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(54) **METHOD AND DEVICE FOR MEASURING DYNAMIC PARAMETERS OF PARTICLES**

VERFAHREN UND VORRICHTUNG ZUR MESSUNG DYNAMISCHER PARTIKELPARAMETER

PROCEDE ET DISPOSITIF POUR MESURER DES PARAMETRES DYNAMIQUES DE PARTICULES

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(56) References cited:
WO-A-03/077552 WO-A-2004/013732
GB-A- 2 130 718 US-A- 5 116 125
US-A- 5 517 298

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Description

Background of invention

[0001] The present invention refers to a method and a device for measuring dynamic parameters of particles, e.g. spermatozoa, such as the translation speed and the rotational speed of particles. In addition, also the number of particles can be evaluated.

[0002] The present invention is used for establishing parameters related to the dynamics/mobility of particles in a solution, in particular biological entities, e.g. cells and cell organelles including spermatozoa. Analysing the dynamic parameters and number of spermatozoa in semen is of importance in order to characterise spermatozoa, and constitutes an important tool for evaluation of male fertility.

[0003] By translation speed (velocity) is meant a directed motion of particles of one or more directions in a detection area or volume such that the path length is significantly longer than the observation length. In contrast to the translation speed there is e.g. Brownian motion signifying stochastic motion of the particles in all directions, i.e. the path length is significantly shorter than the observation length.

[0004] A method for determining the motility of spermatozoa is disclosed in US pat no. 5116125, said analyser being based on dynamic laser light scattering.

[0005] Another method for measuring the motility of spermatozoa is to monitor the spermatozoa with a video camera and analyse the movements of the spermatozoa with computer based analysis of individual trajectories. Although this type of computer aided analysis generates fitness parameters rather quickly compared to manual testing, the analysis has several drawbacks particularly a significant variance with respect to the obtained parameters which increases with increased concentration of the spermatozoa.

[0006] Object of the present invention is to provide a method and device which provide substantially improved quality of dynamic parameters of particles in a solution. In addition, the particle concentration can also be accurately established. The present invention is specifically advantageous for measuring dynamic parameters of particles in a solution comprising the particles in low as well as in high concentrations.

[0007] Other advantages are apparent from the text below.

Summary of the invention

[0008] According to the present invention, there is provided a method and a device for measuring dynamic parameters and the concentration of particles in a solution, such as spermatozoa, as defined by the claims.

[0009] More specifically, the present invention refers to a method for measuring the concentration and dynamic or mobility parameters of particles, a method compris-

ing detecting events in a detection field, the events generated by the particles, and calculating time correlation function based on fluctuations of these events. The method comprises applying correlation analysis on temporal fluctuations of the particles with respect to a detection area of a digital picture. The invention furthermore refers to a method for measuring the dynamic parameters of particles, comprising detecting the particles by a detector capable of generating a digital picture, and applying correlation analysis based on temporal fluctuation of the particles with respect to a detection area of the digital picture. Another method according to the invention refers to a method for measuring dynamic parameters of particles comprising providing the particles in a solution, a light source, a detector capable of generating a digital picture, computational means, and applying correlation analysis, i.e. calculating the time correlation function or functions, based on temporal fluctuation of the particles with respect to a detection area of the digital picture.

[0010] The invention also encompasses a device for measuring the dynamic or mobility parameters of particles, the device comprising a sample compartment comprising a solution comprising the particles, a light source, a position sensitive detector comprising at least one detection field, computational means for processing signals from the detector, wherein the events generated by the particles are detected in the detection field and the time resolved correlation function is calculated. The device comprises a sample compartment comprising a particle solution, a light source, an image sensor, computational means for processing signals from the sensor, detecting the particles on a digital picture generated by the image sensor, and applying correlation analysis, i.e. calculating the (time) correlation function, based on temporal fluctuation of the particles with respect to a detection area of the digital picture. Apart from measuring dynamic parameters of particles in a solution or in another physical environment the method and device can also be used for calculating the number of particles present and hence the concentration in e.g. the sample solution.

[0011] In the present invention, fluctuations of events representing the particles of interest may be detected by a detection field. Preferably, temporal fluctuations of particles in a digital moving picture are analysed by using correlation analysis which generates a correlation function or correlation functions, said correlation function(s) giving information about dynamic parameters of the particles such as diffusion times, translation speed, rotational frequency, etc. and the number of particles in a volume element, i.e. the concentration. With the above mentioned dynamic parameters the mobility of the particles can be evaluated. Any particles or entities present in a solution can be evaluated according to the present invention with a size ranging from molecules to the macroscopic level. If the particles represent spermatozoa a number of important dynamic parameters can be calculated, such as swimming speed referred to as motility, rotational frequency and sperm concentration.

[0012] A novel feature of the present invention is to apply correlation analysis on fluctuation of particles present in a digital moving picture. A digital moving picture of a solution containing the particles of interest is generated by inter alia using a suitable detector capable of generating a digital picture such as an image sensor connected to computational means. The digital picture may suitably by computational means be divided into several measurement/detection areas capable of generating adequate time dependent (temporal) fluctuation of the number of particles or concentration of the particles over the detection area boundary. Thus, the number of particles in the detection area is randomly changing around the average number. The fluctuation of the number of particles with respect to a detection area of the digital picture is analysed by the correlation function of the fluctuation signal, the signal representing the particles. Hence, the fluctuations are the basis for calculating a (time) correlation function or (time) correlation functions for generating the dynamic parameters and the number of the particles. Over time the particles will move in the digital picture, that is in-out motion across the detection area boundary. An image sensor of the present invention is a sensor capable of generating digital images of moving particles having a resolution in the image plane, i.e. in x- and y-axis, spatial resolution. As the image sensor generates a series of digital images (frames) having a resolution in time, a moving picture is obtained which is further analysed by applying correlation analysis. By the present invention dynamic parameters of particles in a solution can be evaluated at the same time as the digital images (the digital moving picture) are created, though, the method can also be applied on a digital moving picture stored on a suitable storage means.

[0013] The information on the digital picture generated by the image sensor stems from inter alia transmitted, scattered or emitted light from the particles. Normally, events not linked to the particles of interest are filtered away, suitably by using any type of digital imaging analysis. Having applied suitable imaging analysis tools to suppress the unwanted information from the image sensor, the time correlation function based on the registered fluctuation of the particles is calculated. In case there are several detection areas present in the digital image, several time correlation functions can be calculated simultaneously each correlation function generated from one and the same detection area.

[0014] The fluctuations of events and/or the number of particles in a detection area of the digital picture are monitored at a plurality of times (τ_1 , τ_2 , τ_3 , etc) during a specific time interval. The obtained time correlation function is basically a function describing the self similarity of events on a time scale, i.e. the detected particles are temporally correlated. Simplified, the time correlation function

$G'(\tau)$ is given by:

$$G'(\tau) = \langle I(t) * I(t+\tau) \rangle$$

5 whereby the angular brackets $\langle \dots \rangle$ denote average over time, and possibly also over a plurality of detection areas, and I denotes the signal created by the detected particles.

[0015] Having calculated the correlation function, the concentration can be derived from the value of the correlation function at $\tau =$ zero, which represent the inverse of the number of particles present in the detection area.

[0016] In case optical elements are positioned in the light beam between the object plane, i.e. sample compartment, and the image plane (plane of the image sensor) the digital picture will not only indicate particles located in an area element of the solution but all particles which are in focus. Hence, the digital picture gives information about a volume element of the sample, given inter alia by the focal depth (depth of field) of the optical element(s).

[0017] In general terms the time correlation function $G'(\tau)$ normalised by the mean square intensity $\langle I^2 \rangle$ is related to the concentration and the mobility by:

$$G(\tau) = 1 + 1/N [f(\text{mobility})].$$

With

$$G(\tau) = G'(\tau) / \langle I^2 \rangle$$

[0018] In the above equation, the amplitude term $1/N$ depends on the inverse number of particles per volume element N and a function $f(\text{mobility})$ which describes translational as well as rotational motion. As revealed by fluctuation theory, fluctuations increase with a decreasing average particle number.

[0019] The fluctuation of the events and/or the particles in the digital picture during the relevant time frame should be of such a magnitude that an accurate time correlation function can be calculated. Hence, the detection area or detection areas of the digital picture must be dimensioned such that proper fluctuation across the detection area boundary is achieved. Generally, higher fluctuation generates better time correlation function. The digital moving picture is suitably divided into a plurality of detection areas which all can have identical or varying size and shape.

[0020] Assume that by computational means the digital picture is divided into a plurality, preferably ≥ 10 , e.g. 100 detection areas, each area representing several pixel elements of the image sensor. At several times τ_1 , τ_2 , etc. (i.e. image frames at times τ_1 , τ_2 , etc.) during a time interval the intensities I_1 , I_2 , etc. representing the particles of interest from the detection area A_1 are used for calcu-

lating the correlation function G_1 with respect to area A_1 . Simultaneously another 99 correlation functions are calculated G_2 up to G_{100} . Having several correlation functions the average correlation function can easily be established.

[0021] The digital picture is preferably generated by a detector capable of generating a digital picture, such as an image sensor. Any sensor/detector capable of generating a digital output which can be used to extract dynamic parameters of moving particles by applying inter alia correlation analysis on the digital output is suited for the present invention. The picture might be captured and stored in an analogue format and subsequently be transformed to a digital format which is then processed using correlation analysis. By digital picture is meant a digital moving picture suitably obtained by a multitude of time resolved images, i.e. image frames. The image sensor is characterised by the capability of rapid image capturing, generating digital images ready for computer analysis, having spatial and temporal resolution, and high sensitivity to a whole spectrum. Suitable image sensors are solid state image sensors/detectors and so called tube-tube type detectors exemplified by vidicon tube detector cameras. Solid state type image sensors are especially preferred. A solid state image sensor is a silicon chip comprising a multitude of photosensitive diodes referred to as photosites. In the short instant that the shutter is open each pixel records the intensity or brightness of the light falling on said pixel by accumulating a charge. The time resolved images of the digital picture contain a high number of picture elements or pixels, usually a few microns in size, where each pixel corresponds to a photosite of the image sensor. Accordingly, the resolution of the image is to a large extent given by the number of photosites on the image sensor. The resolution of the image sensor is not an important factor for the present invention and can vary within a wide range from about one thousand up to about 20 million. A higher resolution of the image sensor may be favourable.

[0022] The most common solid state image sensors are charge coupled devices (CCD) and complementary metal oxide semiconductor detectors (CMOS). Both classes of solid state image sensors are silicon semiconductors designed to capture photons and convert them into electrons. CCD and CMOS image sensors are similar with respect to basic design, but differ in terms of how the charges (electrons) of the photosites are extracted from the sensor. If high sensitivity is needed CMOS avalanche photodiode image sensors can be used.

[0023] Solid state image sensors, e.g. silicon photodiodes, are sensitive to light in a wide spectral range of from about 200 nm up to about 1200 nm.

[0024] The resolution of the digital image is basically governed by the resolution of the image sensor, thus, each pixel of the image sensor represents one pixel in the digital image. However, the resolution can also be improved by software adding pixels to the digital image. By introducing optical means, e.g. one or more optical

elements such as lens systems including objectives and oculars, the particles in the solution can be magnified. Thus, the particles in the image plane (plane of image sensor) are magnified with respect to the physical particles positioned in the object plane. However, the optical means may also have the properties to make the particles in the plane of the image smaller than in the object plane. Such an arrangement might be considered if the particles have a projected area similar to the total area of the image sensor. The smallest detection area of the digital picture/image is given by an area of the image representing an individual pixel element of the image sensor up to in principle an area comprising all pixel elements. Typically, the size/shape of a detection area or areas of the digital picture is/are larger than the projected area of the particles of interest in the digital picture. The analysed volume within the sample is inter alia given by the optical means applied. The optical means can suitably be a microscope, such as a compound microscope comprising an objective and a projection lens having a light source rendering transmitted, scattered or emitted light having a wavelength matching the image sensor. When having optical means in the light path between the object plane and the image plane (image sensor), the analysed volume within the sample is in principle given by the objective and its magnification, both restricting the volume in radial direction with respect to the light path. Furthermore, by the focal depth of the optical means the measurement volume is in principle restricted in axial direction.

[0025] The source of light can be of any wavelength as long as the energy of the electromagnetic radiation does not significantly influence the particles to be analysed and can be detected by the image sensor. The light can have a wide range of wavelengths such as visible light, e.g. wavelengths from about 200 nm up to about 1200 nm, or have a narrower range of wavelengths down to monochromatic light. Either the light source emits monochromatic light or light having a narrow wavelength, alternatively, suitable filters, i.e. monochromatic filters, are applied if the source of light has a wider range of wavelengths. Also light sources producing coherent light, suitably monochromatic, can preferably be used. Examples of coherent light sources are laser light sources. Suitable light sources are inter alia xenon, high and low pressure mercury, tungsten, halogen light sources, light emitting diodes (LED) such as blue diodes, lasers and laser diodes. For the analysis of certain types of motion (rotational motion) it is an advantage if the light is polarised. To choose a specific type of illumination is not important to the present invention, hence, any illuminating type can be applied as long as an accurate time correlation function can be generated.

[0026] Examples of suitable illumination techniques if a microscope is used as optical means includes but are not limited to Köhler illumination, phase contrast, differential interference contrast, darkfield, reflected (scattered) and emitted light (fluorescence), Hoffman modulation contrast, Rheinberg illumination.

[0027] If emitted light from the particles, e.g. fluorescence, is captured by the image sensor, optical means in the light path between the object plane and the image plane are usually fitted including additional filters in addition to a microscope, in order to separate the excitation light from the emitted light. With an incident light fluorescence microscope, the sample is illuminated with excitation light through the objective lens. A dichroic beam splitter placed in the optical path between the objective and the image sensor, transmits or reflects light depending on the wavelength. The light source used for excitation is commonly a laser. Any laser can be used which is capable of exciting the fluorescent particles of interest including e.g. argon- or argon krypton lasers, single-line He-Ne lasers, laser diodes, etc. If larger volume elements of excitation are sufficient, also non coherent light sources as mercury high pressure lamps or halogen lamps can be used. If fluorescence is detected it is preferred to have a favourable signal to noise ratio. Fluorescence which is out of focus appears as flares and reduces the signal significantly. Hence, the measurement volume within the sample, in principle given by the aperture and its magnification of the objective in radial direction, is also restricted in axial direction. This restriction of the volume in axial direction is preferably obtained by applying an aperture (pin-hole), which is conjugated to the object and the image plane. However, the image sensor consisting of individual photosites (pixels) per se can also function as an aperture and detector provided the detector is localised in the image plane conjugate to the object plane. The incident epi illuminent fluorescence microscope is preferred if the particles analysed are small with a molecular weight of below 100 000 mega Daltons. Examples of such small particles are biomolecules such as vesicles or cell organelles. In addition, the volume element has suitable a volume from about 10^{-16} litre up to about 10^{-10} litre, preferably from about 10^{-15} up to about 10^{-12} . In order to obtain these small measurement volumes, the numeric aperture of the objective is suitably above about 0.7, more preferably above 1.0.

[0028] Furthermore, in case fluorescence is measured specific properties related to the expression of specific gene products such as surface proteins, which are important for fertilization processes and/or with other important functional properties can be calculated by using fluorescence intensity fluctuation analysis. In addition, the percentage of spermatozoa in relation of the total sperm count as well as the specific expression level (expressed molecules per spermatozoa) can be measured. Similarly, even the nucleic acid content can be analysed.

[0029] The original light source may preferably be divided into a plurality of light sources which is obtained by positioning a diffractive optical element in the beam path. The number of individual beam paths is preferably matched by an equal number of detection areas in the digital picture.

Brief description of the drawings:

[0030]

- 5 Fig. 1 shows one embodiment of the present invention where the intensity fluctuation of the transmitted and scattered light is measured.
 Fig. 2 indicates another embodiment of the present invention based on fluorescent light emitted from particles in the sample container.
 10 Fig. 3 outlines a yet another embodiment of the invention, the device containing two detectors for analysing fluorescent light signals.
 Fig. 4 shows the device of fig. 3 with a diffractive optical element.
 15 Fig. 5 describes a device which is integrated into the sample chip.

[0031] The sample container can have a wide variety of shapes both closed as well as open as long as the characteristic of the particle solution is not significantly changed over time. Preferably, the sample holding means is fitted with means for keeping the temperature at a specific level. To keep the solution at a predetermined temperature can be of importance if biological systems are analysed, e.g. spermatozoa. Preferably, the size of the sample container in the direction of the light path equals or is smaller than the focal depth of the optical means, provided optical means are situated in the light path between the sample container and the image sensor.
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[0032] The device according to the present invention can have more than just a detector capable of generating a digital picture (e.g. different detector types such as a CCD type or CMOS type sensor) especially if emitted light from the particles such as fluorescence is to be captured. A second detector is suitably adapted to capture phenomena with short response time, e.g. a detector having a high sensitivity such as an avalanche photo diode (APD) detector, e.g. a CMOS APD detector. Having two or more detectors, at least one other beam splitter is located in the beam path (Fig. 3).
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[0033] By using diffractive optical means the light source can be divided into a plurality of light sources generating a plurality of measurement volumes. The dynamic parameters of the particles present in each volume element can be calculated by applying an image sensor having a number of measurement fields suitably corresponding to the number of generated light sources by a diffractive optical element. A suitable position sensitive detector can be any of those mentioned above. The diffractive optical element is suitably positioned between the light source and the beam splitter, e.g. as shown in Fig. 4.
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[0034] Spermatozoa, or semen, can be measured by the present invention. The fitness of the spermatozoa is given by the swimming speed and rotation frequency, also the concentration can be calculated as the number of sperms is given by the amplitude of the correlation
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function at $\tau =$ zero. The total concentration of spermatozoa includes also dead or immobile spermatozoa, the number of which within a detection field can also be measured by rendering them mobile to the detection system by moving the detection field, either mechanically or computationally. Furthermore, by using an image sensor also a visual image is generated of the spermatozoa. The spermatozoa may also be labelled by fluorescent dye or dye conjugated markers, e.g. antibodies with specificity for certain properties of spermatozoa, e.g. DNA, surface based receptors, proteins related to the head, mid-piece or tail of the spermatozoa. Fluorescent labelling of spermatozoa is usually performed by binding of fluorescent markers, e.g. antibodies to the spermatozoa which is known to the person skilled in the art. Suitable fluorescent dyes are those having the absorption maxima in the visible spectrum. i.e. from 350 nm to 750 nm, exemplified by rhodamines such as rhodamine green, TMR, rhodamine B and 6G, cyanines like Cy2, Cy3 and Cy5, texas red. In order to visualise (create specific emission) nucleic acids like DNA, the gene material, dyes interacting specifically with DNA such as ethidium bromide, propidium iodide, acridin dyes and other nucleic specific molecules (e.g. gene probes) can be used.

[0035] According to one embodiment of the invention, the device comprises a phase contrast compound microscope and an image sensor such as a CCD or a CMOS detector. When spermatozoa/semen is measured the total magnification of the microscope (object versus intermediate image) suitably is in the range of from about 10 to about 30 times. Thus, the head of a sperm measuring approx. 3 μm would in the intermediate image plane have a size from 30 μm to 90 μm . The image sensor is suitably positioned in the plane of the intermediate image. The digital image of the image sensor is divided into a plurality of separate detection areas. The detection area can preferably have a size which is equal or greater than the size of the particles in the digital image suitably up to an area giving sufficient fluctuations of the number of particles for calculating accurate dynamic parameters using correlation analysis. The shape of the detection area is not important as long as an accurate correlation function can be calculated. The area can have any shape, e.g. circular or rectangular. The image sensor is furthermore connected to computational means comprising suitable software and hardware for video image analysis. With the computer screen a digital moving picture of the sample is generated. By applying appropriate image analysis, noise, e.g. background such as different categories of non-relevant particles, can be filtered away such that only the fluctuation of the relevant particles form the basis for the calculation of the correlation function. Furthermore, the registering of particles by the image sensor and the calculation of the time correlation function is performed essentially simultaneously.

[0036] A preferred embodiment of the invention is exemplified by Fig.1 outlining a device comprising a light source (1), a sample compartment comprising a solution

of the particles (3), optical means such as a microscope (5), and an image sensor (7). The light beam is preferably focussed on the sample compartment using suitable lenses (2). The light source can be any of the above mentioned types. The optical means (5) is suitably situated at a distance from the sample compartment such that the focal point (4) is within the sample compartment (3). The image sensor is suitably either of CCD or CMOS array type and preferably positioned in the image plane (6) conjugate to the focal point of the objective. With the image sensor in the image plane (Fig.1) the fluctuations of the particles in the digital picture is analysed. Not shown in Fig. 1 is the means for the signal processing, which is suitably a personal computer.

[0037] According to another embodiment of the invention (Fig. 2), the device comprises a light source (10), sample compartment comprising a solution of the particles (8), an optical means (9), a beam-splitter (11), an emission (cut-off) filter (12), and a detector (14). The light beam is reflected 90 deg. by the beam splitter and focussed by the optical means (9) such that the focal point is situated within the sample solution. The beam splitter can be any optical means which is capable of sufficiently separating the reflected light or excited light from the emitted light emanating from the light source, such as a wave length dependent (dichroic) beam splitter. One common optical means is a dichroic mirror when fluorescence is used. Usually, a cut-off filter (12) or a plurality of cut off filters is/are positioned in the beam path between the beam splitter and the pin hole or image sensor in order to improve the signal-to-noise ratio. The particles to be measured can be non-fluorescent or fluorescent, alternatively the solution may comprise both fluorescent and non-fluorescent particles. This embodiment of the invention can both be used for back-scattering measurement and/or for fluorescence measurement.

[0038] According to yet another embodiment of the invention (Fig 3), the device comprises two beam splitters, a first (18) and a second (19) beam splitter and two image sensors (20, 22). Apart from the two image sensors and the two beam splitters the layout is similar to the device shown in Fig. 2, further comprising a sample compartment (15), and an optical means (16). The sensors may be positioned in the image planes conjugate to the focal point of the objective (21, 23). Alternatively, an aperture (pinhole) is positioned in the image planes. One of the sensors (20, 22) is an image sensor, e.g. a CCD or CMOS detector. The other detector may be a detector having a fast time response exemplified by multiple APDs, CMOS APDs or CMOS APD arrays.

[0039] Fig. 4 describes an embodiment of the present invention comprising a diffractive optical element (24) positioned between the light source and the beam splitter.

[0040] Fig. 5 describes another embodiment of the present invention where the image sensor and the illumination is integrated with the measurement cell. A waveguide (25) preferably illuminated by light emitting diodes is positioned opposite one or more image sensors

(26). In the cavity (27) between the waveguide and the image sensor the solution comprising the particles is positioned. All the components are small and they consume little energy, thus, this type of device has preferably very compact dimensions.

Claims

1. A method for measuring dynamic parameters of particles, **characterised in that** the method comprises applying correlation analysis on temporal fluctuations of the number of particles (in 3; 8; 15; 27) with respect to a detection area of a digital picture, wherein a time correlation function or functions is/are calculated based on the temporal fluctuations of the number of particles.
2. The method according to claim 1, **characterised in that** the digital picture is generated by an image sensor (7; 14; 20, 22; 26).
3. The method according to claim 1, **characterised in that** the method comprises detecting the particles (in 3; 8; 15; 27) by a detector (7; 14; 20, 22; 26) capable of generating the digital picture.
4. The method according to claim 1, **characterised in that** the method comprises providing the particles (in 3; 8; 15; 27) in a solution, a light source (1; 10; 17; 25), an image sensor (7; 14; 20, 22; 26), computational means, detecting the particles on a digital picture generated by the image sensor, and calculating the correlation function based on the temporal fluctuations of the number of particles with respect to the detection area of the digital picture.
5. The method according to claim 4, **characterised in that** optical means (5; 9, 11, 12; 16, 18, 19) are positioned in the light path between the object plane and the plane of the image sensor (7; 14; 20, 22; 26).
6. The method according to claims 4 and 5, **characterised in that** the correlation function is based on fluctuations of particles with respect to a plurality of detection areas.
7. The method according to claims 4 to 6, **characterised in that** the image sensor (7; 14; 20, 22; 26) is of solid state detector type.
8. The method according to any of the preceding claims, wherein the method further comprises background subtraction.
9. The method according to any of the preceding claims, wherein the particles are spermatozoa.

10. The method according to any of the preceding claims, wherein the method further comprises a measurement of the particle concentration.
- 5 11. A device for measuring dynamic parameters of particles in a solution, **characterised in that** the device comprises a sample compartment (3; 8; 15; 27), a light source (1; 10; 17; 25), an image sensor (7; 14; 20, 22; 26), computational means adapted for processing signals from the image sensor, detecting the particles on a digital picture generated by the image sensor, and calculating the correlation function based on temporal fluctuations of the number of particles with respect to a detection area of the digital picture, wherein a time correlation function or functions is/are calculated based on the temporal fluctuations of the number of particles.
- 10 12. The device according to claim 11, **characterised in that** the image sensor (7; 14; 20, 22; 26) is of solid state type.
- 15 13. The device according to claim 11 or 12, **characterised in that** optical means (5; 9, 11, 12; 16, 18, 19) are positioned in the light path between the object plane and the plane of the image sensor (7; 14; 20, 22; 26).
- 20 14. The device according to any one of claims 11-13, **characterised in that** the correlation function or functions is based on fluctuations of particles with respect to a plurality of detection areas.
- 25 15. The device according to any one of claims 11-14, **characterised in that** the particles are spermatozoa.
- 30 16. The device according to any one of claims 11-14, **characterised in that** it comprises means for measuring the particle concentration.
- 35 17. Use of a device as defined by any one of claims 11-16 for measuring dynamic parameters.
- 40 18. The use according to claim 17 for further measuring the particle concentration.
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Patentansprüche

1. Verfahren zum Messen von dynamischen Parametern von Partikeln, **dadurch gekennzeichnet, dass** das Verfahren umfasst: Anwenden einer Korrelationsanalyse auf zeitliche Fluktuationen der Anzahl von Partikeln (in 3; 8; 15; 27) in Bezug auf einen Detektionsbereich eines digitalen Bilds, worin eine Zeitkorrelationsfunktion oder -funktionen basierend auf den zeitlichen Fluktuationen der Anzahl von Par-

- tikeln berechnet wird/werden.
2. Das Verfahren gemäß Anspruch 1, **dadurch gekennzeichnet, dass** das digitale Bild von einem Bildsensor (7; 14; 20, 22; 26) erzeugt wird. 5
 3. Das Verfahren gemäß Anspruch 1, **dadurch gekennzeichnet, dass** das Verfahren umfasst: Detektieren der Partikel (in 3; 8; 15; 27) mit einem Detektor (7; 14; 20, 22; 26), der in der Lage ist, das digitale Bild zu erzeugen. 10
 4. Das Verfahren gemäß Anspruch 1, **dadurch gekennzeichnet, dass** das Verfahren umfasst: Bereitstellen der Partikel (in 3; 8; 15; 27) in einer Lösung, einer Lichtquelle (1; 10; 17; 25) eines Bildsensors (7; 14; 20, 22; 26), Rechenmitteln, Detektieren der Partikel auf einem vom Bildsensor erzeugten digitalen Bild, und Berechnen der Korrelationsfunktion basierend auf den zeitlichen Fluktuationen der Anzahl von Partikeln in Bezug auf den Detektionsbereich des digitalen Bilds. 15
 5. Das Verfahren gemäß Anspruch 4, **dadurch gekennzeichnet, dass** optische Mittel (5; 9, 11, 12; 16, 18, 19) in einem Lichtweg zwischen der Objektebene und der Ebene des Bildsensors (7; 14; 20, 22; 26) angeordnet sind. 20
 6. Das Verfahren gemäß den Ansprüchen 4 und 5, **dadurch gekennzeichnet, dass** die Korrelationsfunktion auf Fluktuationen von Partikeln in Bezug auf eine Mehrzahl von Detektionsbereichen beruht. 25
 7. Das Verfahren gemäß den Ansprüchen 4 bis 6, **dadurch gekennzeichnet, dass** der Bildsensor (7; 14; 20, 22; 26) vom Festzustanddetektortyp ist. 30
 8. Das Verfahren gemäß einem der vorangehenden Ansprüche, worin das Verfahren ferner eine Hintergrund-Subtraktion umfasst. 35
 9. Das Verfahren gemäß einem der vorangehenden Ansprüche, worin die Partikel Spermatozoen sind. 40
 10. Das Verfahren gemäß einem der vorangehenden Ansprüche, worin das Verfahren ferner eine Messung der Partikelkonzentration umfasst. 45
 11. Vorrichtung zum Messen von dynamischen Parametern von Partikeln in einer Lösung, **dadurch gekennzeichnet, dass** die Vorrichtung umfasst: einen Probenraum (3; 8; 15; 27), eine Lichtquelle (1; 10; 27; 25), einen Bildsensor (7; 14; 20, 22; 26), Rechenmittel, ausgelegt zum Bearbeiten von Signalen von dem Bildsensor, Detektieren der Partikel auf einem vom Bildsensor erzeugten digitalen Bild, und Berechnen der Korrelationsfunktion basierend auf zeitlichen Fluktuationen der Anzahl von Partikeln in Bezug auf einen Detektionsbereich des digitalen Bilds, worin eine Zeitkorrelationsfunktion oder -funktionen basierend auf zeitlichen Fluktuationen der Anzahl von Partikeln berechnet wird/werden. 50
 12. Die Vorrichtung gemäß Anspruch 11, **dadurch gekennzeichnet, dass** der Bildsensor (7; 14; 20, 22; 26) vom Festzustanddetektortyp ist. 55
 13. Die Vorrichtung gemäß Anspruch 11 oder 12, **dadurch gekennzeichnet, dass** optische Mittel (5; 9, 11, 12; 16, 18, 19) in einem Lichtweg zwischen der Objektebene und der Ebene des Bildsensors (7; 14; 20, 22; 26) angeordnet sind.
 14. Die Vorrichtung gemäß einem der Ansprüche 11 bis 13, **dadurch gekennzeichnet, dass** die Korrelationsfunktion oder -funktionen auf Fluktuationen von Partikeln in Bezug auf eine Mehrzahl von Detektionsbereichen beruht/beruhen.
 15. Die Vorrichtung gemäß einem der Ansprüche 11 bis 14, **dadurch gekennzeichnet, dass** die Partikel Spermatozoen sind.
 16. Die Vorrichtung gemäß einem der Ansprüche 11 bis 14, **dadurch gekennzeichnet, dass** sie Mittel zum Messen der Partikelkonzentration aufweist.
 17. Verwendung einer Vorrichtung, wie durch einen der Ansprüche 11 bis 16 definiert, zum Messen von dynamischen Parametern.
 18. Die Verwendung gemäß Anspruch 17 zum weiteren Messen der Partikelkonzentration.

Revendications

1. Procédé de mesure des paramètres dynamiques de particules, **caractérisé en ce que** le procédé comprend l'application d'une analyse de corrélation sur les fluctuations temporelles du nombre de particules (dans 3 ; 8 ; 15 ; 27) par rapport à une zone de détection d'image numérique, où une fonction ou des fonctions de corrélation dans le temps est/sont calculée(s) à partir des fluctuations temporelles du nombre de particules.
2. Procédé selon la revendication 1, **caractérisé en ce que** l'image numérique est générée par un capteur d'image (7 ; 14 ; 20 ; 22 ; 26).
3. Procédé selon la revendication 1, **caractérisé en ce que** le procédé comprend la détection des particules (dans 3 ; 8 ; 15 ; 27) par un détecteur (7 ; 14 ; 20 ; 22 ; 26) capable de générer l'image numérique.

4. Procédé selon la revendication 1, **caractérisé en ce que** le procédé comprend la fourniture des particules (dans 3 ; 8 ; 15 ; 27) dans une solution, d'une source de lumière (1 ; 10 ; 17 ; 25), d'un capteur d'image (7 ; 14 ; 20 ; 22 ; 26), d'un moyen de calcul, la détection des particules sur une image numérique générée par le capteur d'image et le calcul de la fonction de corrélation à partir des fluctuations temporelles du nombre de particules par rapport à la zone de détection de l'image numérique. 5
5. Procédé selon la revendication 4, **caractérisé en ce que** des moyens optiques (5 ; 9 ; 11 ; 12 ; 16 ; 18 ; 19) sont positionnés dans le trajet de lumière entre le plan objet et le plan du capteur d'image (7 ; 14 ; 20 ; 22 ; 26). 15
6. Procédé selon les revendications 4 et 5, **caractérisé en ce que** la fonction de corrélation est basée sur les fluctuations de particules par rapport à une pluralité de zones de détection. 20
7. Procédé selon les revendications 4 à 6, **caractérisé en ce que** le capteur d'image (7 ; 14 ; 20 ; 22 ; 26) est du type détecteur à semi-conducteur. 25
8. Procédé selon l'une quelconque des revendications précédentes, dans lequel le procédé comprend en outre une soustraction de fond. 30
9. Procédé selon l'une quelconque des revendications précédentes, dans lequel les particules sont des spermatozoïdes. 35
10. Procédé selon l'une quelconque des revendications précédentes, dans lequel le procédé comprend en outre une mesure de la concentration de particules. 40
11. Dispositif de mesure des paramètres dynamiques de particules dans une solution, **caractérisé en ce que** le dispositif comprend un compartiment d'échantillon (3 ; 8 ; 15 ; 27), une source de lumière (1 ; 10 ; 17 ; 25), un capteur d'image (7 ; 14 ; 20 ; 22 ; 26), un moyen de calcul adapté pour traiter les signaux du capteur d'image, détecter les particules sur une image numérique générée par le capteur d'image et calculer la fonction de corrélation à partir des fluctuations temporelles du nombre de particules par rapport à une zone de détection de l'image numérique, où une fonction ou des fonctions de corrélation dans le temps est/sont calculée(s) à partir des fluctuations temporelles du nombre de particules. 45 50
12. Dispositif selon la revendication 11, **caractérisé en ce que** le capteur d'image (7 ; 14 ; 20 ; 22 ; 26) est du type à semi-conducteur. 55
13. Dispositif selon la revendication 11 ou 12, **caractérisé en ce que** des moyens optiques (5 ; 9 ; 11 ; 12 ; 16 ; 18 ; 19) sont positionnés dans le trajet de lumière entre le plan objet et le plan du capteur d'image (7 ; 14 ; 20 ; 22 ; 26).
14. Dispositif selon l'une quelconque des revendications 11 à 13, **caractérisé en ce que** la fonction ou les fonctions de corrélation est/sont basée(s) sur les fluctuations de particules par rapport à une pluralité de zones de détection.
15. Dispositif selon l'une quelconque des revendications 11 à 14, **caractérisé en ce que** les particules sont des spermatozoïdes.
16. Dispositif selon l'une quelconque des revendications 11 à 14, **caractérisé en ce qu'**il comprend un moyen de mesure de la concentration de particules.
17. Utilisation d'un dispositif selon l'une quelconque des revendications 11 à 16, pour mesurer les paramètres dynamiques.
18. Utilisation selon la revendication 17, pour mesurer en outre la concentration de particules.

Figure 1

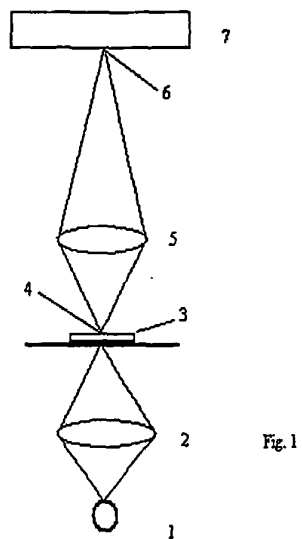


Figure 2

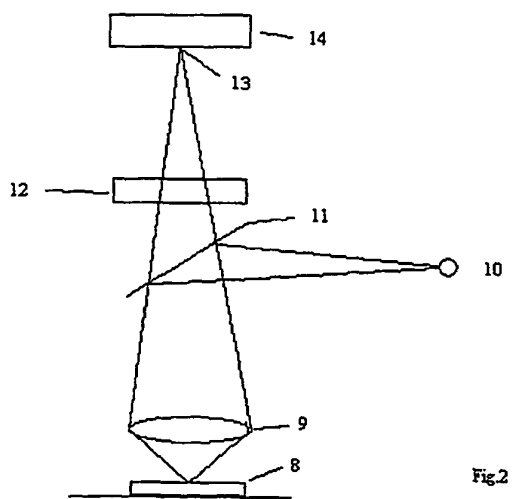


Figure 3

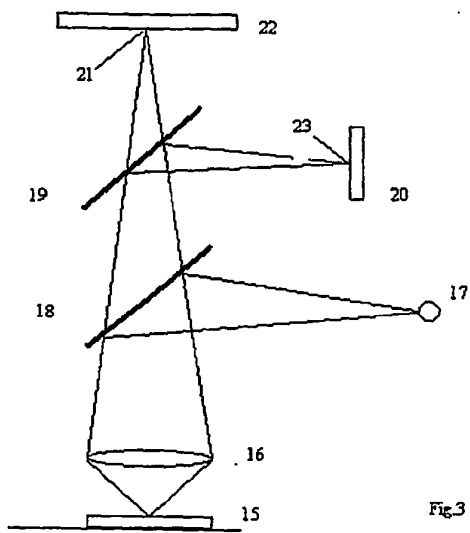


Figure 4

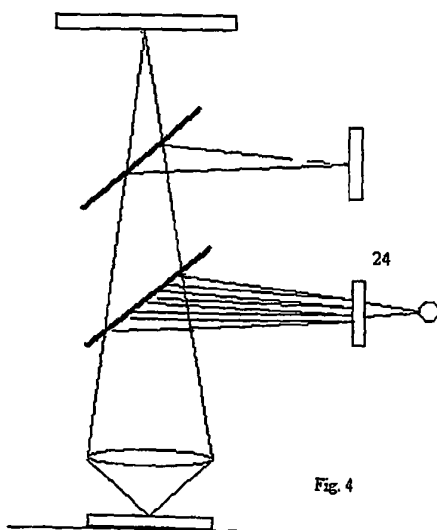
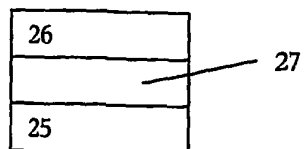


Figure 5



REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5116125 A [0004]