de la pieza de media sombra descrita anteriormente puede ser la construcción ilustrada por la figura 25D. Las mismas dos partes hemisféricas son proyectadas sobre el plano imagen pero la manera en que éstas son generadas es diferente. Un espejo (57) rompe la luz (44) en partes semicirculares, y recombina las mismas nuevamente a su lugar de origen. Cada una de las mitades atraviesa un medio separado para controlar su estado de polarización. Una ventaja de esta realización es que los ángulos de polarización pueden ser controlados continuamente e independientemente, facilitando de esta manera el ajuste de la configuración. Los materiales utilizados en esta realización pueden ser suministrados por firmas de suministro de ópticas estándar, y pueden ser montados en los materiales de montaje similar utilizando la configuración tal como es usada para las ópticas Interferométricas.

Haciendo ahora referencia a la figura 26, para corregir los artefactos introducidos que tienen la luz que pasa a través de una región no plana de material transparente, tal como una corriente de fluido cilíndrica sustancialmente plana pero que incluye otras geometrías también, las realizaciones de la presente invención divulgan la

228279

incorporación de un componente similar en forma a la región no plana, pero opuesta en términos de índices refractivos relativos. En el caso específico de un citómetro de flujo esta forma se aproxima a un cilindro. Para corregir los artefactos introducidos por el hecho de que los objetos a ser evaluados son ubicados dentro de la corriente de agua cilíndrica, es la incorporación de un componente óptico (58) el cual puede estar en la forma de un cilindro transparente, ubicado dentro del material transparente (59) de un índice refractario mayor. Puede ser preferido que la imagen de la corriente y del elemento de compensación caigan uno sobre el otro en el plano de la imagen. Esto puede ser realizado por medio de la ubicación del elemento de compensación entre las lentes objetivas y el plano de imagen, y por medio de la incorporación de lentes auxiliares.

Una realización del componente óptico (58) puede ser ubicada dentro de una tajada fina de material transparente de un índice refractivo mayor (59) por ejemplo, vidrio o Perspex, con un orificio cilíndrico perforado a través del mismo. El Perspex tiene una ventaja que puede ser fácilmente perforado alrededor de un canal dentro del mismo. El orificio cilíndrico puede ser relleno por medio de un material transparente, el índice refractivo

229 8/22

el cual es menor que aquel del material que lo rodea. La diferencia en el índice refractivo entre la sustancia y el material que lo rodea puede ser la misma pero es opuesto a la diferencia en el índice refractivo entre el agua en la corriente y el aire que lo rodea para ciertas aplicaciones. Puede no ser necesario tener el cilindro del mismo tamaño que la corriente de agua, siempre y cuando la ampliación de los lentes utilizados haga que las imágenes resultantes en el plano de la imagen sean del mismo tamaño. En algunas aplicaciones puede ser deseado o necesario ajustar la diferencia de índice refractivo para compensar esta ampliación. La fabricación de tal elemento fuera del Perspex puede ser muy simple, y puede ser realizado por cualquier taller mecánico que tiene experiencia con el mecanizado de Perspex o del material seleccionado. Puede ser realizado en tales dimensiones que coinciden en aparatos de montaje óptico estándar para facilitar la incorporación dentro de las ópticas.

La coincidencia exacta de los índices refractivos puede ser difícil. Una realización de la invención que facilita el ajuste puede ser realizar la sustancia dentro del Perspex u otro material seleccionado, un fluido de índice refractivo transparente (58), como ejemplo un aceite orgánico o mezcla de aceite que tiene un índice refractivo

1/2
230

cercano al deseado Debido al hecho de que el índice refractivo de la mayoría de los fluidos cambia con la temperatura, mucho más que en los sólidos o los vidrios, puede ser posible el ajustar con mucha precisión la diferencia en el índice refractivo por la temperatura Esto puede ser realizado por medio de la incorporación de un controlador de temperatura (60)

El componente óptico (58) de fluidos transparentes o los fluidos de índice refractivo pueden ser suministrados por firmas de suministros químicos Estas firmas generalmente tienen los datos con respecto al índice refractivo de sus fluidos fácilmente disponible Algunas firmas incluso ofrecen fluidos que son especialmente fabricados para servir como fluidos de índice refractivo, y presentan una garantía e índice refractivo estable Los controladores de temperatura y termostato son suministrados por varias firmas Una manera práctica de aplicar calor al fluido de índice refractivo puede ser el utilizar un montaje hueco hecho de material de conducción de calor, tal como metal que contiene el fluido del índice refractivo Utilizando un ciclo termostático de inmersión convencional, encontrado en varios laboratorios, el agua puede ser bombeada a través del montaje manteniendo de esta manera el elemento en una temperatura controlable y fija

La discusión incluida en esta solicitud PCT está dirigida para servir como una descripción básica. El lector debe darse por enterado que la discusión específica no describe explícitamente todas las realizaciones posibles, dado que varias alternativas son implícitas. Además, puede no explicar en su totalidad la naturaleza genérica de la invención y puede no mostrar explícitamente cómo cada característica o elemento puede actualmente ser representativo de una función más amplia o de una gran variedad de alternativas o elementos equivalentes. Nuevamente, estos son implícitos incluidos en esta divulgación. En donde la invención es descrita en terminología orientada en funcionalidad, cada aspecto de la invención está realizado por medio de un dispositivo, subrutina o programa. Las reivindicaciones de aparato pueden no sólo ser incluidas para los dispositivos descritos, pero además las reivindicaciones de métodos o procesos pueden ser incluidos para dirigir las funciones de la invención y la realización de cada elemento. Ni la descripción ni la terminología presentan la intención de limitar el alcance de las reivindicaciones las cuales son incluidas.

Además, cada uno de los varios elementos de la invención y reivindicaciones pueden además ser realizados de una variedad de maneras. Esta divulgación debe ser

132
232

entendida para abarcar cada una de tales variaciones, siendo una variación de una realización de cualquier realización de aparatos, un método o realización de proceso, o incluso meramente una variación de cualquiera de estos elementos. Particularmente debe entenderse que mientras que la divulgación se refiere a los elementos de la invención, las palabras para cada elemento pueden ser expresados por términos de aparatos equivalentes o términos de métodos, incluso si sólo la función o resultado es el mismo. Tales términos genéricos amplios o incluso más genéricos deben ser considerados para que abarquen en la descripción para cada elemento o acción. Tales términos pueden ser sustituidos en donde se desee para hacer más explícita la cobertura amplia implícita para lo cual la invención está asignada. Como un ejemplo, debe entenderse que todas las acciones pueden ser expresadas por medio de tomar aquella acción o como un elemento el cual causa esa acción. Similarmente, cada elemento físico divulgado debe ser entendido para que abarque una divulgación de la acción en la cual ese elemento físico facilita. Con respecto a este último aspecto, como ejemplo la divulgación de un separador de gota A debe entenderse para que abarque la divulgación del acto de la separación de gotas, ya sea discutido explícitamente o no, y en cambio, en donde sólo la divulgación del hecho de cubrir el líquido gaseoso tal

como se divulga debe ser entendido para que abarque la divulgación de un separador de gota e incluso un medio para separar gotas Tales cambios y términos alternativos son destinados para ser explícitos incluidos en la descripción

Adicionalmente, varias combinaciones y permutaciones de todos los elementos o aplicaciones pueden ser creados y presentados Todo puede ser realizado para optimizar el diseño o performance en una aplicación específica

Cualquier acto de ley, estatuto, regulación o reglas mencionadas en esta solicitud para patente, o patentes, publicaciones u otras referencias mencionadas en esta solicitud para la patente son aquí incorporados a modo de referencia Especialmente las solicitudes de patentes norteamericanas U S No 60/267571, 60/239 752 y 60/203 089 que son incorporadas a la presente a modo de referencia incluyendo aquí cualquier figura o adjunto, cada una de las referencias en la siguiente tabla de referencias son incorporadas a la presente a modo de referencia

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248

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250
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términos alternativos y sinónimos tales como los contenidos en el diccionario Unabridged Webster House Random, segunda edición que son aquí incorporados a modo de referencia Sin embargo, para cada uno de los mencionados anteriormente, hacia la extensión de que tal información o divulgación incorporada por referencia debe ser considerada inconsistentemente con el patentamiento de estas invenciones, tales divulgaciones son expresadas para no ser consideradas como realizadas por el solicitante

Además, a no ser que el contexto requiera lo contrario, debe entenderse que el término "comprende" o variaciones tales como "comprendiendo" o "comprenden" están dirigidas para otorgar la inclusión de un elemento mencionado o paso o grupo de elementos o pasos pero no la exclusión de cualquier otro elemento o paso o grupo de elementos o pasos Tales términos deben ser interpretados en su forma más extensa para afrontar la cobertura amplia del solicitante legalmente permisible en países tales como Australia y lo similar

De esta manera, el solicitante debe entender que tiene el soporte para reivindicar al menos i) cada uno de los dispositivos de conversión líquido a gas descritos aquí, ii) los métodos relacionados divulgados y descritos, iii) variaciones implícitas similares, equivalentes, para cada uno de estos dispositivos y métodos, iv) aquellos

251 2/2

diseños alternativos los cuales alcanzan cada una de las funciones ilustradas tales como son divulgadas y descritas, v) aquellos diseños alternativos y métodos los cuales realizan cada una de las funciones ilustradas tales como son implícitas para realizar aquello que está divulgado y descrito, vi) cada característica componente y paso ilustrado como dimensión independiente y separada, vii) las aplicaciones mejoradas para los varios sistemas o componentes divulgados, viii) los productos resultantes producidos por tales sistemas o componentes, ix) métodos y aparatos sustancialmente descritos anteriormente y con referencia a cualquiera de los ejemplos acompañantes y x) las varias combinaciones y permutaciones de cada uno de los elementos divulgados

Además, a no ser que el contexto requiera lo contrario, debe entenderse que el término "comprende" o variaciones tales como "comprendiendo" o "comprenden" está dirigido para otorgar la inclusión de un elemento mencionado o paso o grupo de elementos o pasos pero no la exclusión de cualquier otro elemento o paso o grupo de elementos o pasos Tales términos deben ser interpretados en su forma más extensa para afrontar la cobertura amplia del solicitante legalmente permisible en países tales como Australia y lo similar

252 ~~252~~
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Las reivindicaciones mencionadas en esta solicitud son aquí incorporadas a modo de referencia como parte de esta descripción de la invención, y el solicitante se reserva expresamente el derecho y el uso de la totalidad de o una porción de tal contenido incorporado de tales reivindicaciones como descripción adicional para soportar cualquiera o la totalidad de las reivindicaciones o cualquier elemento o componente del mismo, y el solicitante además se reserva expresamente el derecho de mover cualquier porción o la totalidad del contenido incorporado de tales reivindicaciones o cualquier elemento o componente del mismo a partir de la descripción dentro de las reivindicaciones o viceversa si es necesario para definir la materia para la cual la protección es traída por medio de esta solicitud o por medio de cualquier continuación subsecuente, división o solicitud de continuación en parte de la misma, o para obtener cualquier beneficio de la reducción en las tasas perseguidas, o para cumplir con las leyes de patente, reglas o regulaciones de cualquier país o tratado, y tal contenido incorporado por referencias deben sobrevivir durante la totalidad del trámite de esta solicitud incluyendo cualquier continuación subsecuente, división o solicitud de continuación en parte de la misma o cualquier reemisión o extensión de la misma

253/292

REIVINDICACIONES

1 Un método para aislar células de esperma que contiene cromosoma X y células de esperma que contiene cromosoma Y, que comprende los pasos de

a recolectar las células de esperma de un macho de una especie de mamífero,

b determinar la característica de diferenciación de dichas células de esperma,

c diferenciar entre dichas células de esperma basadas sobre dicha característica de diferenciación de sexo,

d separar las células de esperma diferenciadas en una población que contiene cromosoma X y una población que contiene cromosoma Y, y

e producir separadamente las poblaciones que contienen el cromosoma X y el cromosoma Y de células de esperma, cada una presentando una puridad mayor que 90%

2 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 1, en donde dicho paso de diferenciar entre dichas células de esperma basadas sobre dicha característica de

diferenciación de sexo comprende evaluar una cantidad de ADN dentro de un núcleo de dichas células de esperma

3 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 1, en donde dicho paso de diferenciar dichas células de esperma basadas sobre dicha característica de diferenciación de sexo, comprende evaluar un volumen de una cápsula que rodea dicha cantidad de ADN

4 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 3, en donde dicho paso de evaluar dicho volumen de dicha cápsula que rodea dicha cantidad de ADN comprende determinar un volumen de un núcleo dentro de dichas células de esperma

5 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 3, en donde dicho paso de evaluar el volumen de una cápsula que rodea dicho ADN comprende determinar el volumen de la cabeza de esperma

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255

6 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 5, en donde dicho paso de evaluar dicho volumen de dicha cápsula que rodea dicha cantidad de ADN comprende además los pasos de

a generar un rayo de luz que presenta formas de ondas características,

b atravesar dicho volumen de dicha cápsula que rodea dicha cantidad de ADN con dicho rayo de luz,

c alterar dichas formas de onda características atravesando dicho volumen de dicha cápsula, y

d analizar las formas de onda características alteradas

7 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 6, en donde dicho paso de alterar dichas características de formas de onda por medio del atravesado de dicho volumen de dicha cápsula comprende cambiar la fase de dicha onda inicial de dicho rayo de radiación electromagnética

8 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen

cromosoma Y tal como se describe en la reivindicación 6, que comprende además el paso de superponer dichos rayos de luz presentando una frecuencia alterada en dicho rayo de luz que tiene dichas formas de ondas características

9 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 8, que comprende además el paso de comparar la intensidad de la radiación electromagnética superpuestos con dicho rayo de radiación electromagnética con la intensidad de dicho rayo de radiación electromagnética que tiene dichas formas de ondas características

10 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 9, que comprende además el paso de determinar dicho volumen de dicha cápsula que contiene dicha cantidad de ADN basado sobre una diferencia en dicha intensidad de luz entre dichos rayos superpuestos de radiación electromagnética y dicho rayo de radiación electromagnética que tiene formas de ondas características

257

11 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 2, que además comprende los pasos de

a introducir dichas células de esperma dentro de una corriente de fluido,

c formar gotas una pluralidad teniendo una de dichas células de esperma transportada,

b analizar dichas células de esperma transportadas en dicha corriente de flujo,

d cargar cada una de dichas gotas diferencialmente basado sobre dicha característica de diferenciación de sexo de dichas células de esperma transportadas en dichas gotas,

e desviar cada una de dichas gotas,

f diferencialmente recolectar cada una de dichas gotas basadas sobre dichas características de diferenciación de sexo de dichas células de esperma transportadas en dichas gotas

12 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 11, que comprende además el paso de mantener dicha cantidad de ADN dentro de dicho núcleo de dicha célula de esperma sin mancha

2582

13 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 11, que comprende además el paso de manchar dicha cantidad de ADN dentro de dicho núcleo de dicha célula de esperma

14 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 13, que comprende además el paso de irradiar el ADN manchado dentro de dicho núcleo de dicha célula de esperma

15 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 14, que comprende además el paso de detectar la luz fluorescente emitida desde el ADN manchado irradiado dentro del núcleo de dicha célula de esperma

16 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 15, en donde dicho paso de detectar la luz fluorescente desde el ADN manchado irradiado dentro de dicho núcleo de dicha

259
8/2

célula de esperma comprende generar una señal con un tubo fotomultiplicador

17 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 16, que comprende además el paso de operar dicho tubo fotomultiplicador fuera de una típica operación de rango de voltaje

18 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 14, en donde dicho paso de irradiar el ADN manchado dentro del núcleo de dicha célula de esperma comprende además generar un patrón de rayo de irradiación que presenta una altura reducida

19 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 18, en donde dicha altura reducida comprende una altura casi igual a la longitud de dicha célula de esperma a lo largo de un eje longitudinal hasta alrededor de tres veces la

260 29

longitud de dicha célula de esperma a lo largo de el eje longitudinal

20 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 19, en donde dicha célula de esperma tiene un eje longitudinal de alrededor de nueve micrómetros de largo, y dicha altura de dicho patrón de rayo de irradiación es de alrededor de 20 micrómetros

21 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 18, en donde dicho paso de formar las gotas comprende formar gotas que presentan un tamaño suficiente para encapsular sustancialmente la totalidad de las células de esperma

22 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 21, que comprende además el paso de expulsar dicha corriente de fluido desde una boquilla presentando un orificio, en donde dicho orificio tiene un diámetro de 70 micrómetros

09/2
261

23 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 22, que comprende además los pasos de

a orientar dichas células de esperma con respecto a un detector,

b detectar la luz que presenta características diferenciales sensibles a la orientación de las células de esperma hacia dicho detector,

c convertir dicha luz que presenta características diferenciales sensibles a dicha orientación de célula de esperma dentro de al menos una señal que contiene la información de la orientación de célula, y

d determinar la orientación de dicha célula de esperma con respecto a dicho detector

24 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 23, que comprende además el paso de diferencialmente colectando dichas gotas basadas en una orientación determinada de dicha célula de esperma con respecto a dicho detector

25 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen

cromosoma Y tal como se describe en las reivindicaciones 1, 6, 12, 13, 17, 18, 21 o 23, en donde dicha puridad de dicho cromosoma X y dicho cromosoma Y que presenta la población de células de esperma es seleccionado del grupo que consiste de entre alrededor de 90% a alrededor de 100%, entre alrededor de 91% a alrededor de 100%, entre alrededor de 92% a alrededor de 100%, entre alrededor de 93% a alrededor de 100%, entre alrededor de 94% a alrededor de 100%, entre alrededor de 95% a alrededor de 100%, entre alrededor de 96% a alrededor de 100%, entre alrededor de 97% a alrededor de 100%, entre alrededor de 98% a alrededor de 100%, entre alrededor de 99% a alrededor de 100%

26 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en las reivindicaciones 1, 6, 12, 13, 17, 18, 21 o 23, que comprende además el paso de establecer una velocidad de evento separable del grupo que consiste de al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos separables por segundo, al menos

263 262

13 000 eventos separables por segundo, al menos 14 000 eventos separables por segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000 eventos separables por segundo, al menos 19 000 eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 21 000 eventos separables por segundo

27 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en las reivindicaciones 1, 6, 12, 13, 18, 21 o 23 en donde dicho paso de separar dichas células de esperma comprende una velocidad de separación seleccionada del grupo que consiste de al menos 500 separaciones por segundo, al menos 1000 separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo

28 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en las reivindicaciones 1, 6, 12, 13, 18, 21 o 23, en donde dicho paso de formar gotas teniendo cada una de dichas células de esperma transportadas que comprende una velocidad de formación de gotas seleccionada del grupo que consiste de al menos 10 000 gotas por segundo, al menos 20 000 gotas por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo

29 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 1, en donde dicha especie de mamífero comprende un mamífero bovino

30 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 1, en donde dicha especie de mamífero comprende un mamífero equino

31 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 1, en donde dicha especie de mamífero comprende un mamífero ovino

32 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y, que comprende

a células de esperma que presentan cabezas de esperma, en donde dichas cabezas de esperma tienen una diferencia en el volumen entre las células de esperma que contienen cromosoma X y las células de esperma que contienen cromosoma Y,

b un rayo de radiación electromagnética que presenta características de formas de onda iniciales diferencialmente sensible a dicha diferencia en el volumen entre las células de esperma que contienen cromosoma X y las células de esperma que contienen cromosoma Y,

c un detector sensible a dichas características de formas de onda de dicho rayo de radiación electromagnética,

d un analizador acoplado a dicho detector, en donde dicho analizador diferencia entre dichas células de esperma que contienen cromosoma X y dichas células de

esperma que contienen cromosoma Y basado sobre dicha alteración de las características de forma de onda

33 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 32, que comprende además una corriente de fluido dentro del cual dichas células de esperma son introducidas

34 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 33, que además comprende gotas que se destruyen desde dicha corriente de fluido cada una presentando una de dichas partículas transportadas

35 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 34, en donde dichas gotas que se destruyen desde dicha corriente de fluido tienen el tamaño suficiente para encapsular una de dichas células de esperma

36 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen

267 286

cromosoma Y tal como se describe en la reivindicación 35, que comprende además una boquilla que presenta un orificio de alrededor de 100 micrómetros en diámetro

37 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 36, que comprende además un cargador de gotas acoplado a dicho analizador, en donde dichas gotas reciben una carga diferencial basada sobre dicha diferencia en el volumen entre las células de esperma que contienen cromosoma X y las células de esperma que contienen cromosoma Y

38 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 37, que comprende además un separador de gota, en donde dicho separador de gota separa dicha gota basado sobre la carga de dichas gota

39 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 38, que comprende además al menos un contenedor de recolección en el cual las gotas que contienen dichas células de

268 ¹/₆ ~

esperma que contienen cromosoma X son recolectadas como una población que contiene cromosoma X

40 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 38, que comprende además al menos un contenedor de recolección en el cual las gotas que contienen células de esperma que contienen cromosoma Y son recolectadas como una población que contiene cromosoma Y

41 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en las reivindicaciones 38 o 39, en donde dicha población que contiene cromosoma X y dicha población que contiene cromosoma Y de dichas células de esperma presentan una pureza seleccionada del grupo que consiste de entre 90% a alrededor de 100%, entre alrededor de 91% a alrededor de 100%, entre alrededor de 92% a alrededor de 100%, entre alrededor de 93% a alrededor de 100%, entre alrededor de 94% a alrededor de 100%, entre alrededor de 95% a alrededor de 100%, entre alrededor de 96% a alrededor de 100%, entre alrededor de 97% a alrededor de 100%, entre alrededor de 98% a alrededor de 100%, entre alrededor de 99% a alrededor de 100%

42 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 41, que comprende además una velocidad de evento separable en donde dicha velocidad de evento separable seleccionada del grupo que consiste de al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos separables por segundo, al menos 13 000 eventos separables por segundo, al menos 14 000 eventos separables por segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000 eventos separables por segundo, al menos 19 000 eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 21 000 eventos separables por segundo

43 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 42,

270
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que comprende además una velocidad de separación seleccionada del grupo que consiste de al menos 500 separaciones por segundo, al menos 1000 separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo, al menos 11 000 separaciones por segundo

44 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 43, que comprende además una velocidad de formación de gotas seleccionada del grupo que consiste de al menos 10 000 gotas por segundo, al menos 20 000 gotas por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo

06/2
27/162

45 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 44, en donde dicho detector comprende al menos un tubo fotomultiplicador en donde dicho al menos un tubo fotomultiplicador convierte dicha luz coherente en al menos una señal, y en donde dicho tubo fotomultiplicador tiene un rango de voltaje de operación típico, y en donde dicho tubo fotomultiplicador es operado fuera de dicho rango de voltaje de operación típico

46 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 45, en donde dicho rango de voltaje de operación típica de dicho tubo multiplicador es desde alrededor de 400 voltios a alrededor de 999 voltios

47 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 46, en donde dicho tubo fotomultiplicador es operado en un rango de alrededor de 0 voltios a alrededor de 300 voltios

272 ¹/₂

48 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 41, en donde dicha especie de mamífero comprende un mamífero bovino

49 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 41, en donde dicha especie de mamífero comprende un mamífero equino

50 Un aparato para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 41, en donde dicha especie de mamífero comprende un mamífero ovino

51 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y, que comprende los pasos de

a recolectar células de esperma de un macho de una especie de mamífero,

b evaluar un volumen de una cápsula que contiene el ADN nuclear de dicha célula esperma

293 2

c diferenciar entre dichas células de esperma que contienen cromosoma X y dichas células de esperma que contienen cromosoma Y basado sobre el volumen de dicha cápsula que contiene dicho ADN nuclear de dichas células de esperma

52 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 51, en donde dicho paso de evaluar el volumen de una cápsula que contiene dicho ADN comprende determinar un cambio de fase en dicha radiación electromagnética

53 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 52, en donde dicho paso de evaluar el volumen de una cápsula que rodea dicho ADN comprende

a generar un rayo de radiación electromagnética que presenta características de forma de onda inicial,

b atravesar dicho volumen de dicha cápsula que contiene dicho ADN con dicho rayo de radiación electromagnética que presenta características de forma de onda inicial,

274 ²/₂

c alterar dichas características de forma de onda inicial por medio de atravesar dicho volumen de dicha cápsula, y

d analizar las características de forma de onda alterada de dicho rayo de radiación electromagnética

54 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 53, en donde dicho paso de alterar dichas características de forma de onda por medio del atravesado del volumen de dicha cápsula comprende cambiar la fase de dichas formas de onda características iniciales de dicho rayo de radiación electromagnética

55 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 54, que comprende además el paso de superponer dichas características de forma de onda inicial y el cambio de fase de las características de forma de onda

56 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 55,

275

que comprende además el paso de comparar una intensidad de dichas características de forma de onda inicial con la superposición de características de forma de onda

57 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 56, que comprende además el paso de determinar dicho volumen de dicha cápsula que contiene dicho ADN basado sobre la diferencia de dicha intensidad entre dichos rayos superpuestos características de forma de onda y dichas características iniciales de forma de onda

58 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 57, que comprende además los pasos de

a introducir dichas células de esperma dentro de una corriente de fluido,

b formar gotas una pluralidad que presenta de dichas células de esperma transportadas,

c cargar cada una de dichas gotas diferencialmente basadas sobre el volumen evaluado de dicha cápsula que rodea el ADN nuclear de dichas células de esperma,

d desviar cada una de dichas gotas, y

e recolectar cada una de dichas gotas basadas sobre dicho volumen de dicha cápsula que rodea dicho ADN, y
f generando la población que contiene cromosoma X y cromosoma Y de dichas células de esperma

59 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 58, que comprende además el paso de mantener dicha cantidad de ADN dentro del núcleo de dichas células de esperma sin manchar

60 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 59, que comprende además el paso de formar gotas que presentan el tamaño suficiente para encapsular dichas células de esperma

61 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 60, que comprende además el paso de expulsar dicha corriente de fluido desde una boquilla que presenta un orificio, en donde dicho orificio tiene un diámetro de 100 micrómetros

277

62 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 58, que comprende además los pasos de

a detectar una señal que presenta características de orientación de células de esperma,

b comparar dicha señal presentando las características de orientación de células de esperma hacia una señal que presenta características de células de esperma orientadas

c determinar que dichas células de esperma tienen características de células de esperma sin orientar, y

d diferencialmente recolectar dichas células de esperma que presentan características de células de esperma sin orientar

63 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 58 o 62, en donde la puridad de dicha población que contiene cromosoma X y cromosoma Y de dichas células de esperma es seleccionada del grupo que consiste de alrededor de 90% a alrededor de 100%, entre alrededor de 91% a alrededor de 100%, entre alrededor de 92% alrededor de 100%, entre

278²

alrededor de 93% a alrededor de 100%, entre alrededor de 94% a alrededor de 100%, entre alrededor de 95% a alrededor de 100%, entre alrededor de 96% a alrededor de 100%, entre alrededor de 97% a alrededor de 100%, entre alrededor de 98% a alrededor de 100%, entre alrededor de 99% a alrededor de 100%

64 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 63, que comprende además el paso de establecer una velocidad de evento separable en donde dicha velocidad de evento separable es seleccionada del grupo que consiste de al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos separables por segundo, al menos 13 000 eventos separables por segundo, al menos 14 000 eventos separables por segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000 eventos separables por segundo, al menos 19 000

279 ²/₂

eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 21 000 eventos separables por segundo

65 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 64, en donde dicho paso de separar dichas células de esperma comprende una velocidad de separación seleccionada del grupo que consiste de al menos 500 separaciones por segundo, al menos 1000 separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo

66 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 65, en donde dicho paso de formar gotas que tienen cada una dichas células de esperma transportadas comprende una velocidad de formación de gotas seleccionada del grupo que

280-271

consiste de al menos 10 000 gotas por segundo, al menos 20 000 gotas por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo

67 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 51, en donde dicha especie de mamífero comprende un mamífero bovino

68 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 51, en donde dicha especie de mamífero comprende un mamífero equino

69 Un método para aislar células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y tal como se describe en la reivindicación 51, en donde dicha especie de mamífero comprende un mamífero ovino

281
08/2

70 Un método para diferenciar partículas, que comprende los pasos de

- a recolectar partículas asimétricas,
- b establecer dicha corriente de fluido con un citómetro de flujo,
- c introducir dichas partículas asimétricas dentro de dicha corriente de fluido,
- d orientar dichas partículas asimétricas con respecto a un detector,
- e detectar la luz que presenta características diferenciales sensibles a la orientación de partículas asimétricas hacia dicho detector,
- g convertir dicha luz que presenta características diferenciales sensibles a la orientación de partículas asimétricas dentro de al menos una señal que contiene la información de orientación de partícula asimétrica,
- h analizar la información de orientación de partícula asimétrica, y
- i determinar la orientación de dichas partículas asimétricas con respecto a dicho detector

71 Un método para diferenciar partículas tal como se describe en la reivindicación 70, en donde dicha corriente de fluido comprende una vaina de fluido

282
182

72 Un método para diferenciar partículas tal como se describe en la reivindicación 70, en donde dicho detector comprende un tubo fotomultiplicador

73 Un método para diferenciar partículas tal como se describe en la reivindicación 72, en donde dicha luz comprende luz fluorescente

74 Un método para diferenciar partículas tal como se describe en la reivindicación 73, en donde dicha luz fluorescente emite desde un material de emisión de luz unido a dichas partículas asimétricas

75 Un método para diferenciar partículas tal como se describe en la reivindicación 70, en donde dicho paso de analizar la información de orientación de partícula asimétrica comprende los pasos de

a trazar una señal electrónica por un período de tiempo,

b integrar las áreas de los trazos correspondientes a dicha señal electromagnética, y

c comparar las conformaciones de dichos trazos con las conformaciones de partículas asimétricamente orientadas

76 Un método para diferenciar partículas tal como se describe en la reivindicación 70, que comprende además remover dichas partículas asimétricas desde dicha corriente de fluido basado sobre la orientación determinada de dichas partículas asimétricas con respecto a dicho detector

77 Un método para diferenciar partículas tal como se describe en la reivindicación 70, en donde dichas partículas asimétricas son células de esperma de un macho de una especie de mamífero

78 Un método para diferenciar partículas tal como se describe en la reivindicación 70, que comprende además los pasos de

a evaluar el volumen de una cápsula que rodea el ADN nuclear de dichas células de esperma, y

b diferenciar entre dichas células de esperma que contienen cromosoma X y dichas células de esperma que contienen cromosoma Y basado sobre el volumen de dicha cápsula que rodea el ADN nuclear de dicha célula de esperma

79 Un método para diferenciar partículas tal como se describe en la reivindicación 78, en donde dicha cápsula que rodea dicho ADN comprende una cabeza de esperma

284 2/3

80 Un método para diferenciar partículas tal como se describe en la reivindicación 78, en donde dicho paso de evaluar el volumen de una cápsula que rodea dicho ADN comprende

a generar un rayo de luz coherente que presenta características de forma de onda inicial,

b atravesar dicho volumen de dicha cápsula que rodea dicho ADN con dicho rayo de luz coherente que presenta características de forma de onda inicial,

c alterar dichas características de forma de onda de dicho rayo coherente de luz por medio de atravesar dicho volumen de dicha cápsula, y

d analizar las características de forma de onda alterada

81 Un método para diferenciar partículas tal como se describe en la reivindicación 80, en donde dicho paso de alterar dichas características de forma de onda por medio de atravesar dicho volumen de dicha cápsula comprende cambiar la fase de dichas características de forma de onda inicial

82 Un método para diferenciar partículas tal como se describe en la reivindicación 81, que comprende además

285

el paso de superponer dichas características de forma de onda inicial y dichos cambios de fase de las características de forma de onda

83 Un método para diferenciar partículas tal como se describe en la reivindicación 82, que comprende además el paso de comparar una intensidad de dichas características de forma de onda inicial y superposición de las características de forma de onda

84 Un método para diferenciar partículas tal como se describe en la reivindicación 83, que comprende además el paso de determinar dicho volumen de dicha cápsula que rodea dicho ADN basado sobre la diferencia de dicha intensidad

85 Un método para diferenciar partículas tal como se describe en la reivindicación 80, que comprende además los pasos de

a formar gotas que presentan cada una de dichas células de esperma transportadas,

b cargar cada una de dichas gotas diferencialmente basado sobre el volumen determinado de dicha cápsula que rodea el ADN nuclear de dichas células de esperma,

c desviar cada una de dichas gotas, y

286
SP2

d recolectar cada una de dichas gotas basado sobre la carga de dicha gota, en donde la recolección de dichas gotas y,

e generando al menos poblaciones que comprenden cromosoma X y cromosoma Y de dichas células de esperma

86 Un método para diferenciar partículas tal como se describe en la reivindicación 77, que comprende además los pasos de

a formar gotas que presenta cada una de dichas células de esperma transportadas,

b cargar cada una de dichas gotas diferencialmente basado sobre una cantidad de ADN nuclear de dichas células de esperma,

c desviar cada una de dichas gotas, y

d recolectar cada una de dichas gotas basado sobre la carga de dicha gota, en donde la recolección de dichas gotas y,

e generando al menos poblaciones que comprenden cromosoma X y cromosoma Y de dichas células de esperma

87 Un método para diferenciar partículas tal como se describe en la reivindicación 85 u 86, en donde dicho paso de formar una pluralidad de gotas que tienen cada una dichas células de esperma transportadas comprende la

287

formación de gotas que tienen el tamaño suficiente para encapsular en su totalidad dichas células de esperma, en donde dicha célula de esperma total comprende una célula de esperma viva intacta que presenta una cola

88 Un método para diferenciar partículas tal como se describe en la reivindicación 87, que comprende además el paso de expulsar dicha corriente de fluido desde una boquilla que presenta un orificio, en donde dicho orificio tiene un diámetro de 100 micrómetros

89 Un método para diferenciar partículas tal como se describe en la reivindicación 88, en donde dicha población que contiene cromosoma X y cromosoma Y de células de esperma presenta una puridad seleccionada del grupo que consiste de entre alrededor de 90% a alrededor de 100%, entre alrededor de 91% a alrededor de 100%, entre alrededor de 92% a alrededor de 100%, entre alrededor de 93% a alrededor de 100%, entre alrededor de 94% a alrededor de 100%, entre alrededor de 95% a alrededor de 100%, entre alrededor de 96% a alrededor de 100%, entre alrededor de 97% a alrededor de 100%, entre alrededor de 98% a alrededor de 100%, entre alrededor de 99% a alrededor de 100%

Jb
288^c

90 Un método para diferenciar partículas tal como se describe en la reivindicación 88, que comprende además el paso de establecer una velocidad de evento separable donde dicha velocidad de evento separable es seleccionada del grupo que consiste de al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos separables por segundo, al menos 13 000 eventos separables por segundo, al menos 14 000 eventos separables por segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000 eventos separables por segundo, al menos 19 000 eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 21 000 eventos separables por segundo

91 Un método para diferenciar partículas tal como se describe en la reivindicación 88, en donde dicho paso de separar dichas células de esperma comprende una velocidad de separación seleccionada del grupo que consiste de al menos 500 separaciones por segundo, al menos 1000

289

separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo

92 Un método para diferenciar partículas tal como se describe en la reivindicación 88, en donde dicho paso de formar gotas que tiene cada una dichas células de esperma transportadas comprende una velocidad de formación de gotas seleccionada del grupo que consiste de al menos 10 000 gotas por segundo, al menos 20 000 gotas por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo

93 Un método para diferenciar partículas tal como se describe en la reivindicación 77, en donde dicha especie de mamífero comprende un mamífero bovino

290
1820

94 Un método para diferenciar partículas tal como se describe en la reivindicación 77, en donde dicha especie de mamífero comprende un mamífero equino

95 Un método para diferenciar partículas tal como se describe en la reivindicación 77, donde dicha especie de mamífero comprende un mamífero ovino

96 Un aparato de diferenciación de partícula que comprende

a al menos una partícula asimétrica que presenta características de orientación dentro de una corriente de fluido,

b una fuente de irradiación que genera un rayo de irradiación sensible a dicha partícula asimétrica,

c ópticas para enfocar dicho rayo de irradiación,

d un material de emisión de luz acoplado a dicha partícula asimétrica, en donde dicho material de emisión de luz emite luz en respuesta a dicho rayo de irradiación,

e un detector sensible diferencialmente a dicha luz emitida desde dicho material de emisión de luz basado sobre dichas características de orientación de partícula, y

f un analizador acoplado a dicho detector, en donde dicho analizador diferencia dichas partículas

29/2

asimétricas basadas sobre la orientación dentro de dicha corriente de fluido

97 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 96, en donde dicha al menos una partícula asimétrica comprende al menos una célula de esperma

98 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 96, en donde dichas ópticas enfocan un patrón de rayo que presenta una altura de alrededor igual a la longitud de dicha partícula asimétrica a lo largo del eje longitudinal alrededor de dos veces la longitud de dicha partícula asimétrica a lo largo del eje longitudinal

99 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 98, en donde dicha al menos una célula de esperma tiene una cabeza que presenta una longitud a lo largo del eje longitudinal de alrededor de 9 micrómetros, y en donde dicho patrón de irradiación tiene una altura de alrededor de 20 micrómetros

100 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 96, en donde dicha al

292²

menos una partícula asimétrica presenta características de orientación que comprende además al menos una característica de diferenciación de partícula

101 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 96, en donde dicho material de emisión de luz unido a dicha al menos una partícula asimétrica comprende una mancha unida al ADN nuclear de la célula de esperma de un macho de una especie de mamífero

102 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 101, en donde dicha característica de diferenciación de partícula comprende una diferencia en cantidad de dicha mancha unida a dicho ADN nuclear de las células de esperma que contienen cromosoma X y dicho ADN nuclear de las células de esperma que contienen cromosoma Y

103 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 96, en donde dicho detector comprende al menos un tubo fotomultiplicador, y en donde dicho tubo fotomultiplicador tiene un rango de voltaje de operación típico, en donde dicho tubo

fotomultiplicador está operado fuera de dicho rango de voltaje de operación típico

104 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 103, en donde dicho rango de voltaje de operación típico de dicho tubo fotomultiplicador es desde alrededor de 400 voltios a alrededor de 999 voltios

105 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 104, en donde dicho tubo fotomultiplicador está operado en un rango de alrededor de 0 voltios a alrededor de 300 voltios

106 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 96, que comprende además gotas que se destruyen desde dicha corriente de fluido que presenta cada una al menos una de dichas partículas asimétricas transportadas

107 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 106, en donde dichas gotas que se destruyen desde dicha corriente de fluido presentan un tamaño suficiente para encapsular una de dichas células de esperma, en donde dichas células de

342
294

esperma comprenden células de esperma vivas intactas que presentan al menos una cabeza y un cuello y una cola

108 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 107, que comprende además una boquilla que presenta un orificio de un diámetro de alrededor de 100 micrones

109 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 106, que comprende además un cargador de gotas acoplado a dicho analizador, en donde dichas gotas reciben una carga diferencial basada sobre dicha diferencia en la cantidad de dicha mancha unida al ADN nuclear de las células de esperma que contienen cromosoma X y del ADN nuclear de las células de esperma que contienen cromosoma Y

110 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 109, que comprende además un cargador de gota acoplado a dicho analizador, en donde dichas gotas reciben una carga diferencial basada sobre dicha diferencia en el volumen de dichas partículas, y en donde dicha diferencia en volumen de dichas partículas comprende una diferencia entre las células de esperma que

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295

contienen cromosoma X y las células de esperma que contienen cromosoma Y

111 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 110, que comprende además un separador de gotas, en donde dicho separador de gotas separa dichas gotas basada sobre la carga de dicha gota

112 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 111, que comprende además al menos un contenedor de recolección en el cual las gotas que contienen dichas células de esperma que contienen cromosoma X son recolectadas como una población que contiene cromosoma X

113 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 111, que comprende además al menos un contenedor de recolección en el cual las gotas que contienen dichas células de esperma que contienen cromosoma Y son recolectadas como una población que contiene cromosoma Y

114 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 112 o 113, en donde

296

dicha población que contiene cromosoma X y cromosoma Y de dichas células de esperma es seleccionada del grupo que consiste de entre 90% a alrededor de 100%, entre 91% a alrededor de 100%, entre 92% a alrededor de 100%, entre 93% a alrededor de 100%, entre 94% a alrededor de 100%, entre 95% a alrededor de 100%, entre 96% a alrededor de 100%, entre 97% a alrededor de 100%, entre 98% a alrededor de 100%, entre 99% a alrededor de 100%

115 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 114, que comprende además el paso de establecer una velocidad de evento separable en donde dicha velocidad de evento separable es seleccionada del grupo que consiste de al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos separables por segundo, al menos 13 000 eventos separables por segundo, al menos 14 000 eventos separables por segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000

eventos separables por segundo, al menos 19 000 eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 21 000 eventos separables por segundo

116 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 115, en donde dicho paso de separar dichas células de esperma comprende una velocidad de separación seleccionada del grupo que consiste de al menos 500 separaciones por segundo, al menos 1000 separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo, al menos 11 000 separaciones por segundo

117 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 116, en donde dicho paso de formar gotas que tiene cada una de dichas células de esperma transportadas comprende una velocidad de formación de gotas seleccionada del grupo que consiste de al menos 10 000 gotas por segundo, al menos 20 000 gotas

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298

por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo

118 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 102, en donde dichas especies de mamífero comprende un mamífero bovino

119 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 102, en donde dichas especies de mamífero comprende un mamífero equino

120 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 102, en donde dichas especies de mamífero comprende un mamífero ovino

121 Un aparato de diferenciación de partícula, que comprende

- a una corriente de fluido,
- b células de esperma vivas intactas introducidas dentro de dicha corriente de fluido,

299 8

c una boquilla que presenta un orificio a través del cual dicha corriente de fluido sale,

d un oscilador que genera oscilaciones sensibles a dicha corriente de fluido, y

e gotas que se excluyen desde dicha corriente de fluido, en donde cada una de dichas gotas transportan dichas células de esperma vivas intactas, y en donde dichas gotas presentan el tamaño suficiente para encapsular una de dichas células de esperma vivas

122 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 121, en donde dicho orificio tiene un diámetro de 100 micrones

123 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 122, en donde dichas gotas que se destruyen desde dicha corriente de fluido se destruyen a una velocidad seleccionada del grupo que consiste de al menos 10 000 gotas por segundo, al menos 20 000 gotas por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo

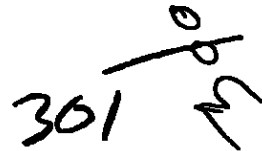
124 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 123, que comprende además una fuente de emisión de luz que genera luz

125 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 124, que comprende además un detector sensible a dicha luz

126 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 125, en donde dicha al menos una célula de esperma viva intacta tiene al menos una característica de diferenciación de sexo

127 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 126, en donde dicha al menos una característica de diferenciación de sexo comprende una diferencia de volumen de cabeza de célula de esperma de dicha célula de esperma viva intacta

128 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 127, en donde dicha fuente de emisión de luz emite luz coherente, y en donde dicha luz coherente atraviesa dicho volumen de dicha cabeza de célula de esperma, y en donde dicha luz coherente tiene

301 

características de forma de onda diferenciales sensible a dicha diferencia en volumen de dicha cabeza de célula de esperma

129 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 128, que comprende además un analizador sensible a dicho detector, en donde dicho analizador diferencia entre dichas células de esperma vivas intactas basadas sobre dicha diferencia de volumen de dichas cabezas de células de esperma

130 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 129, en donde dicha diferencia de volumen de dichas cabezas de células de esperma en volumen comprenden una diferencia de células de esperma intactas vivas que contienen cromosoma X y cromosoma Y

131 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 126, en donde una fuente de irradiación genera un rayo de irradiación sensible a dichas células de esperma vivas intactas

132 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 131, que comprende

además ópticas, en donde dichas ópticas enfocan un patrón de rayo de irradiación que presenta una altura igual a alrededor de la longitud de dichas cabezas de esperma a lo largo del eje longitudinal hacia alrededor de dos veces la longitud de dicha cabeza de esperma de dichos a lo largo del eje longitudinal

133 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 132, en donde dichas cabezas de esperma tienen una longitud a lo largo del eje longitudinal que es de alrededor de 9 micrómetros, y en donde dicho patrón de irradiación tiene una altura de alrededor de 20 micrómetros

134 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 131, en donde dicha al menos una característica de diferenciación de sexo comprende una diferencia en cantidad de ADN nuclear de dichas células de esperma vivas intactas, y en donde dicha fuente de emisión de luz comprende un material de emisión de luz unido al ADN nuclear, y en donde dicho material de emisión de luz emite luz diferencialmente basado sobre dicha diferencia en la cantidad de dicho ADN nuclear, y en donde dicho detector genera al menos una señal en respuesta a dicha luz

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135 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 134, que comprende además un analizador sensible a dicho detector, en donde dicho analizador diferencia entre dicha al menos una célula de esperma viva intacta basada sobre dicha diferencia en la cantidad de ADN nuclear

136 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 135, en donde dicha diferencia en dicha cantidad de dicho ADN nuclear comprende una diferencia entre las células de esperma que contienen cromosoma X y las células de esperma que contiene cromosoma Y

137 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 136, que comprende además un cargador de gota acoplado a dicho analizador, en donde dicho cargador de gota genera una carga en dichas gotas diferencialmente basadas sobre dicha diferencia en la cantidad de dicho ADN nuclear

138 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 129, que comprende además un cargador de gota acoplado a dicho analizador, en

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donde dicho cargador de gota genera una carga en dichas gotas diferencialmente basada sobre dicha diferencia de volumen de dichas cabezas de células de esperma, y en donde dicha diferencia de volumen de dichas cabezas de células de esperma comprende una diferencia entre células de esperma que contienen cromosoma X y células de esperma que contienen cromosoma Y

139 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 137 o 138, que comprende además un separador de gotas, en donde dicho separador de gotas separa dichas gotas basado sobre la carga de dichas gotas

140 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 139, que comprende además al menos un contenedor de recolección en el cual las gotas que contienen las células de esperma que contienen cromosoma X son recolectadas como población que contiene cromosoma X

141 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 139, que comprende además al menos un contenedor de recolección en el cual las gotas que contienen las células de esperma que contienen

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cromosoma Y son recolectadas como población que contiene cromosoma Y

142 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 125, en donde dicho detector comprende al menos un tubo fotomultiplicador, y en donde dicho al menos un tubo fotomultiplicador convierte la luz desde dicha fuente de emisión de luz en al menos una señal, y en donde dicho tubo fotomultiplicador tiene un rango de voltaje de operación típico, y en donde dicho tubo fotomultiplicador es operado fuera de dicho rango de voltaje de operación típico

143 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 142, en donde dicho rango de voltaje de operación típico de dicho tubo fotomultiplicador es de alrededor de 400 voltios a alrededor de 999 voltios

144 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 143, en donde dicho tubo fotomultiplicador es operado en un rango de alrededor de 0 voltios a alrededor de 300 voltios

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145 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 140 o 141, en donde dicha población que contiene cromosoma X y cromosoma Y de dichas células de esperma presentan una puridad seleccionada del grupo que consiste de entre 90% a alrededor de 100%, entre 91% a alrededor de 100%, entre 92% a alrededor de 100%, entre 93% a alrededor de 100%, entre 94% a alrededor de 100%, entre 95% a alrededor de 100%, entre 96% a alrededor de 100%, entre 97% a alrededor de 100%, entre 98% a alrededor de 100%, entre 99% a alrededor de 100%

146 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 145, que comprende además el paso de establecer una velocidad de evento separable en donde dicha velocidad de evento separable seleccionado del grupo que consiste de al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos separables por segundo, al menos 13 000 eventos separables por segundo, al menos 14 000 eventos separables por

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segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000 eventos separables por segundo, al menos 19 000 eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 21 000 eventos separables por segundo

147 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 146, en donde dicho paso de separar dichas células de esperma comprende una velocidad de separación seleccionada del grupo que consiste de al menos 500 separaciones por segundo, al menos 1000 separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo, al menos 11 000 separaciones por segundo

148 Un aparato de diferenciación de partícula, que comprende

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a partículas que presentan al menos una característica de diferenciación de partícula,

b una fuente de emisión de luz diferencialmente sensible a dichas características de diferenciación de partícula,

c al menos un tubo fotomultiplicador, en donde dicho al menos un tubo fotomultiplicador convierte la luz desde dicha fuente de emisión de luz en al menos una señal, y en donde dicho tubo fotomultiplicador tiene un rango de voltaje de operación típico, y en donde dicho tubo fotomultiplicador es operador fuera de dicho rango de voltaje de operación típico, y

d un analizador sensible a dicha al menos una señal la cual diferencia las partículas basadas sobre dichas características de diferenciación de partícula

149 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 148, en donde dicho rango de voltaje de operación típico de dicho tubo fotomultiplicador es desde alrededor de 400 voltios a alrededor de 999 voltios

150 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 149, en donde dicho

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tubo fotomultiplicador es operado en un rango desde alrededor de 0 voltios a alrededor de 300 voltios

151 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 148, que comprende además una fuente de irradiación que genera un rayo de irradiación sensible a dichas partículas

152 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 151, en donde dicha fuente de emisión de luz comprende un material de emisión de luz unido a dichas partículas las cuales emiten dicha luz en respuesta a dicho rayo de irradiación

153 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 152, en donde dicho material de emisión de luz está unido a dichas partículas comprende una mancha unida al ADN nuclear de las células de esperma

154 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 153, en donde dicha característica de diferenciación de partícula comprende una diferencia en la cantidad de dicha mancha unida al ADN nuclear de las células de esperma que contienen cromosoma X

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y el ADN nuclear de las células de esperma que contienen cromosoma Y

155 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 152, en donde dicha partícula comprende una partícula asimétrica

156 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 155, que comprende además ópticas para enfocar dicho rayo de irradiación sensible a dicha partícula, en donde dichas ópticas enfocan un patrón de irradiación que presenta una altura casi igual a la longitud de dicha partícula asimétrica a lo largo del eje longitudinal hacia casi el doble de la longitud de dicha partícula asimétrica a lo largo del eje longitudinal

157 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 156, que comprende además una corriente de fluido dentro de la cual dichas partículas son introducidas

158 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 157, en donde dicha al menos una característica de diferenciación de partícula comprende la orientación de dicha partícula asimétrica

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dentro de dicha corriente de fluido, y en donde dicho tubo fotomultiplicador es sensible diferencialmente a dicha luz emitida desde dicho material de emisión de luz basado sobre dichas características de orientación de partícula, y en donde dicho analizador acoplado a dicho tubo fotomultiplicador diferencia entre dichas partículas asimétricas basadas sobre la orientación dentro de dicha corriente de fluido

159 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 158, en donde dichas partículas asimétricas comprenden células de esperma que presentan cabezas de células de esperma

160 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 159, en donde dichas cabezas de células de esperma de dichas células de esperma presentan una longitud a lo largo del eje longitudinal de alrededor de 9 micrómetros, y en donde dicho patrón de irradiación tiene una altura de alrededor de 20 micrómetros

161 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 148, en donde dichas partículas presentan un volumen, y en donde dicha al menos

una característica de diferenciación de partícula comprende una diferencia de volumen de dichas partículas

162 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 161, en donde dicha fuente de emisión de luz emite luz coherente, y en donde dicha luz coherente atraviesa dicho volumen de dicha partícula, y en donde dicha luz coherente tiene características de forma de onda alteradas por medio de dicho volumen de dichas partículas

163 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 162, en donde dicho tubo fotomultiplicador es sensible a dichas características de forma de onda alteradas por dicho volumen de dichas partículas

164 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 163, en donde dicho analizador sensible a dicha al menos una señal, la cual se diferencia de las partículas basadas sobre dichas características de diferenciación de partícula diferencia entre dichas partículas basadas sobre dicha diferencia en volumen

165 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 164, en donde dicha diferencia de volumen comprende una diferencia entre las células de esperma que contienen cromosoma X y las células de esperma que contienen cromosoma Y

166 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 160, que comprende además gotas que se destruyen desde dicha corriente de fluido cada una de dichas gotas presentan una de dichas células de esperma transportada

167 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 165, que comprende además gotas que se destruyen desde dicha corriente de fluido cada una de dichas gotas presenta una de dichas células de esperma transportada

168 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 166, en donde dichas gotas se destruyen desde dicha corriente de fluido que presentan tamaño suficiente para encapsular una de dichas células de esperma

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169 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 168, que comprende además una boquilla que presenta un orificio de alrededor de 100 micrómetros de diámetro

170 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 166, que comprende además un cargador de gotas acoplado a dicho analizador, en donde dichas gotas reciben una carga diferencial basada sobre dicha diferencia en la cantidad de dicha mancha unida al ADN nuclear de las células de esperma que contienen cromosoma X y al ADN nuclear de las células de esperma que contienen cromosoma Y

171 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 167, que comprende además un cargador de gotas acoplado a dicho analizador, en donde dichas gotas reciben una carga diferencialmente basada sobre dicha diferencia de volumen de dichas cabezas de células de esperma, y en donde dicha diferencia en volumen de dichas cabezas de células de esperma comprenden una diferencia entre las células de esperma que contienen cromosoma X y las células de esperma que contienen cromosoma Y

315/3

172 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 170, que comprende además un separador de gota, en donde dicho separador de gota separa dichas gota basado sobre la carga de dicha gota

173 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 171, que comprende además un separador de gota, en donde dicho separador de gota separa dicha gota basado sobre la carga de dicha gota

174 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 172, que comprende además un contenedor de recolección sensible a dicho separador de gota, en donde dichas células de esperma que contienen cromosoma X y dichas células de esperma que contienen cromosoma Y son recolectadas

175 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 173, que comprende además contenedores de recolección sensibles a dicho separador de gota, en donde dichas células de esperma que contienen cromosoma X y dichas células de esperma que contienen cromosoma Y son recolectadas

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176 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 174 o 175, en donde dicha población de células de esperma que contienen cromosoma X y dicha población de células de esperma que contienen cromosoma Y son seleccionadas del grupo que consiste de entre 90% a 100%, entre alrededor de 91% a alrededor de 100%, entre alrededor de 92% a alrededor de 100%, entre alrededor de 93% a alrededor de 100%, entre alrededor de 94% a alrededor de 100%, entre alrededor de 95% a alrededor de 100%, entre alrededor de 96% a alrededor de 100%, entre alrededor de 97% a alrededor de 100%, entre alrededor de 98% a alrededor de 100%, entre alrededor de 99% a alrededor de 100%

177 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 176, que comprende además el paso de establecer una velocidad de evento separable en donde dicha velocidad de evento separable es seleccionada del grupo que consiste en al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos

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separables por segundo, al menos 13 000 eventos separables por segundo, al menos 14 000 eventos separables por segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000 eventos separables por segundo, al menos 19 000 eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 21 000 eventos separables por segundo

178 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 177, en donde dicho paso de separar dichas células de esperma comprende una velocidad de separación seleccionada del grupo que consiste de al menos 500 separaciones por segundo, al menos 1000 separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo, al menos 11 000 separaciones por segundo

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179 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 178, en donde dicho paso de formar gotas que cada una contiene las células de esperma transportadas comprende una velocidad de formación de gota seleccionada del grupo que consiste de al menos 10 000 gotas por segundo, al menos 20 000 gotas por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo

180 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 179, en donde dichas células de esperma comprenden células de esperma de mamífero bovino

181 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 179, en donde dichas células de esperma de mamífero equino

182 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 179, en donde dichas células de esperma comprende células de esperma de mamífero ovino

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183 Un aparato de diferenciación de partícula, que comprende

- a partículas asimétricas,
- b una fuente de irradiación que genera un rayo de irradiación sensible a dichas partículas asimétricas,
- c ópticas para enfocar dicho rayo de irradiación sensible a dichas partículas asimétricas, en donde dichas ópticas enfocan un patrón de rayo que presenta una altura casi igual a la longitud de dichas partículas asimétricas a lo largo del eje longitudinal hacia casi dos veces la longitud de dichas partículas asimétricas a lo largo del eje longitudinal,
- d un material de emisión de luz acoplado a dichas partículas asimétricas, en donde dicho material de emisión de luz emite luz en respuesta a dicho rayo de irradiación,
- e un detector sensible a dicha luz

184 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 183, en donde dicha al menos una partícula asimétrica comprende al menos una célula de esperma

185 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 184, en donde dicha

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al menos una célula de esperma tiene una cabeza que presenta una longitud a lo largo del eje longitudinal de alrededor de 9 micrómetros, y en donde dicho patrón de irradiación tiene una altura de alrededor de 20 micrómetros

186 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 183, en donde dicha al menos una partícula asimétrica tiene al menos una característica de diferenciación de partícula

187 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 186, que comprende además una corriente de fluido

188 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 187, en donde dicha al menos una característica de diferenciación de partícula comprende la orientación de dicha partícula asimétrica dentro de la corriente de fluido, y en donde dicho detector es diferencial sensible a dicha luz emitida desde dicho material de emisión de luz basado sobre dichas características de orientación de partícula

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189 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 188, que comprende además un analizador acoplado a dicho detector

190 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 189, en donde dicho analizador se diferencia entre dichas partículas asimétricas basadas sobre dicha orientación de dichas partículas asimétricas dentro de dicha corriente de fluido

191 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 190, en donde dicho material de emisión de luz unido a dicha al menos una partícula asimétrica comprende una mancha unida al ADN nuclear de las células de esperma

192 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 191, en donde dicha característica de diferenciación de partícula comprende una diferencia en la cantidad de dicha mancha unida al ADN nuclear de las células de esperma que contienen cromosoma X y el ADN nuclear de las células de esperma que contienen cromosoma Y

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193 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 192, en donde dicho detector comprende al menos un tubo fotomultiplicador, y en donde dicho tubo fotomultiplicador tiene un rango de voltaje de operación típico, y en donde dicho tubo fotomultiplicador es operado fuera de dicho rango de voltaje de operación típico

194 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 193, en donde dicho rango de voltaje de operación típico de dicho tubo fotomultiplicador es desde alrededor de 400 voltios a alrededor de 999 voltios

195 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 194, en donde dicho tubo fotomultiplicador es operado en un rango desde alrededor de 0 voltios a alrededor de 280 voltios

196 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 193, que comprende además gotas que se destruyen desde dicha corriente de fluido cada una presentando una de dichas partículas transportada

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197 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 196, en donde dichas gotas que se destruyen desde dicha corriente de fluido presentan el tamaño suficiente para encapsular una de dicha células de esperma, en donde dichas células de esperma comprenden células de esperma vivas intactas que presentan al menos una cabeza, un cuello y una cola

198 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 197, que comprende además una boquilla que presenta un orificio de alrededor de 100 micrómetros de diámetro

199 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 196, que comprende además un cargador de gotas acoplado a dicho analizador, en donde dichas gotas reciben una carga diferencialmente basada sobre dicha diferencia en la cantidad de dicha mancha unida al ADN nuclear de las células de esperma que contienen cromosoma X y el ADN nuclear de las células de esperma que contienen cromosoma Y

200 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 199, que comprende

324 

además un separador de gota, en donde dicho separador de gota separa dicha gota basada sobre la carga de dicha gota

201 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 200, que comprende además al menos un contenedor de recolección en el cual las gotas que contienen dichas células de esperma que contienen cromosoma X son recolectadas como una población que contiene cromosoma X

202 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 200, que comprende además al menos un contenedor de recolección en el cual las gotas que contienen dichas células de esperma que contienen cromosoma Y son recolectadas como una población que contiene cromosoma Y

203 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 201 o 202, en donde dicha población que contiene cromosoma X y cromosoma Y de dichas células de esperma son seleccionadas del grupo que consiste de entre 90% a 100%, entre 91% a 100%, entre 92% a 100%, entre 93% a 100%, entre 94% a 100%, entre 95% a 100%, entre 96% a 100%, entre 97% a 100%, entre 98% a 100%, entre 99% a 100%

325 ³²⁴

204 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 203, que comprende además el paso de establecer una velocidad de evento separable en donde dicha velocidad de evento separable es seleccionada del grupo que consiste de al menos 5000 eventos separables por segundo, al menos 6000 eventos separables por segundo, al menos 7000 eventos separables por segundo, al menos 8000 eventos separables por segundo, al menos 9000 eventos separables por segundo, al menos 10 000 eventos separables por segundo, al menos 11 000 eventos separables por segundo, al menos 12 000 eventos separables por segundo, al menos 13 000 eventos separables por segundo, al menos 14 000 eventos separables por segundo, al menos 15 000 eventos separables por segundo, al menos 16 000 eventos separables por segundo, al menos 17 000 eventos separables por segundo, al menos 18 000 eventos separables por segundo, al menos 19 000 eventos separables por segundo, al menos 20 000 eventos separables por segundo, al menos 21 000 eventos separables por segundo

205 Un aparato de diferenciación de partícula tal como se define en la reivindicación 204, en donde dicho paso de separar dichas células de esperma comprende una

326

velocidad de separación seleccionada del grupo que consiste de al menos 500 separaciones por segundo, al menos 1000 separaciones por segundo, al menos 2000 separaciones por segundo, al menos 3000 separaciones por segundo, al menos 4000 separaciones por segundo, al menos 5000 separaciones por segundo, al menos 6000 separaciones por segundo, al menos 7000 separaciones por segundo, al menos 8000 separaciones por segundo, al menos 9000 separaciones por segundo, al menos 10 000 separaciones por segundo, al menos 11 000 separaciones por segundo

206 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 205, en donde dicho paso de formar gotas que contienen cada una dichas células de esperma transportadas comprende una velocidad de formación de gotas seleccionada del grupo que consiste de al menos 10 000 gotas por segundo, al menos 20 000 gotas por segundo, al menos 30 000 gotas por segundo, al menos 40 000 gotas por segundo, al menos 50 000 gotas por segundo, al menos 60 000 gotas por segundo, al menos 70 000 gotas por segundo, al menos 80 000 gotas por segundo, al menos 90 000 gotas por segundo, al menos 100 000 gotas por segundo

327 

207 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 206, en donde dichas células de esperma comprende una célula de esperma bovina

208 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 206, en donde dichas células de esperma comprenden células de esperma de un mamífero equino

209 Un aparato de diferenciación de partícula tal como se describe en la reivindicación 206, en donde dichas células de esperma comprenden células de esperma de un mamífero ovino

328 ~~40~~ 7

RESUMEN

Poblaciones de espermatozoides que suceden de la
aislación no natural que presentan una alta puridad y
tecnologías para diferenciar espermatozoides basados en las
características tales como masa, volumen orientación, o luz
emitida incluyendo los métodos de análisis y aparatos tales
como ópticas de formación de rayo y detectores

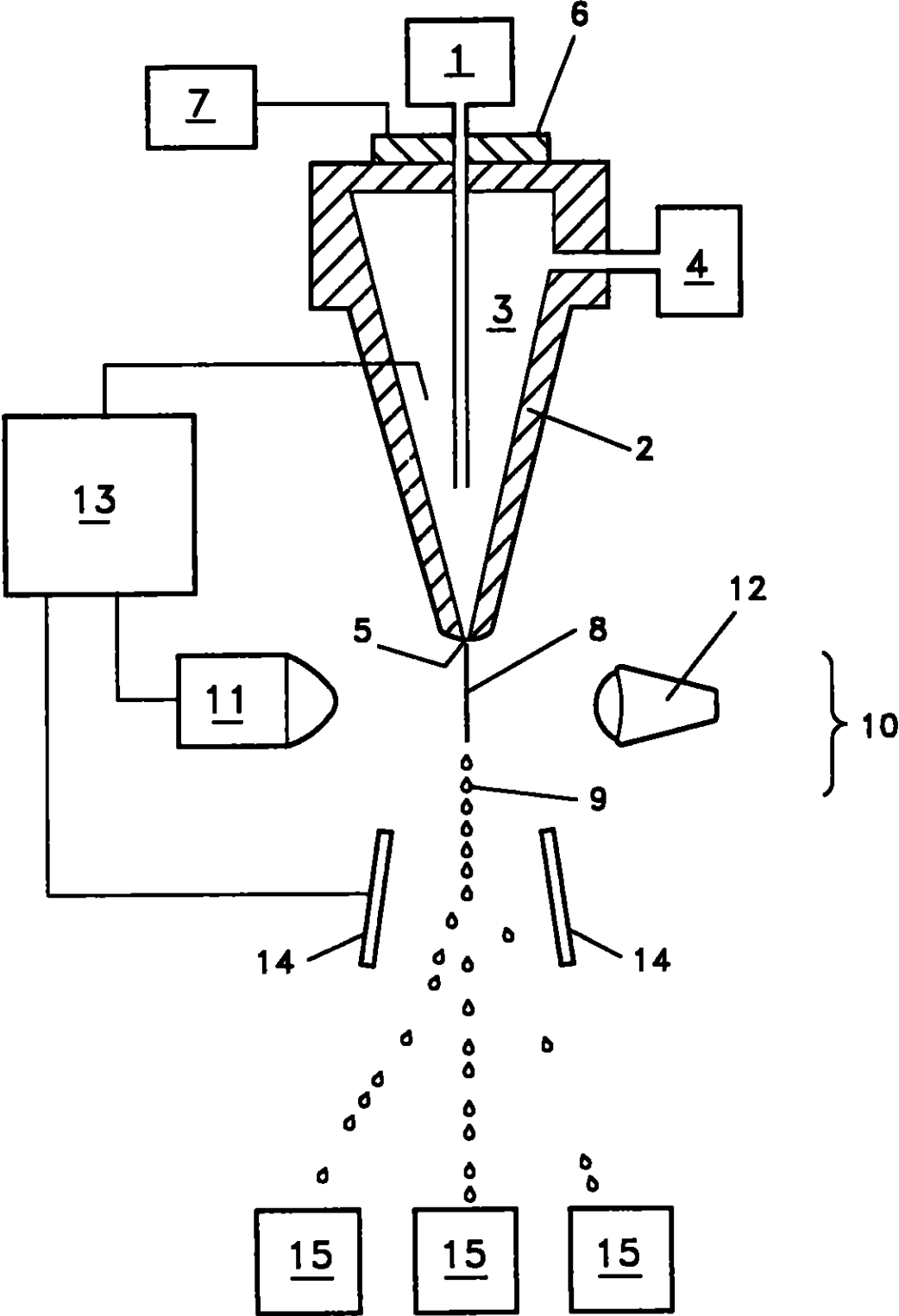


Fig. 1

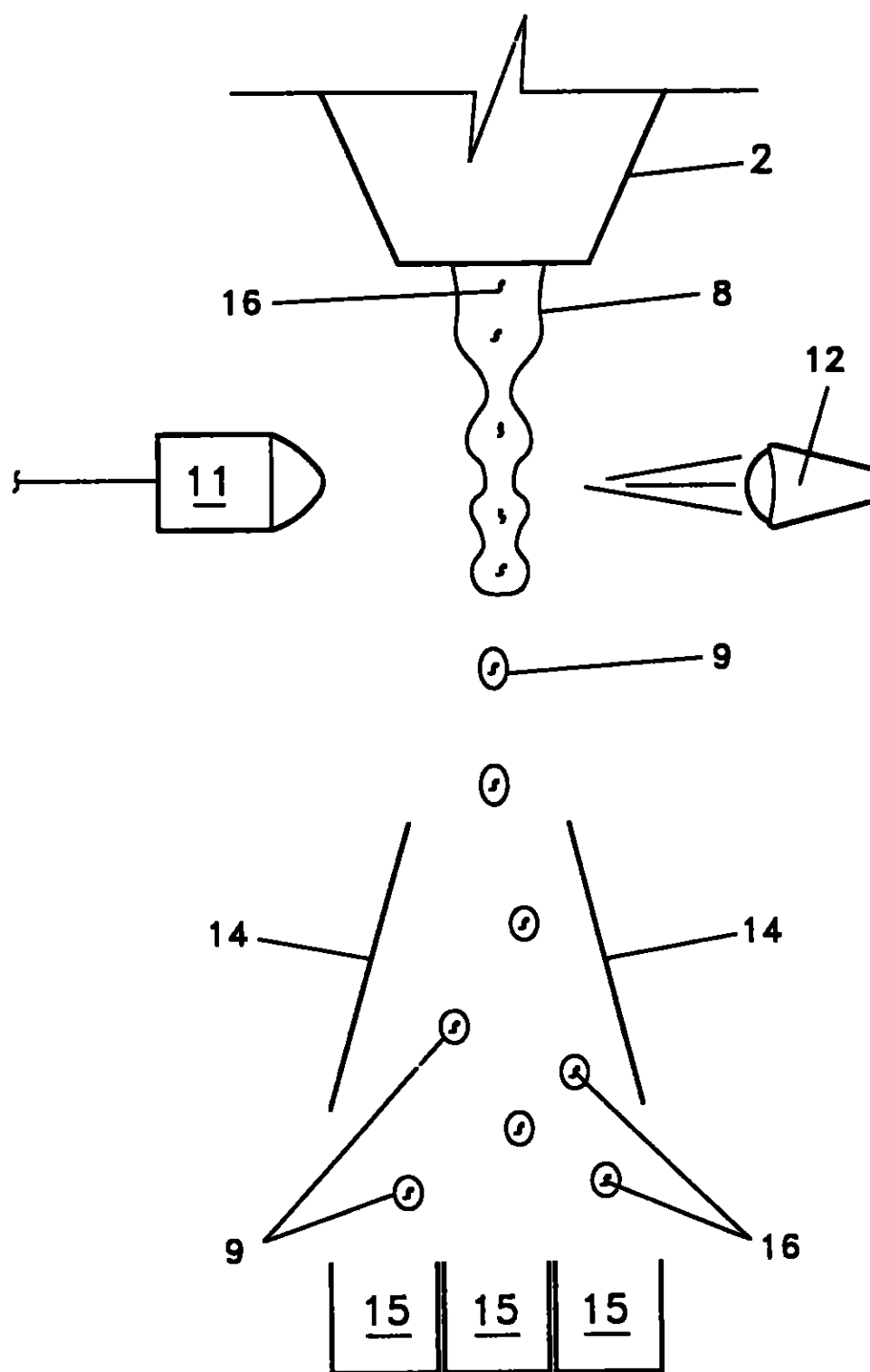


Fig. 2

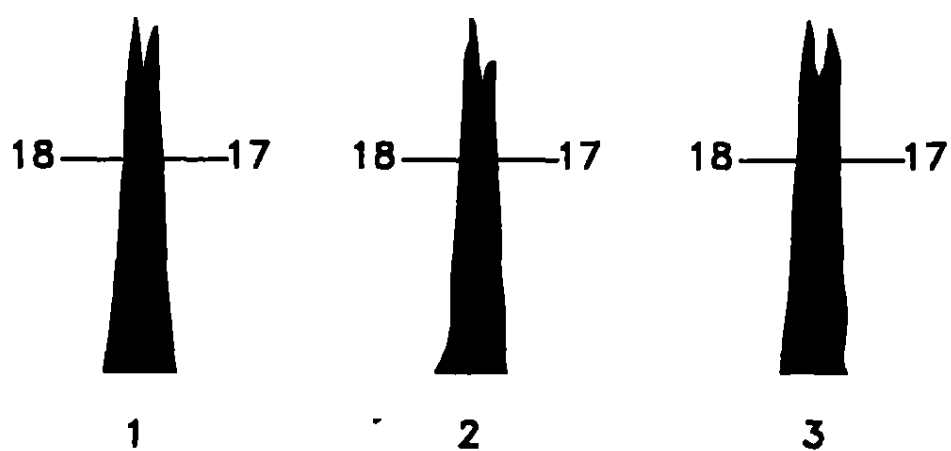


Fig. 3a

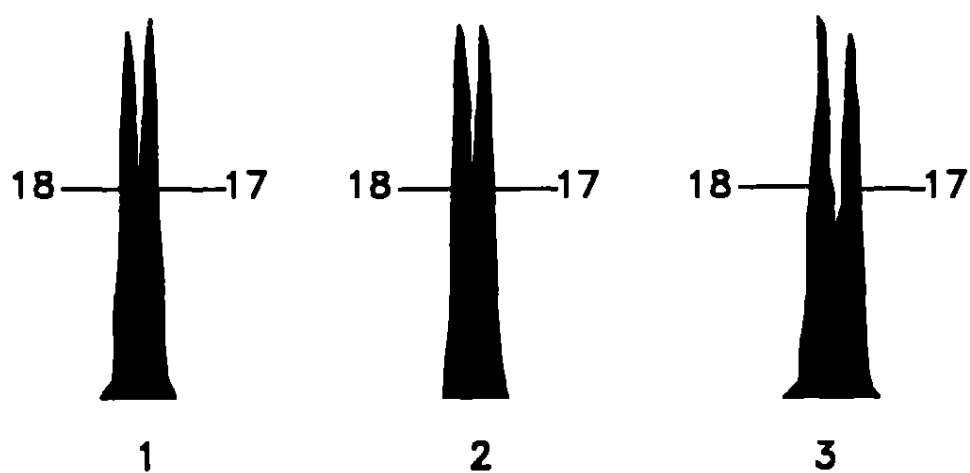


Fig. 3b

Fig. 3

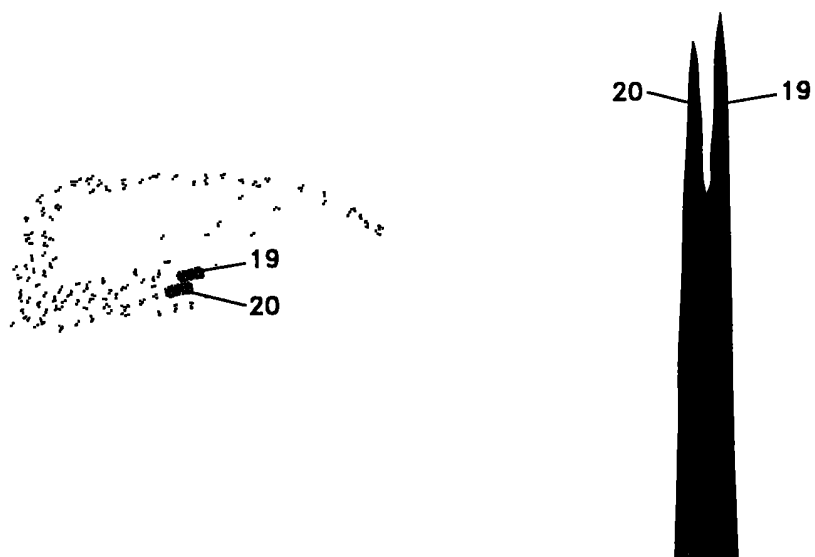


Fig. 4

4/28

4/28

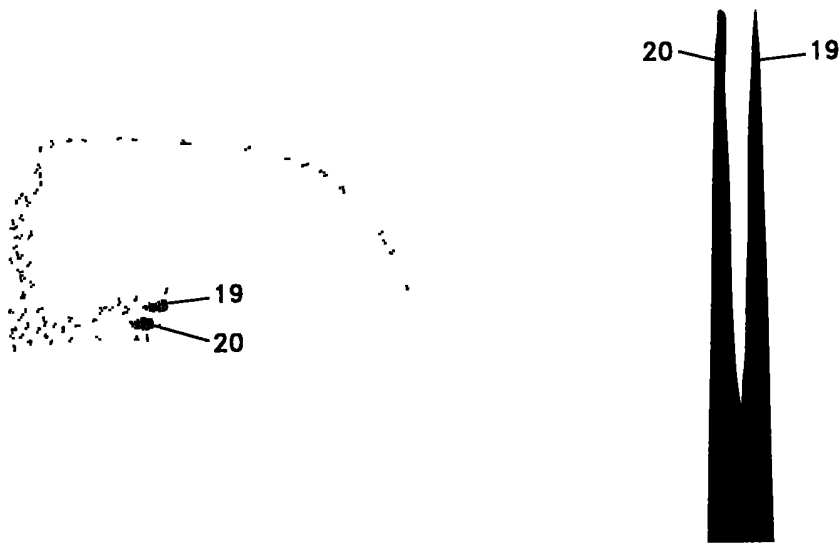


Fig. 5

334

6/28

333

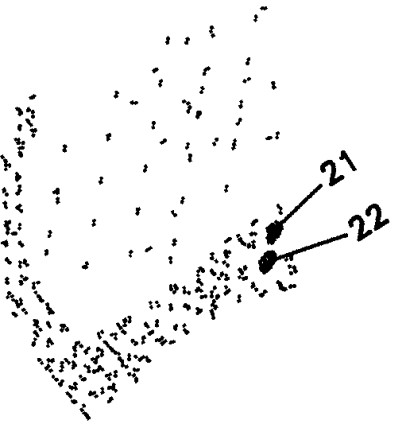
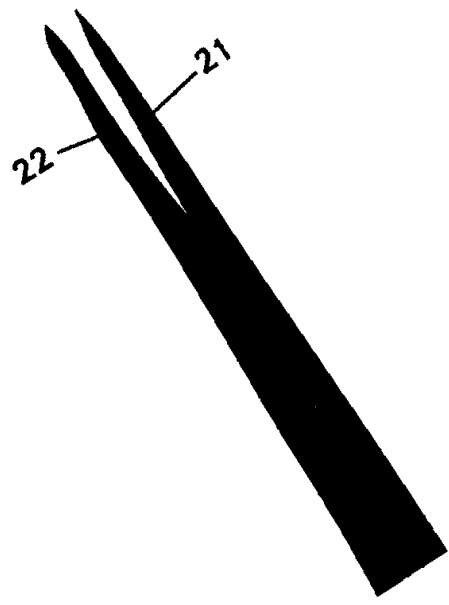


Fig. 6

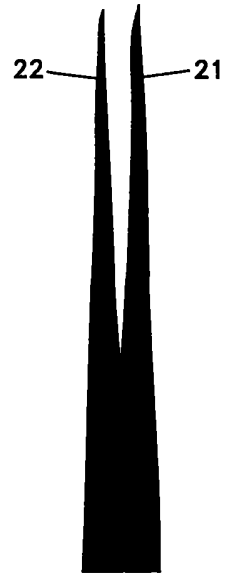
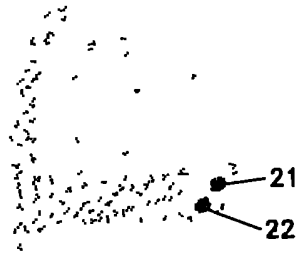


Fig. 7

7/28

534

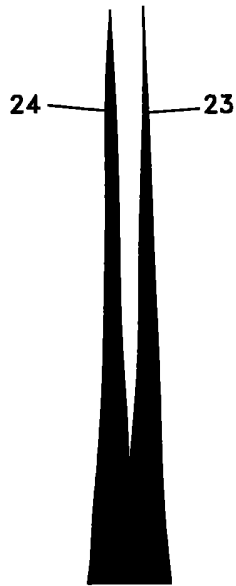
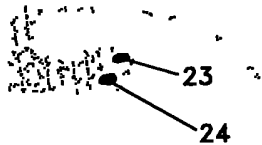


8/28

Fig. 8

8/28

337



9/28

Fig. 9

337

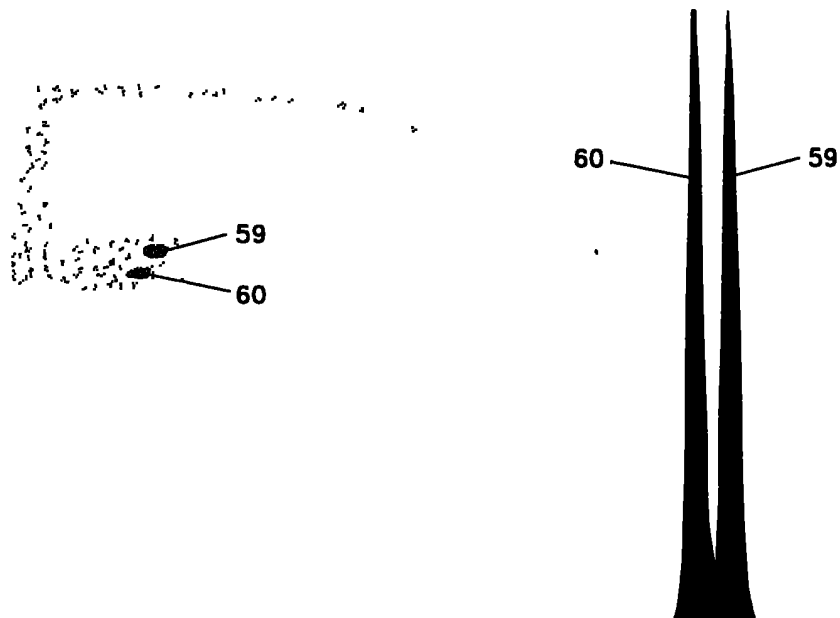


Fig. 10

10/28

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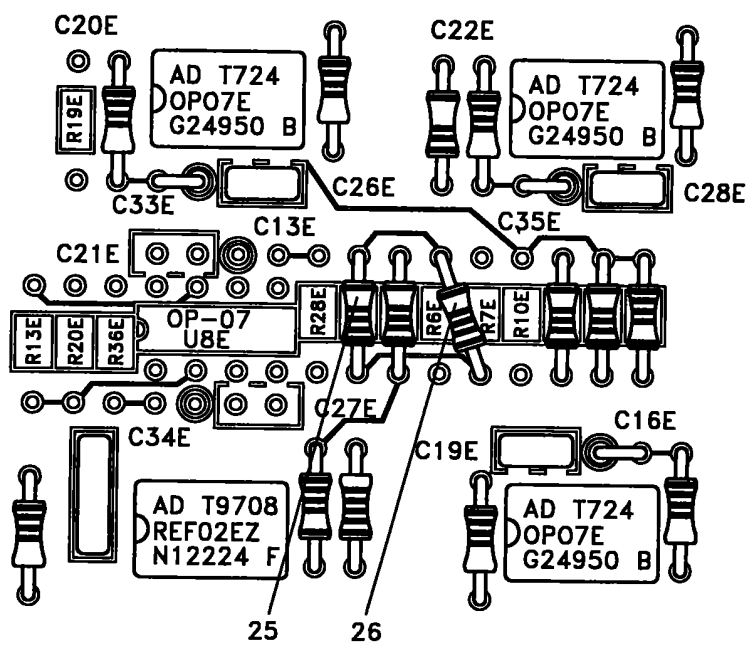
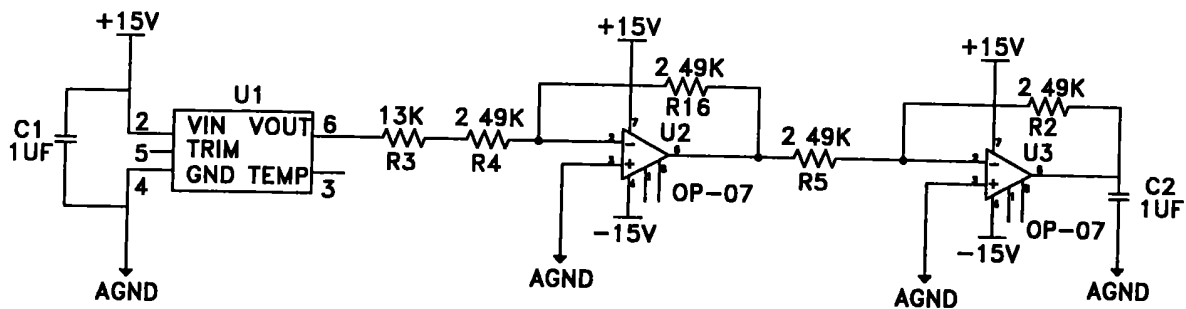


Fig. 11

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12/28

Fig. 12a

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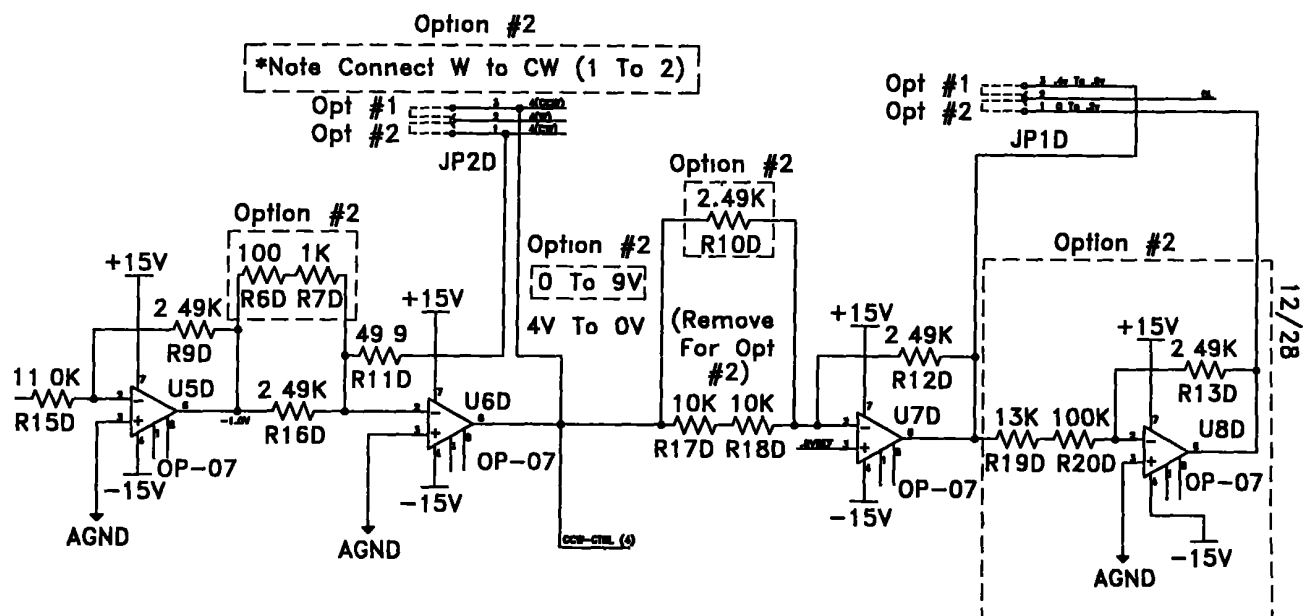


Fig. 12b

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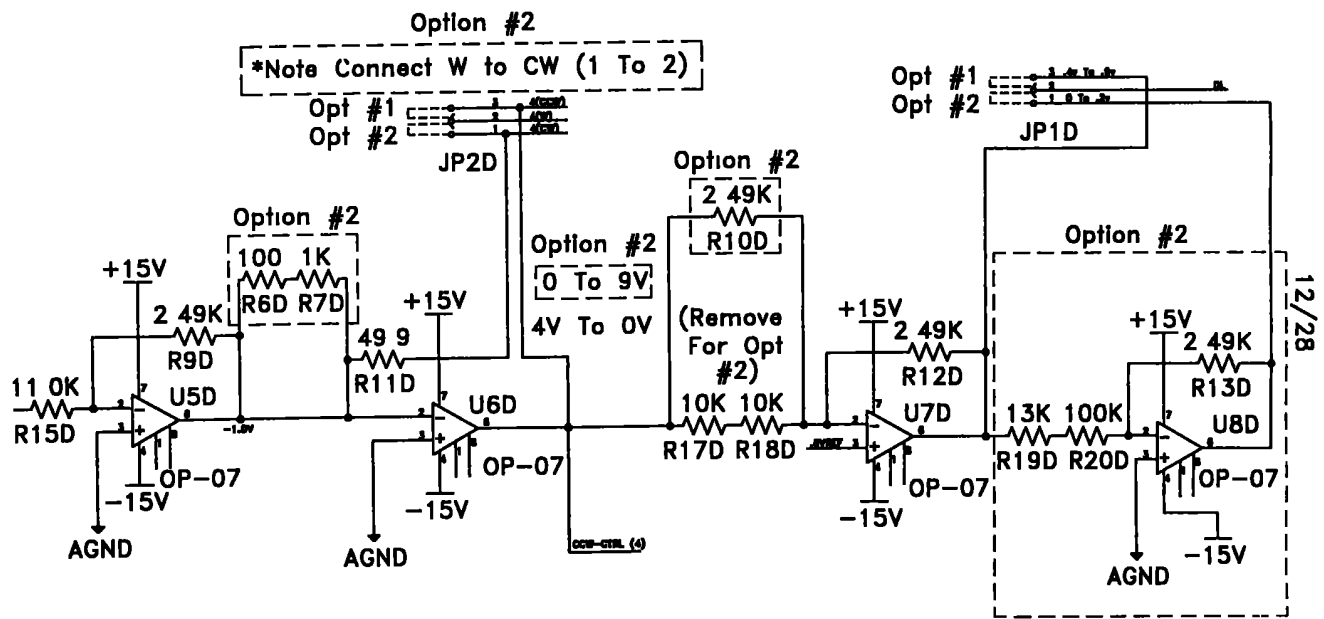


Fig. 12c

341

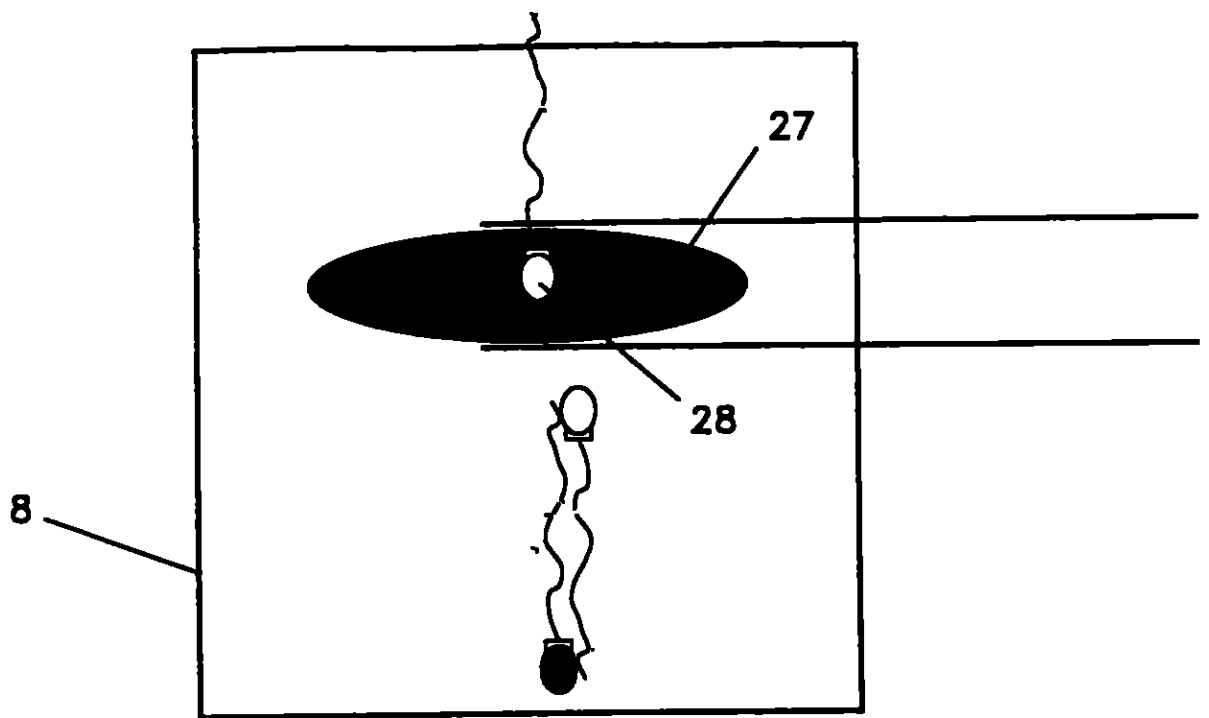


Fig. 13a

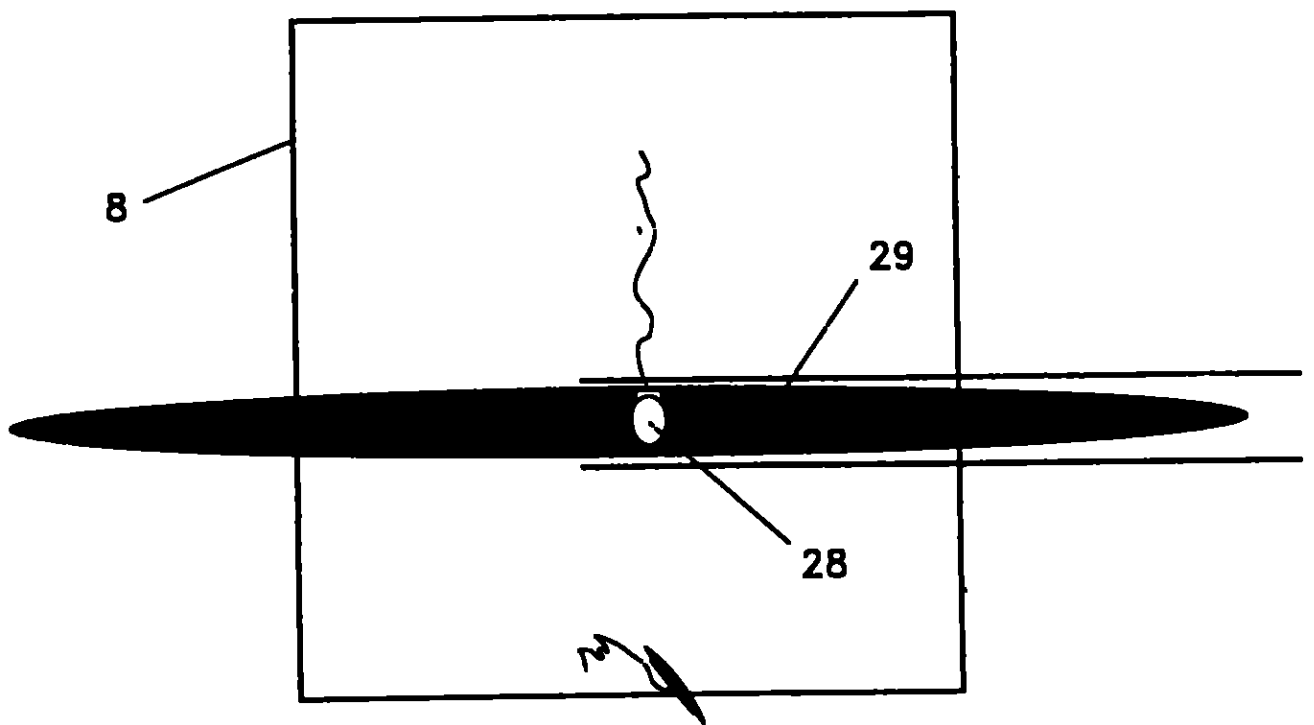


Fig. 13b

Fig. 13

14/28

344

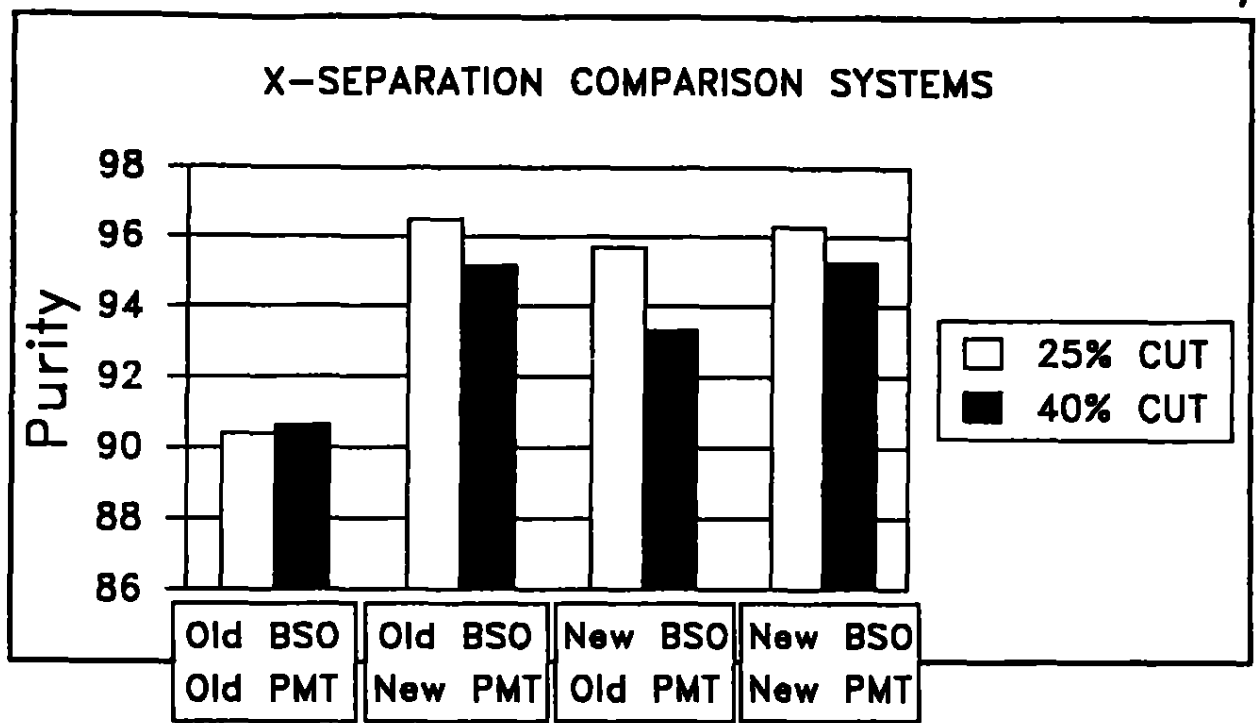


Fig. 14a

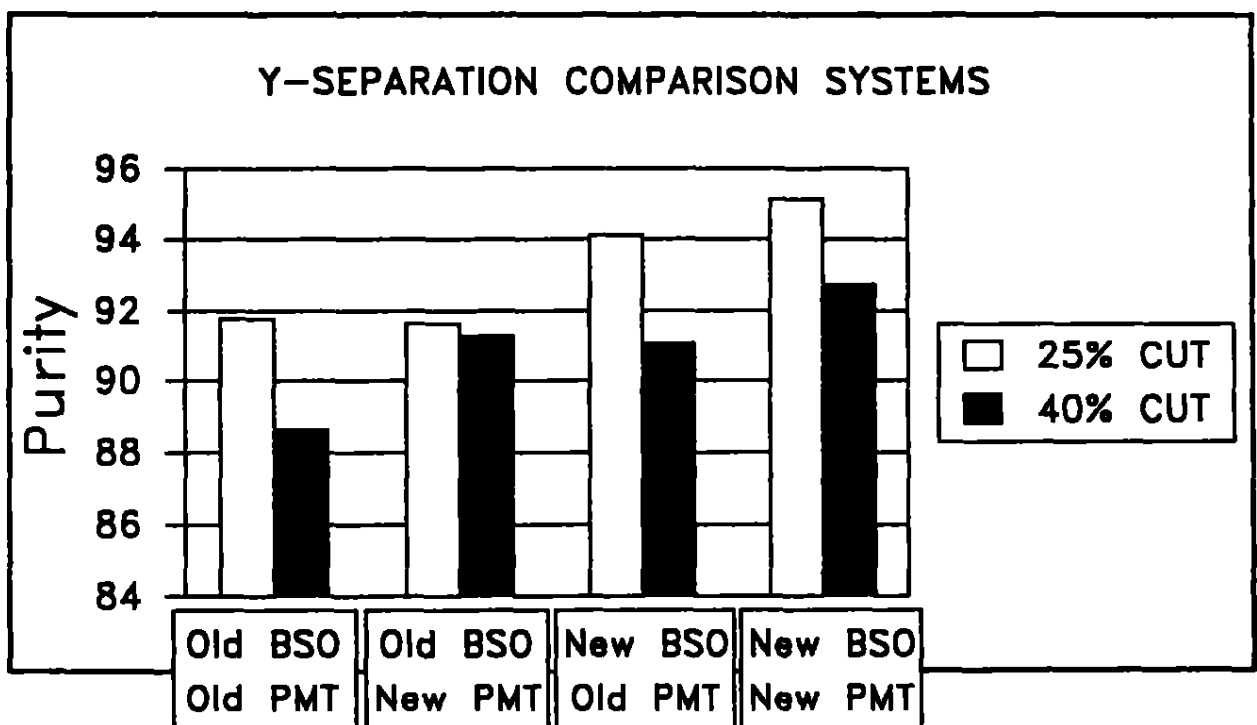


Fig. 14b

Fig. 14

345

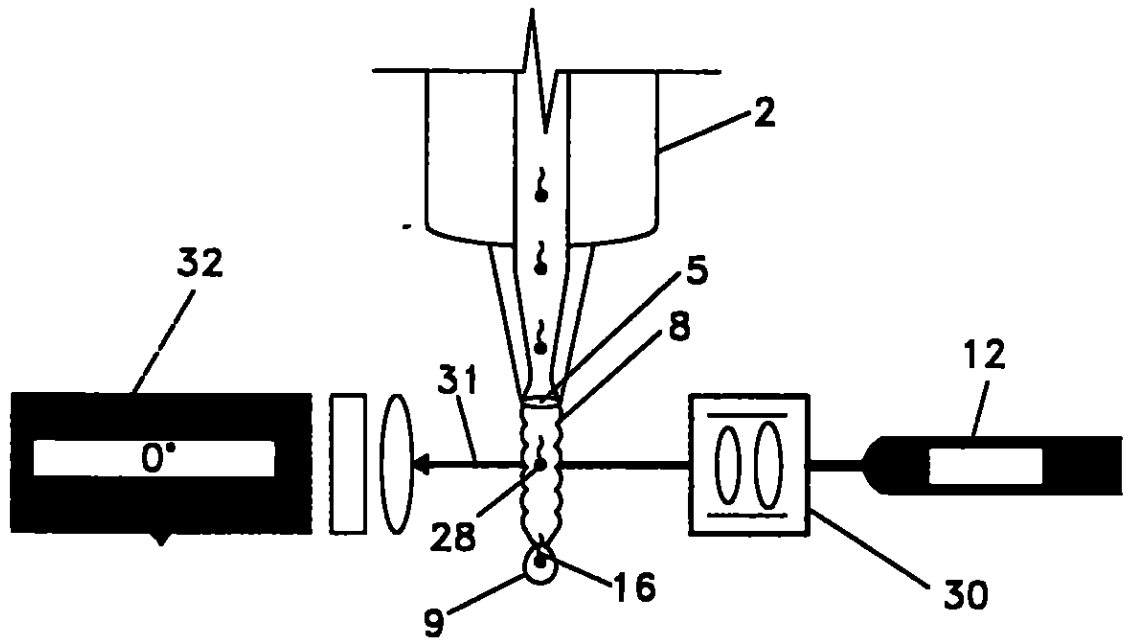


Fig. 15

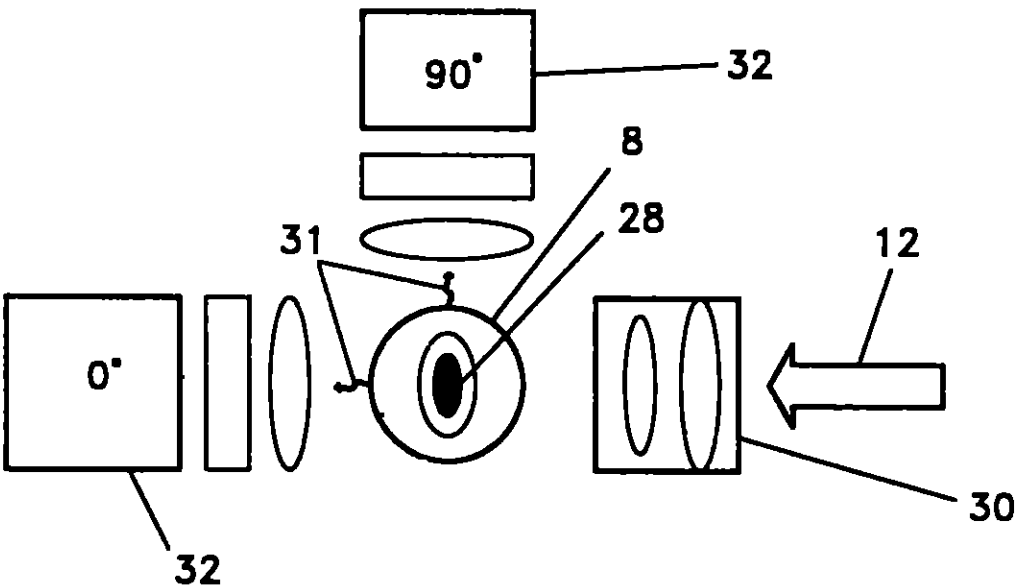


Fig. 16

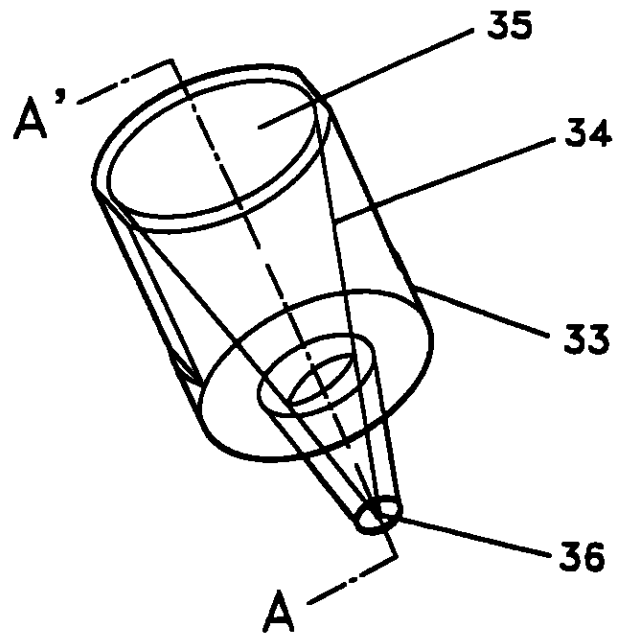
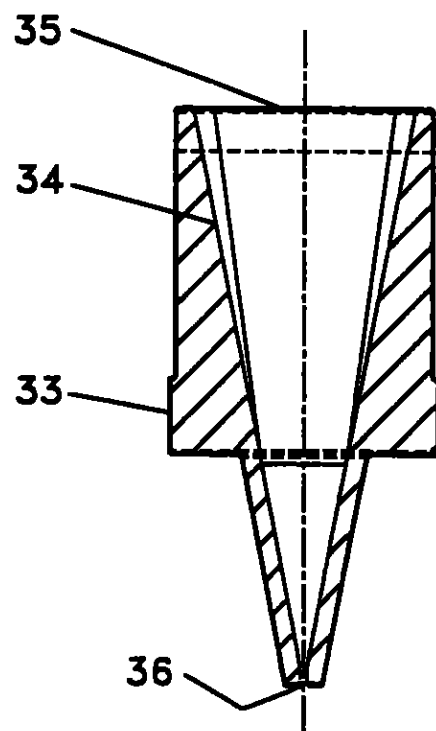


Fig. 17a



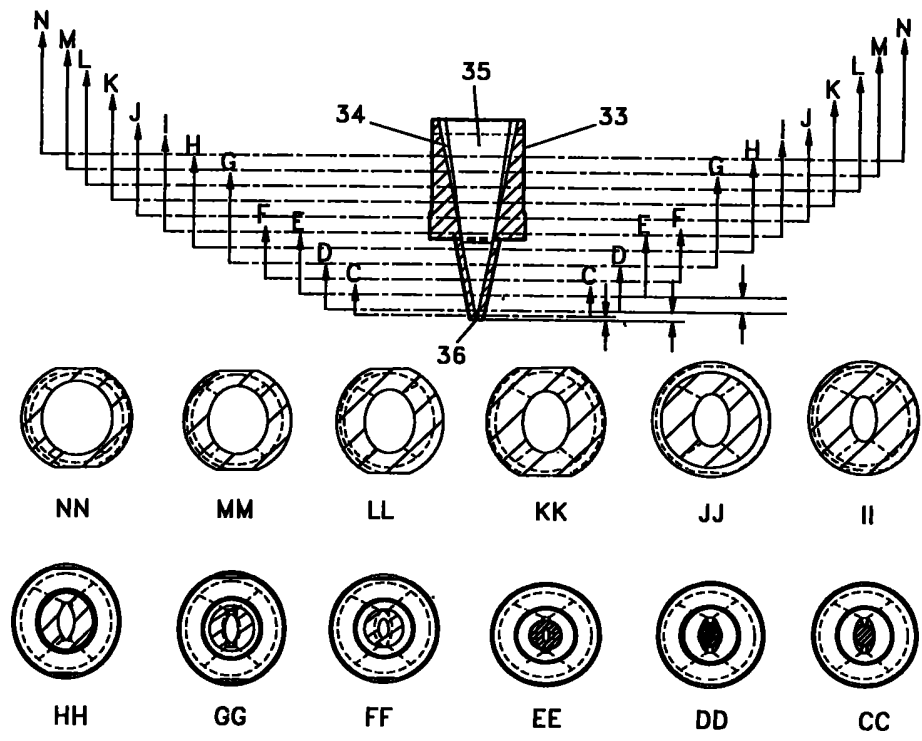
Section A-A'

Fig. 17b

Fig. 17

348

Fig. 18



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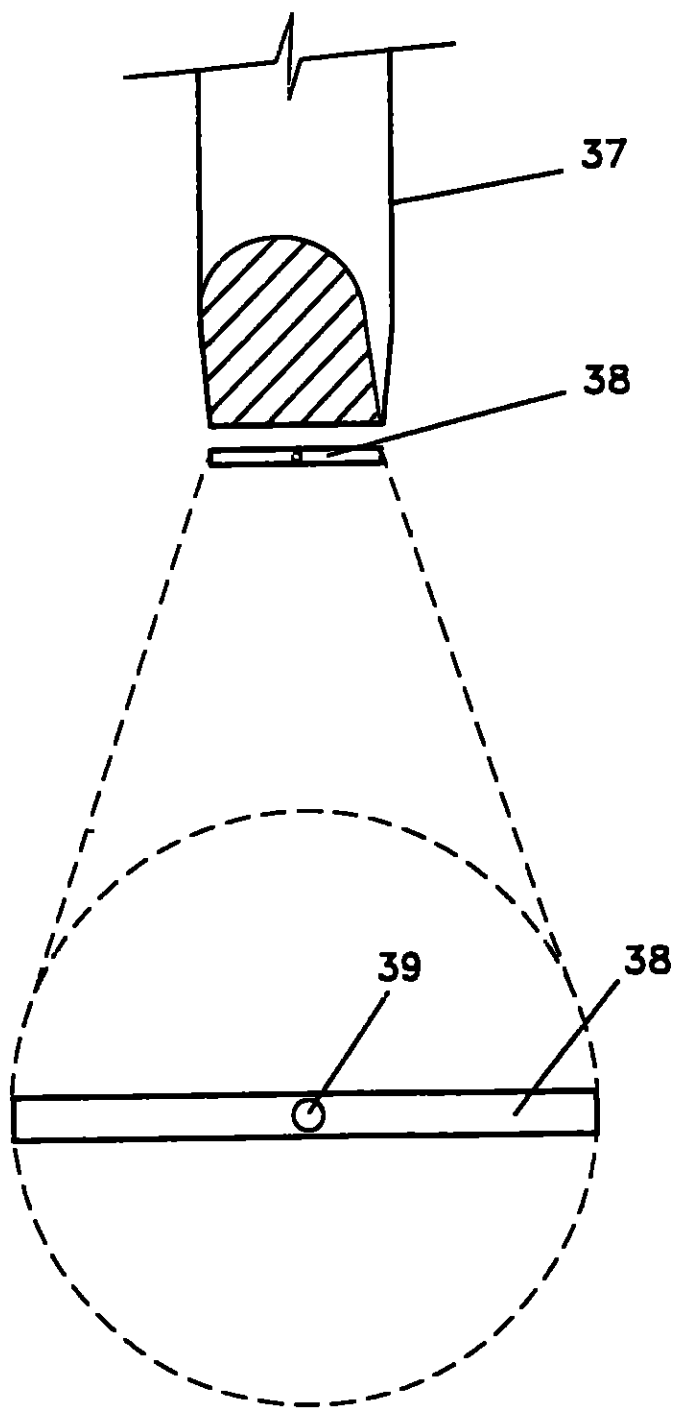


Fig. 19

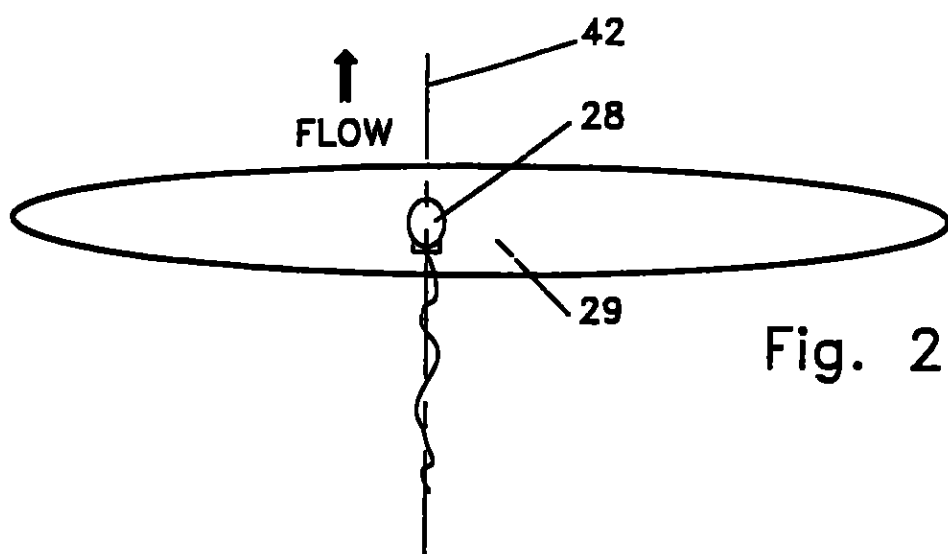


Fig. 20a



Fig. 20b

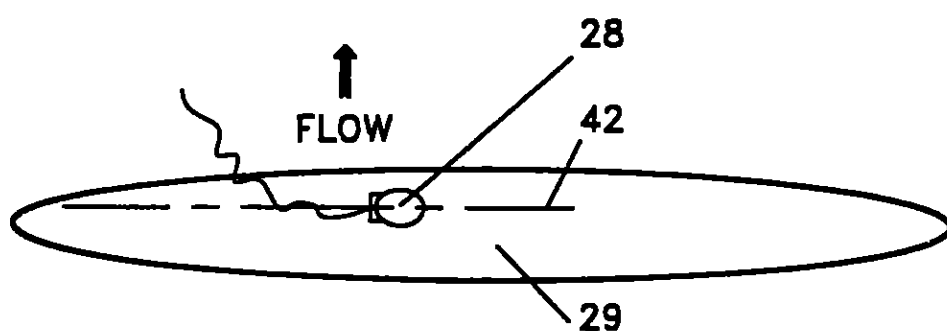


Fig. 20c

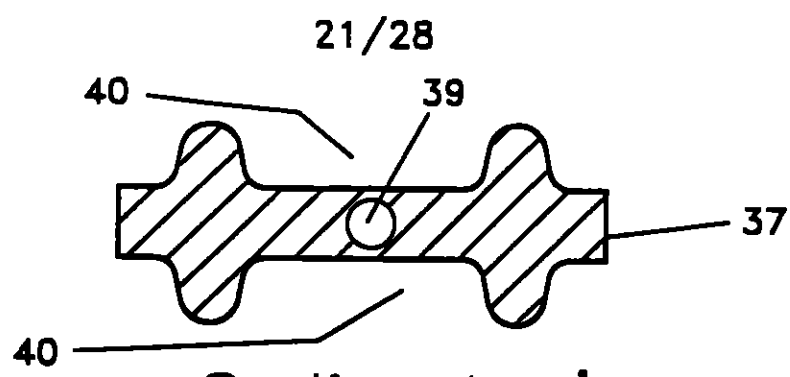


Fig. 20d

Fig. 20

25

351



Section A-A'
Fig. 21b

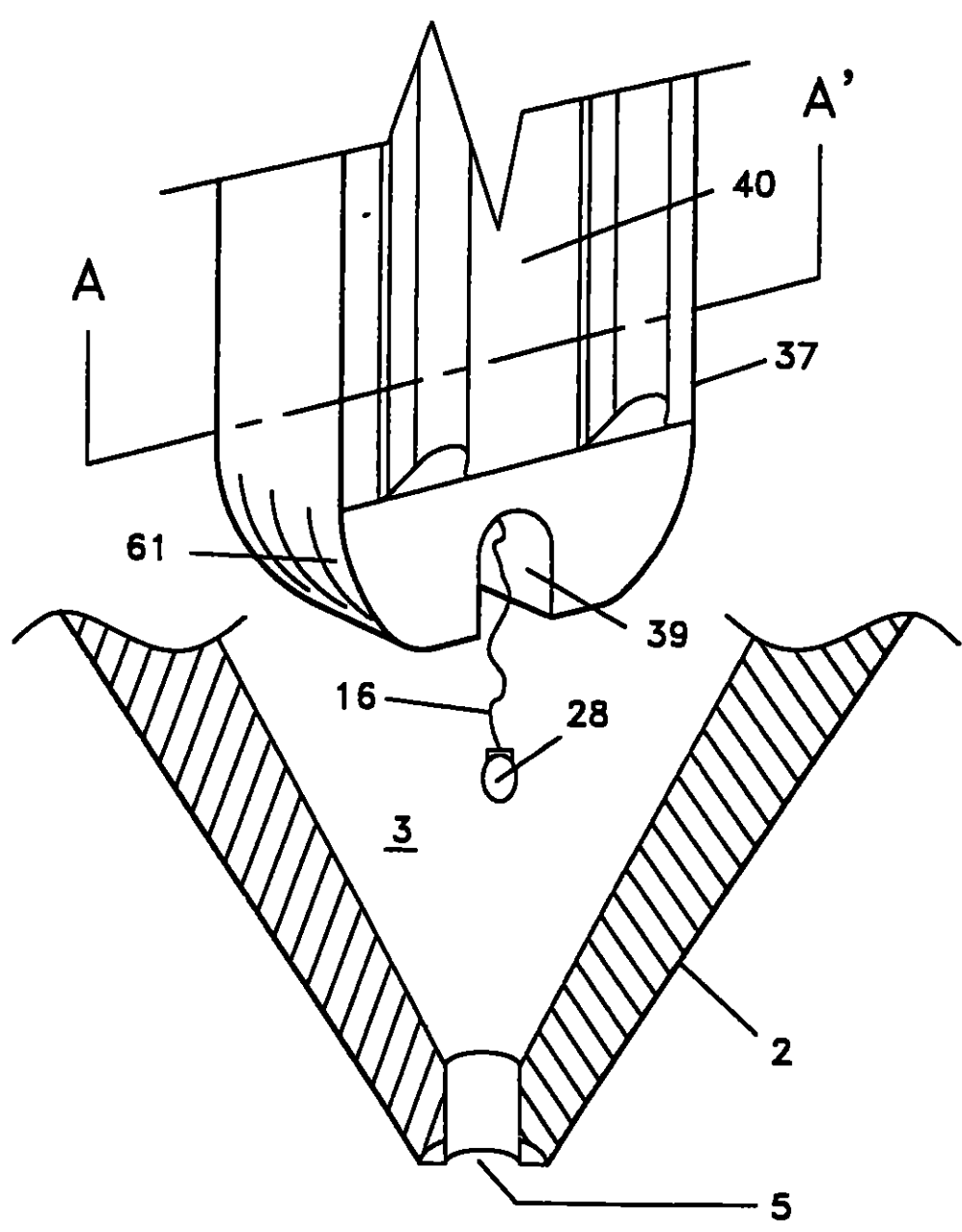
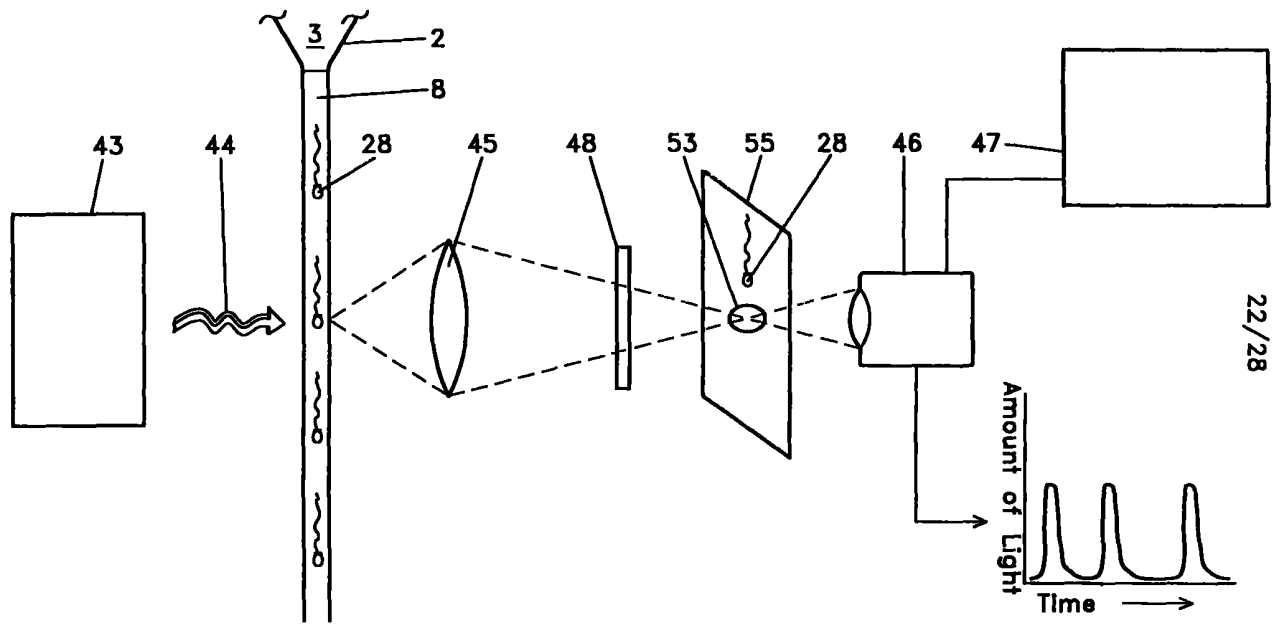


Fig. 21a

Fig. 21



22/28

Fig. 22

5/5

23/28

353

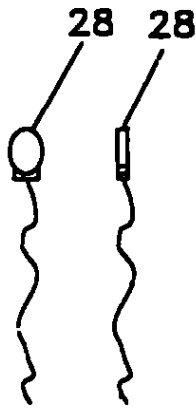
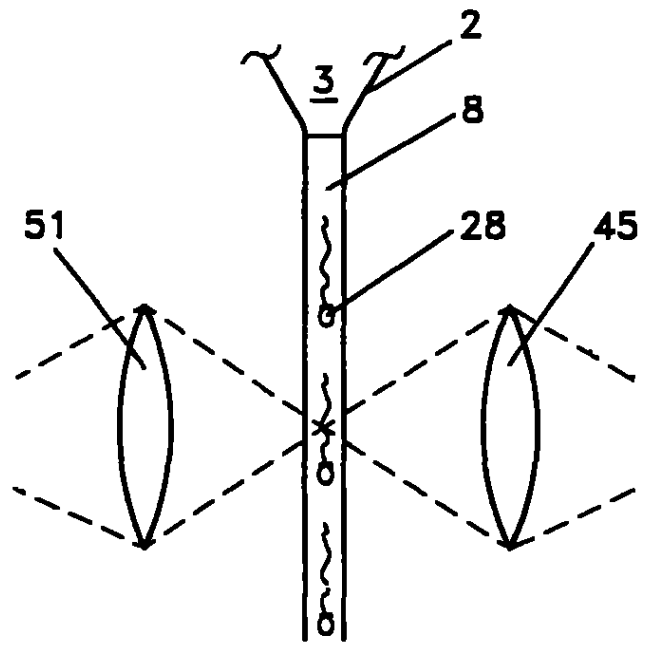
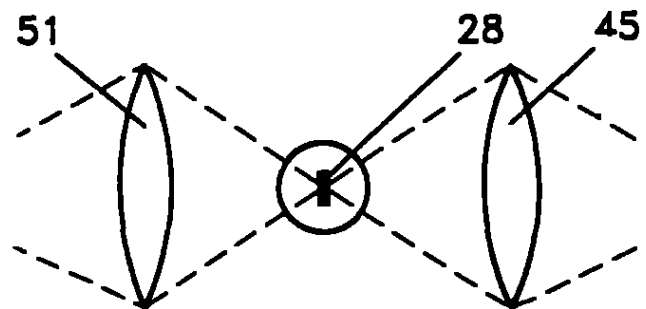


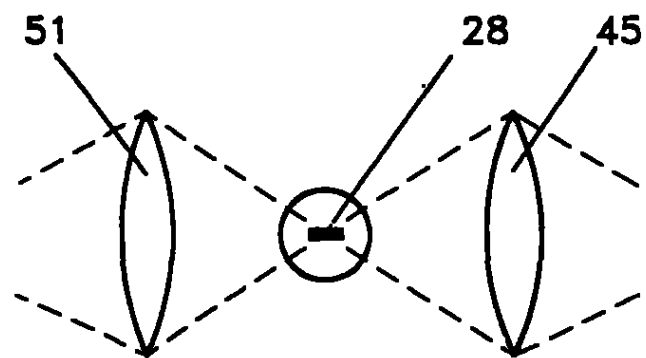
Fig. 23a



Side View



Correct Orientation



Incorrect Orientation
Top View

Fig. 23b

Fig. 23

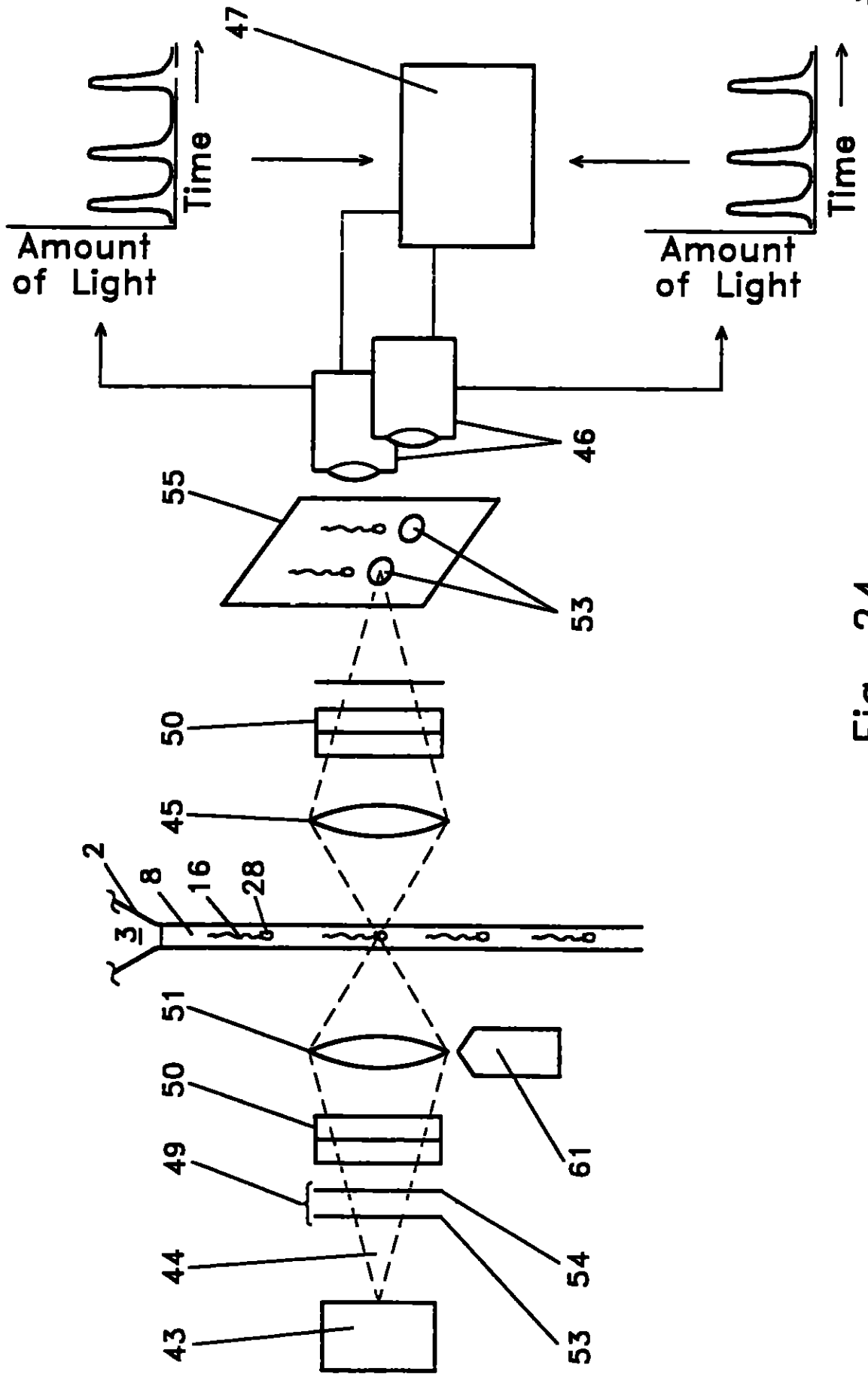


Fig. 24

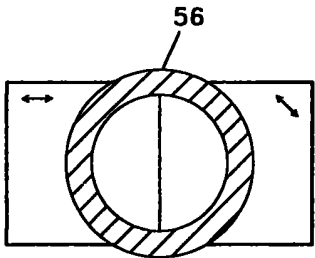


Fig. 25a

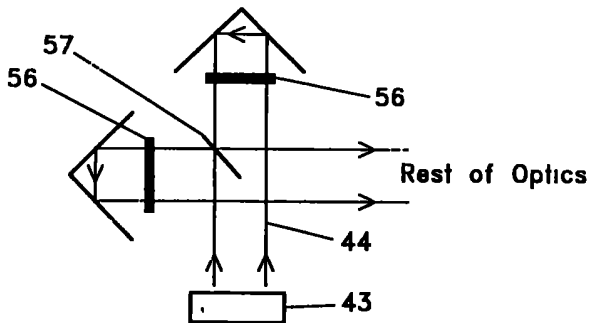


Fig. 25d

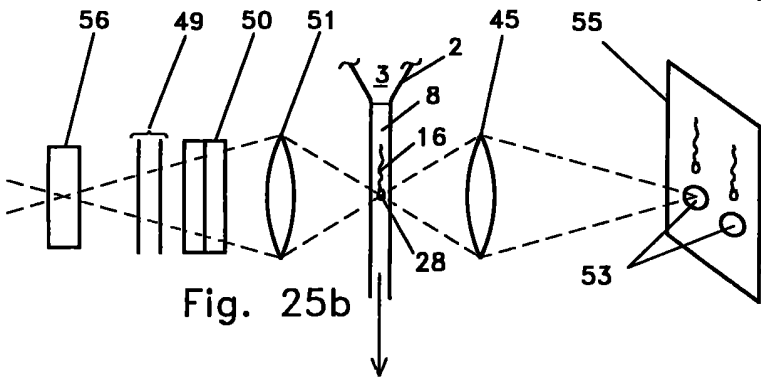


Fig. 25b

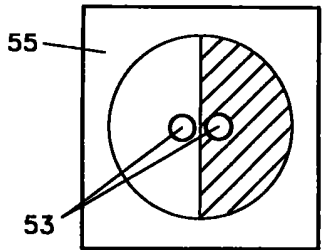


Fig. 25c

Fig. 25

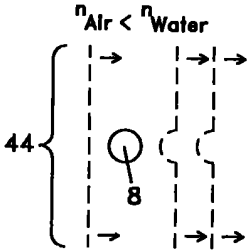
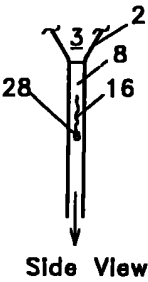


Fig. 26a

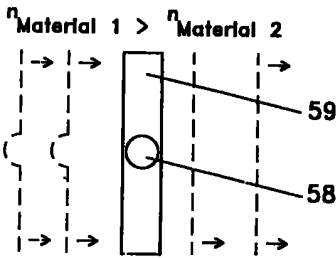


Fig. 26b

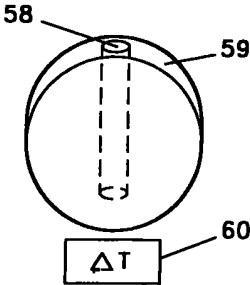


Fig. 26c

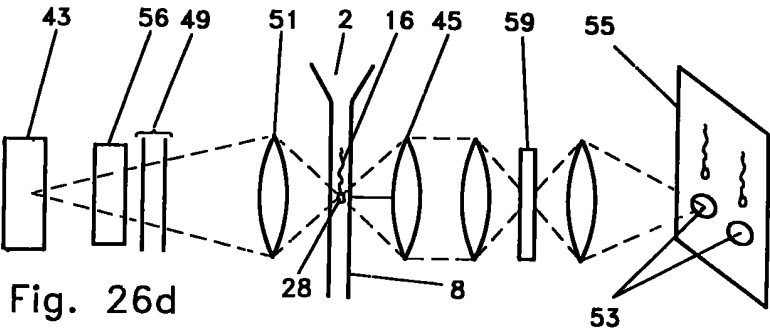
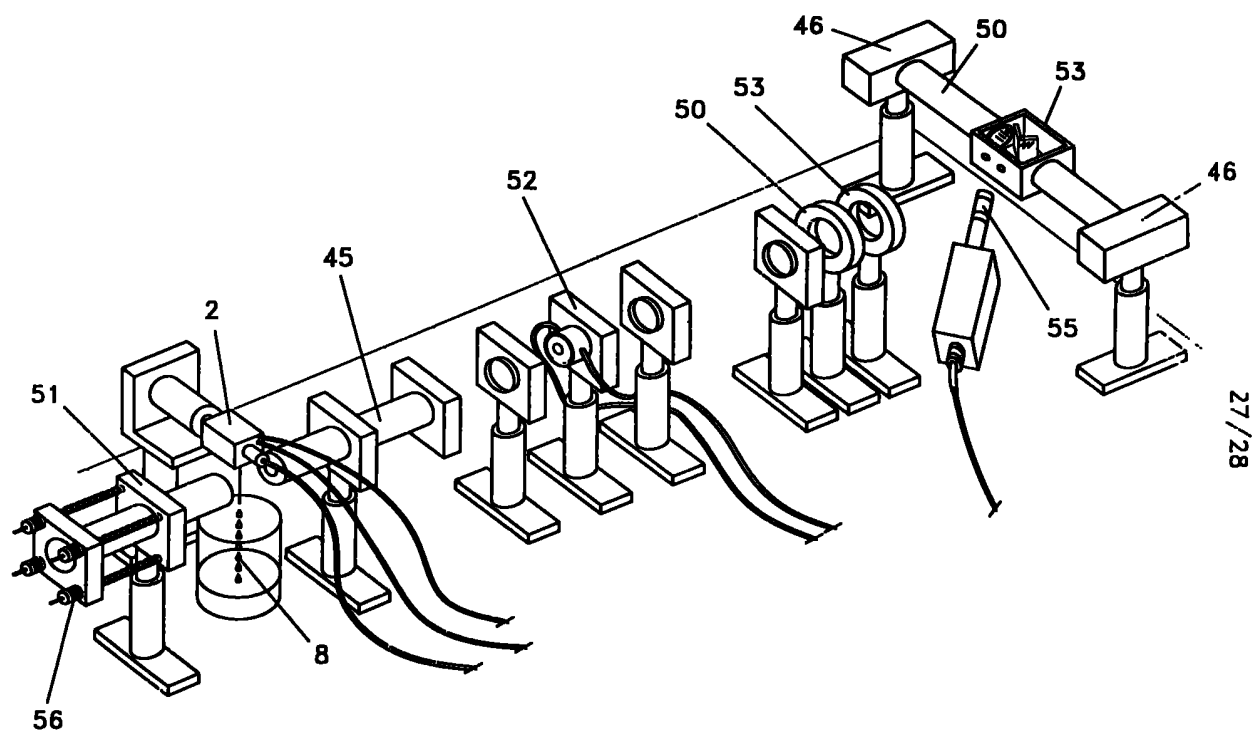


Fig. 26d

Fig. 26

26/28

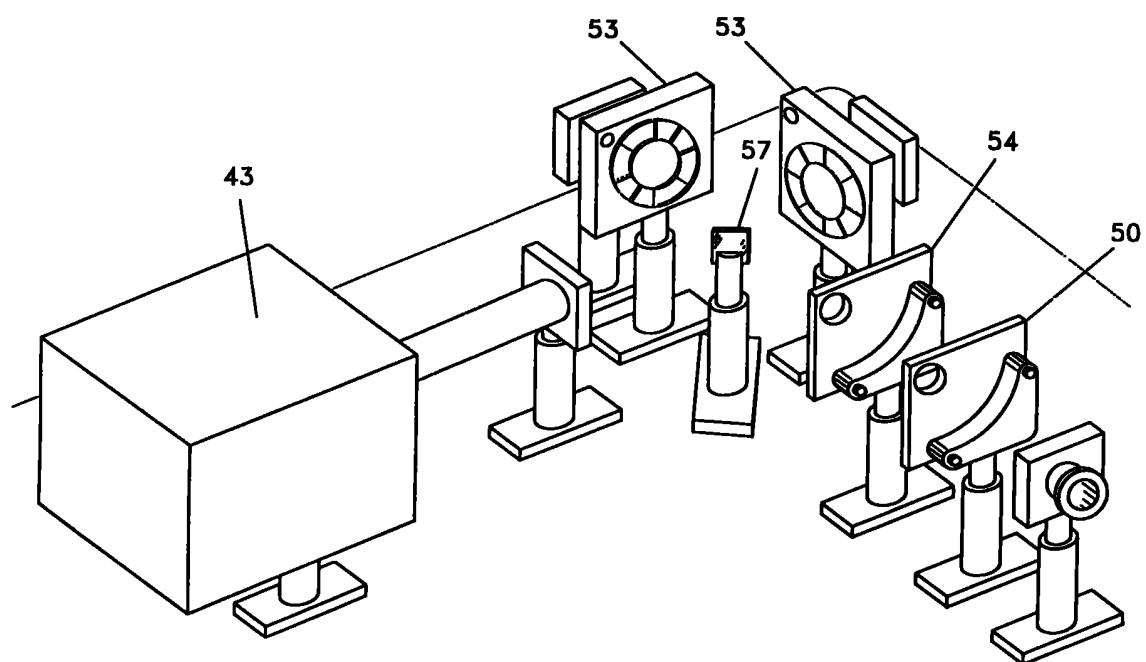
880



27/28

Fig. 27

316



28/28

Fig. 28

358

359

Industria y Comercio
SUPERINTENDENCIA

NIT 800176089-2

RECIBO OFICIAL DE CAJA 02 - 68,672
FECHA : OCTUBRE 11 DE 2002

SUPERINTENDENCIA Y TRIBUNAL
Radicación : 42101971 00000000 Folios: 258
Fecha (HH): 2002-11-08 17:08 58
Trámite : 011 PCT-PATENT 379 FASE HACI 411 PRESENTAR
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No PAGO	FECHA PAGO	Vr PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	7030359	11/10/2002	8,620,000 00

***** CONCEPTO *****

CANT	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
0005-01-01	SOLICITUDES	7 TRAMITES DE SOL DE DISEÑOS INDUS	378,000 00
		TOTAL :	\$ 378,000 00

SON TRESCIENTOS SETENTA Y OCHO MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No _____

Colo 13534-847-001-4

Carrera 13 No 27-00 Pisos 5 7 y 10
Conmutador 382 0840
Fax 350 5220
E-mail info@sic.gov.co
www.sic.gov.co
Bogotá D C Colombia

REQUISITOS NECESARIOS PARA LA PRESENTACION Y AGILIZACION DEL TRAMITE

PATENTE DE INVENCION ☒ MODELO DE UTILIDAD ☐

- | | | |
|---|--------------------------|-------------------------------------|
| - Indicación de que se solicita la concesión de una patente. | ☐ | ☒ |
| - Datos de identificación del solicitante o de la persona que presenta la solicitud | ☐ | ☒ |
| - Descripción de la invención. | ☐ | ☒ |
| - Dibujos de se estos pertinentes | ☐ | ☒ |
| - Comprobante de Pago de las tasas establecidas | ☐ | ☒ |

COMPLETA ☒ INCOMPLETA ☐

DISEÑO INDUSTRIAL ☐

- | | | |
|---|--------------------------|--------------------------|
| - Indicación de que se solicita el registro de Diseño Industrial | ☐ | ☐ |
| - Datos de identificación del solicitante o de la persona que presenta la solicitud | ☐ | ☐ |
| Representación gráfica y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño | ☐ | ☐ |
| Comprobante de pago de las tasas establecidas | ☐ | ☐ |

COMPLETA ☐ INCOMPLETA ☐

ESQUEMA DE TRAZADO ☐

- | | | |
|---|--------------------------|--------------------------|
| - Indicación de que solicita el registro de un Esquema de trazado | ☐ | ☐ |
| - Datos de identificación del solicitante o de la persona que presenta la solicitud | ☐ | ☐ |
| Representación grafica del esquema de trazado | ☐ | ☐ |
| Comprobante de pago de las tasas establecidas | ☐ | ☐ |

COMPLETA ☐ INCOMPLETA ☐

Firma responsable



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