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(54) Title: INTERFEROMETRIC SYSTEM AND METHOD FOR USE WITH BIOLOGICAL CELLS AND ORGANISMS INCLUDING SPERM

(57) Abstract: Method and system for use in sperm analysis are described, for processing measured data comprising at least interferometric phase data of a label-free sperm cell, the processing of the measured data comprising determining topographic optical phase delay (OPD) map of the label-free sperm cell, determining at least one physical parameter of the label-free sperm cell, and generating data indicative of sperm quality for the label-free sperm cell.

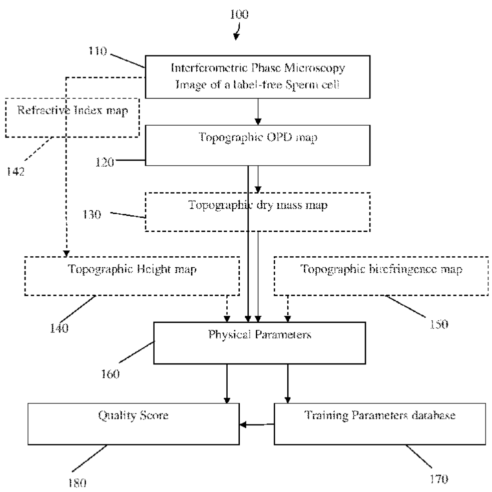

Fig 1A

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INTERFEROMETRIC SYSTEM AND METHOD FOR USE WITH BIOLOGICAL CELLS AND ORGANISMS INCLUDING SPERM

TECHNOLOGICAL FIELD

The invention relates generally to interferometric systems and methods of optical analysis of biological cells and organisms. Specifically, the invention is useful for interferometric analysis of sperm samples.

BACKGROUND

Approximately 70 million people worldwide need assisted reproductive techniques (ART) to bare children. Following the introduction of *in-vitro* fertilization (IVF), several efforts have been directed towards identifying the oocyte's and fetus's morphologies as a prognostic tool for IVF success. Works were also conducted on the ability of sperm cell morphology to predict the success rates of natural fertilization, intra-uterine insemination, IVF and IVF with intra-cytoplasmatic sperm injection (ICSI), in which a single sperm cell is selected, based on its mobility and overall morphology, using high magnification phase contrast microscope and the selected sperm cell is injected directly into the oocyte retrieved from the female partner. It has been found that sperm morphology is a good predictor for fertilization success and patients with abnormal semen analysis had lower probability of successful pregnancies using IVF. ICSI is the only treatment solution, known to date, for severe male factor infertility. In spite of this, the success rate of ICSI (measured by clinical pregnancy or live birth rate) is still very low, only about 20% of patients undergoing IVF with ICSI succeed in achieving pregnancy. Moreover, several clinical studies have shown that the percentage of chromosomal abnormalities in fetuses and babies conceived using ICSI is higher than in the general population, and even compared to other ART procedures. Ideally, the sperm that is chosen for ICSI should have the highest chance of successful fertilization and subsequent embryo growth. The viability of the sperm cell to be used in ICSI procedure is maintained by diagnostic modalities which are label-free and which do not involve any kind of stain.

One of the known and common techniques for evaluating sperm cell morphological parameters is the qualitative, non-quantitative, label-free bright field microscopy (BFM). Typically, sperm cells are imaged optically using BFM and chosen according to the world health organization (WHO) guidelines. Therefore, a morphological examination of about 200 fixated, stained cells is conducted for the evaluation of properties such as the size of the nucleus and the acrosome. The images are then analyzed visually by an experienced embryologist, or using a software (computer assisted sperm analysis (CASA)) that automatically measures the different morphological features. Without staining, sperm cells are nearly transparent under BFM, since their optical properties are only slightly different from their surroundings, resulting in a weak image contrast. Recently, new methods were developed for identifying finer properties of the sperm cell, not seen by BFM (e.g. surface charge selection). Most of these methods involve biochemical preparations that might change the viability of the cells and thus precluding their use in IVF.

Phase imaging methods use the optical interference phenomenon to record the light delays when passing through the sample, and are able to create label-free contrast in the image. Conventional phase-contrast imaging methods for sperm cells were developed, such as Zernike's phase contrast and Nomarski's differential interference contrast (DIC). DIC is the basis for the motile sperm organelle morphology examination (MSOME) technique. DIC enhances the image edges by shadowing, and thus enables to see details not seen on normal label-free BFM. However, both Zernike's phase contrast and DIC techniques are not fully quantitative, since they do not create meaningful contrast on all points of the measured sperm. In addition, these techniques present significant imaging artifacts, especially near the cell edges, which might yield wrong morphological assays.

GENERAL DESCRIPTION

There is a need in the art for a novel technique for quantitatively determining morphological parameters of a biological sample. Specifically, the invention provides a novel technique which is useful in obtaining quantitative and three-dimensional physical analysis, of unstained (so called unlabeled, or label-free) biological samples or cells, with very high accuracy. The invention enables, for example, quantifying morphological parameters of live unlabeled sperm cells and quantitatively scoring of the sperm cells. The technique of the invention would enable better selection of sperm cells for intra-cytoplasmatic sperm injection (ICSI) procedures, resulting in higher pregnancy rates and potentially avoiding genetic disorders in the resulting offspring.

Although the invention is exemplified herein with regard to sperm cells, it should be understood that the principles of the invention are not limited to any specific type of biological samples / cells and may be used for any type of such samples / cells such as cancer cells.

During selection of the best sperm cell for using in ICSI, several physical or morphological parameters, preferably in quantitative measures, are explored, such as the size, length and width of the sperm head, neck, midpiece and acrosome, tail configuration and a general normality of the cell sperm cell head.

When applying optical imaging / measurements to biological samples, e.g. sperm cells, a light beam that passes through the sperm cells is delayed as compared to that of the surroundings, since the cells have a slightly higher refractive index than their surroundings. Accordingly, the refractive index of the biological sample presents an internal optical contrast mechanism which may be exploited during examination procedures of the biological sample / sperm cell. However, regular intensity-based detectors are not fast enough to record this delay directly.

Interferometric phase microscopy (IPM) is capable of providing fully quantitative, phase-contrast image data. IPM is a holographic imaging method, which allows for quantitative measurement of the sample/cell optical thickness (OPD, i.e. the product of refractive index and physical thickness). It should be understood that the terms optical thickness and OPD, used herein, are the same and are used through the text interchangeably. Recently, several initial researches have demonstrated utilization of IPM (also called digital holographic microscopy) for sperm characterization. In one research, IPM was used to evaluate sperm, mainly based on the presence or absence of vacuoles. Since the vacuoles form early in spermatogenesis, they may reflect defects in sperm content, and therefore these cells should be avoided in ICSI. Using IPM, it was shown that vacuolated cells have a decreased volume. Another research compared normal and pathological sperm samples with IPM, and it was shown that abnormal sperm heads have lower maximal optical path difference (i.e. the OPD) compared to normal samples. The present invention provides a method and system for assessing the quality of the sperm cell (e.g. for fertility potential examination) by creating an objective and quantitative quality score based on IPM measurements of a label-free cell, and possibly on additional measurements. The quantitative quality score can be used to identify sperm cells suitable for use in IVF. According to the present invention, IPM-based methods are used to quantify sperm morphology and to identify the cellular compartments which play significant role in the fertility potential (e.g. the acrosome and the nucleus) based on the OPD data, and on the spatial distribution of the dry mass of the sperm cell (the mass of the sperm cell's tissues without water/fluid) between the cellular compartments which is obtained directly from the OPD data.

Thus, IPM can be used to quantify sperm morphology without staining and to differentiate between the acrosomal and the nuclear compartments, and other morphological and physiological structures (e.g. vacuoles) in a live, unlabeled sperm cell. The present invention also provides equivalent information to bright field microscopy (BFM) imaging of stained sperm cells when measuring key morphologic parameters of fixed human spermatozoa according to the World Health Organization (WHO) criteria, thus paving the way for directly selecting label-free sperm cells for IVF and ICSI.

According to the present invention, one of the main parameters that are used to score the sperm cell is a topographic OPD map corresponding to each point on the imaged biological sample/cell, such that the value of each pixel in a digital IPM image corresponds to the OPD for that pixel. Other parameters for scoring the sperm cell may be a topographic dry mass map, topographic height map and topographic birefringence map. The score of the sperm cell may utilize all or some of the aforementioned maps, to assess, by quantification, the quality score of the label-free sperm cell, e.g. for fertility examination procedure.

The topographic height map, i.e. the physical thickness map, may be obtained from the OPD map by knowing the refractive indices of the corresponding different organs/regions of the sperm cell. Alternatively, an atomic force microscope can be used to measure the physical thickness of dehydrated, fixated, un-labeled sperm cells and obtain the average refractive indices of each location within a sperm cell.

The measurements and/or calculation of the dry mass and/or refractive indices allow the construction of a database of dry mass of the several organelles/compartments in the sperm cell, and a data base of refractive indices of normal and abnormal cells and their cellular compartments, enabling to quantify sperm potency for fertilization. It should be noted that for a given physical thickness, the OPD of the cell, and its compartments, is linearly dependent on the refractive index of the cell relative to the surrounding medium. As cells are mainly composed of water, it is possible to calculate the dry mass of the cell (i.e. the average mass of the proteins, carbohydrates, lipids etc. within the cell) using the OPD when the cells are immersed in liquid with a refractive index similar to that of water. Specifically, the human spermatozoa head comprises two general compartments, which differ significantly in the composition and concentration of proteins, nucleic acids and other components: the nucleus and the acrosome. The acrosomal part contains an array of hydrolytic enzymes necessary for digesting the zona pellucida during penetration of the oocyte, whereas the nucleus comprises the DNA, the related proteins, enzymes and lipids. Consequently, the dry mass of these cellular compartments will differ due to their different composition.

The topographic birefringence map may also be included in the quality score of the sperm cell to assess DNA quality. DNA fragmentation may be associated with a poor outcome for fertilization, implantation and fetal development. A recent study demonstrated that patients with a predominance of abnormal acrosomes had a significantly greater percentage of sperm with DNA fragmentation compared with the controls. Optical measurement, using a polarization microscope, demonstrated that the birefringence distribution (total or partial) of sperm heads is correlated with DNA fragmentation. In particular, cells with a total birefringence coverage of the sperm head show DNA fragmentation. As birefringence is related to the spatial order of the proteins and nucleic acids within the organelles, their findings relate to variations in the birefringence of the acrosomal and nuclear parts of the cells, and cells without an acrosome will show a total birefringence.

IPM is advantageous because it requires low power resources and presents high throughput since capturing is done in a single exposure and without scanning. However, IPM setups are usually bulky, expensive, and hard to operate. A simple, portable, compact and inexpensive IPM has been developed by some inventors of the present invention and is described in WO 2013/140396. This interferometric device provides a substantially stable, easy to align common path interferometric geometry, while eliminating a need for controllably changing the optical path of the beam. The interferometric device can be used for acquiring images of live biological cells in a label-free manner with sub-nanometric thickness precision. The device can be easily used with any microscope.

In contrast to other non-quantitative techniques, IPM can yield the cell optical thickness (i.e., physical thickness × refractive index) profile for each point on the measured cell. The present invention provides a fast and effective quantitative analysis of biological organisms, such as sperm cells, without a need for labeling the sample being analyzed, based on a novel data interpretation technique. It should be noted that, generally, the interpretation of the IPM results is difficult due to the decoupling between the refractive index and the physical thickness of the cell.

In addition, the present invention provides other information that can be of interest to the fertilizing potential of the sperm cell. Since IPM measures the optical thickness of the cell, which is the product of the refractive index and the physical thickness, one can extract any of these parameters by assuming the other one. Also, according to the present invention, the dry mass parameter, which can be calculated based on the OPD, is utilized in addition to the OPD, to generate the quality score of the sperm cell that is related to measureable physical properties of the cell and its compartments.

As said, according to the present invention, the quantitative scoring analysis of the sperm cell may use unique morphological quantitative parameters, which are based on the three-dimensional morphology of the sperm cell, as obtained from the OPD measurements. To derive the parameters, IPM measurements may be done firstly on one or more sperm cells which are labeled in order to define the cell organelles transverse locations and a training database is built to obtain a characteristic OPD map which includes, *inter alia*, the characteristic refractive indices of the various organelles inside a sperm cell. According to the invention, the characteristic refractive indices of different organelles, e.g. the vacuoles, are determined, *inter alia*, by an IPM measurement performed on the labeled sperm cells immersed in mediums of known refractive indices. A two-dimensional OPD map defined by the product of the sperm physical thickness and the refractive index map (distribution) allows determining the refractive index and physical thickness (height) in each point (in the image plane) of the sperm and specifically at the different locations of the organelles. The refractive indices of the various organelles in a sperm cell are considered to have relatively constant values. Accordingly, a two-dimensional map of refractive indices for each point in a sperm cell is saved in a database and can be used to obtain a map of the physical thickness (the topographic height map), i.e. a three-dimensional representation, of the whole sperm cell including internal organelles of interest.

Real measurement of the phase map, performed on an unlabeled sperm cell, gives, by using the novel refractive indices map of every point in the sperm cell (i.e. the characteristic refractive index map) stored in database, the physical sperm thickness of the sperm cell, specifically at the different locations of the various organelles.

In some embodiments, based on the sperm transverse locations, a computerized thickness map for the sperm head, the sperm neck or any other organelle of interest, is built. Fitting may be used to correlate the expected computerized thickness map with the measured thickness map.

Immersion of the sperm cell in a medium with a specific refractive index may also enhance the contrast when imaging. It is known that the refractive index of the vacuoles is significantly lower than the refractive index of the other cellular contents. If the refractive index of the immersion medium becomes higher than the refractive index of the vacuole (plus the cellular material located above and below it), the contrast in the vacuole location will be inverted. If the sperm cell is put in a medium with a slightly higher refractive index, then an enhanced contrast may be achieved.

Thus according to a first broad aspect of the present invention, there is provided a method for use in sperm analysis, the method comprising processing measured data comprising at least interferometric phase data of a label-free sperm cell, said processing of the measured data comprising determining topographic OPD map, determining at least one physical parameter of the label-free sperm cell and generating data indicative of sperm quality for the label-free sperm cell.

In some embodiments, the processing of the measured data further comprises generating a topographic dry mass map of the label-free sperm cell.

In some embodiments, the processing of the measured data further comprises generating a topographic height map of the label-free sperm cell. The generating of the topographic height map may comprise utilizing a refractive index map being characteristic of sperm cells.

In some embodiments, the measured data comprises a birefringence image data, and the processing of the measured data comprises generating a topographic birefringence map of the label-free sperm cell.

The at least one physical parameter comprises at least one of the following: head area; total volume of the head; width and length of the head; relative area or volume of the acrosome and nucleus within the head; total dry mass of the head; dry mass of the acrosome and nucleus; volume of vacuoles within the head, and their relative volume; centroid and weighted centroid of each head region and the distance between them; Mean OPD of each head region; variance or standard deviation of each head region; Mean anterior –posterior difference; midpiece width; midpiece length; tail length; presence of cytoplasmic droplets; tail form and head form.

In some embodiments, the data indicative of sperm quality for the label-free sperm cell comprises sperm fertility quality data.

In some embodiments, the data indicative of sperm quality for the label-free sperm cell comprises a quantitative quality score.

In some embodiments, the measured data is obtained for a plurality of label-free sperm cells, and a training data set is built comprising said at least one physical parameter for normal and abnormal sperm cells. The training data set may comprise data indicative of at least one of spatial, spectral and polarization state and birefringence distribution within a sperm cell. The sperm quality being indicative of a plurality of physical parameters is determined by comparing measured physical parameters with physical parameters of said training data set.

In some embodiments, the sperm quality is indicative of at least one of chromosomal aberrations within a nucleus of the cell, DNA fragmentation within the cell and sex of the sperm cell.

In some embodiments, the refractive index map is obtained by processing image data of at least one labeled sperm cell. The processing of the image data indicative of the at least one labeled sperm cell may comprise calculating a sperm refractive index map corresponding to different points in the labeled sperm cell, and generating and storing data indicative of a characteristic sperm refractive index map of said different points in the labeled sperm cell to thereby determine properties of a label-free sperm cell. The properties of a label-free sperm cell may comprise morphological parameters of the label-free sperm cell. The different points in the labeled sperm cell correspond to at least one of the following cellular compartments of the labeled sperm cell: vacuoles, nucleus, acrosome, head, neck region and tail.

In some embodiments, the at least one labeled sperm cell is imaged to obtain the image data.

In some embodiments, the imaging of the labeled sperm cell is made with interferometric phase microscopy (IPM), while sequentially immersing said at least one labeled sperm cell in a first medium having a first refractive index and in a second medium having a second different refractive index, said image data comprising a first and second image data indicative of first and second phase map data, respectively.

In some embodiments, the imaging of the labeled sperm cell is made with interferometric phase microscopy (IPM), while immersing said at least one labeled sperm cell in a medium with a known refractive index and sequentially illuminating said at least one labeled sperm cell with light having first and second different light properties, said image data comprising first and second image data indicative of first and second phase map data, respectively. The light property comprises at least one of wavelength and polarization.

In some embodiments, the imaging of the labeled sperm cell is made with interferometric phase microscopy (IPM), while immersing said at least one labeled sperm cell in a medium with a known refractive index, said image data comprises a first image data indicative of a phase map data and a second image data indicative of physical thickness of said different points in the labeled sperm cell.

In some embodiments, the method comprises receiving and processing of the image data indicative of a plurality of labeled sperm cells, building a training data set comprising a plurality of sperm refractive index maps corresponding to the plurality of labeled sperm cells, and determining a characteristic sperm refractive index map from said plurality of the sperm refractive index maps. Said characteristic sperm refractive index map may be determined by averaging said plurality of sperm refractive index maps.

In some embodiments, the method comprises analyzing said topographical height map data and determining 3D morphology of the label-free sperm cell based on physical thickness distribution. The measured 3D morphology of the label-free sperm cell may be evaluated by correlating between it and theoretical data indicative of a computerized 3D morphology model of the label-free sperm cell. The computerized 3D morphology may be based on at least one characteristic dimension in an image of the label-free sperm cell. The measured data of the label-free sperm cell may comprise IPM data.

According to another broad aspect of the present invention, there is provided a method for use in sperm analysis, the method comprising processing image data indicative of at least one labeled sperm cell, calculating a sperm refractive index map corresponding to different points in the labeled sperm cell, generating and storing data indicative of a characteristic sperm refractive index map of said different points in the labeled sperm cell, to thereby determine properties of a label-free sperm cell.

According to yet another broad aspect of the present invention, there is provided a method for use in analysis of a biological organism, the method comprising imaging at least one labeled biological organism and obtaining image data, processing the image data and calculating a refractive index map corresponding to different points in the labeled biological organism, and generating and storing data indicative of a characteristic refractive index map and of said different points in the labeled biological organism to be used for determining properties of a label-free biological organism.

According to yet another broad aspect of the present invention, there is provided a computerized system for use in sperm analysis, the system comprising: a communication utility for receiving interferometric measured data of a sperm cell; and a processor utility configured and operable for processing said measured data and determining at least one physical parameter of said sperm cell, said processing comprising determining topographic optical phase delay (OPD) map of the label-free sperm cell, determining at least one physical parameter of the label-free sperm cell, and generating data indicative of a fertility quality score for the label-free sperm cell.

In some embodiments, the processor utility is further configured and operable for utilizing the measured data and a characteristic refractive index map of a sperm cell, stored in a memory, calculating a physical thickness distribution of different points in said measured label-free sperm cell, and generating topographic height map for the label-free sperm cell.

In some embodiments, the system comprises a flow chamber configured and operable to trap said sperm cell and to allow imaging thereof. The processor utility may be configured and operable to control at least one of operation of said flow chamber, flow of the fluid inside the chamber, and substitution of different fluids for enhancing contrast of said sperm cell.

In some embodiments, the system comprises a memory utility configured and operable to store at least one of said measured data, results of analysis of the measured data, and characteristic refractive index map of said measured data.

According to yet another broad scope of the invention, there is provided a device for use in analysis and selection of cells, comprising a flow chamber comprising a micro-channel configured and operable to allow applying analysis and imaging to a cell flowing in a medium thereinside, and two switchable gates at both sides of the micro-channel configured and operable to trap a single cell at a time inside said micro-channel. The device may further comprise a flow circuit configured and operable to substitute the medium inside said micro-channel while the cell thereinside, thereby enabling enhancing image contrast for specific organelles in the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to better understand the subject matter that is disclosed herein and to exemplify how it may be carried out in practice, embodiments will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1A illustrates a method for quantitative analysis according to one embodiment of the invention,

Figs. 1B-1D illustrate some examples utilizing the method of the invention,

Fig. 2 illustrates a method for quantitative analysis according to a second embodiment of the invention,

Fig. 3 exemplifies a flow chamber for using in sperm examination and selection procedure, according to one embodiment of the invention, and

Fig. 4 illustrates an example of a system according to one embodiment of the present invention.

DETAILED DESCRIPTION OF EMBODIMENTS

Reference is made to **Fig. 1A** exemplifying a flow diagram **100** of a method of the invention for in-vivo quantitative analysis of unlabeled, i.e. not stained, biological live cell/organism, such as a live sperm cell. The method may be used for effective and improved selection of a label-free sperm cell for intra cytoplasmic sperm injection (ICSI).

The method concerns quantitative IPM-based analysis of label-free sperm cells, taking into account at least the optical thickness (OPD) or the dry mass data of the sperm in all of its points. By utilizing these data, it is possible to evaluate a plurality of physical/morphological parameters of live sperm cells, such as the sperm head volume, neck volume and tail length. The method thus comprises processing images indicative of at least one label-free sperm cell and providing at least one of the optical thickness of the cell, the dry mass of the cell, the birefringence of the cell or a physical thickness of the cell in addition to its motility. The invention thus combines the ability of interferometric phase microscopy to provide non-invasive, label free imaging of several physical (morphological) parameters simultaneously.

In **step 110**, an IPM image of a label-free sperm cell is received. The imaging process itself is not part of the method **100**, however, in some cases, especially when the examination of the sperm cell is done concurrently with the selection process, the system executing the method (such as the system 50 illustrated in **Fig. 4**) may comprise an IPM imaging facility. The image data in the digital era is typically a matrix of pixels that include digit values of the measured entity. In this case, the immediate measured entity by IPM is the optical phase delay (OPD) of the light passing through the sperm cell. Accordingly, the first data used for the acquisition of the physical parameters is the Topographic OPD map (**step 120**), which is a matrix in which the value of each pixel corresponds to the optical phase delay for that pixel..

In **step 130** (dashed outline), a second map, the topographic dry mass map, is generated based on the OPD map **120**. The topographic dry mass map **130** is a matrix in which the value of each pixel in the image corresponds to the dry mass within that pixel. The dry mass is calculated from the OPD as follows:

$$\sigma_{DM}(x,y) = \frac{OPD(x,y)}{\alpha}$$

$$DM = \iint_{Sc} \sigma_{DM}(x,y)dxdy = Sc \times \langle \sigma_{DM}(x,y) \rangle$$

Where Sc is the area of the pixel in the image, and $\langle \sigma_{DM}(x,y) \rangle$ is the average dry mass surface density over that area. α is the specific refractive increment in ml/g (0.18-0.21 ml/g).

Steps 140 and 150 (dashed outline) may be used for obtaining some of the physical (morphological) parameters, as will be further detailed below. Two additional maps may be generated as well: the topographic height map **140**, in which the value of each pixel in the matrix corresponds to the thickness of the cell perpendicular to the optical axis, and the topographic birefringence map **150**, in which the value of each pixel corresponds to the birefringence (in nm) of polarized light passing through the cell. The topographic height map **140** can be extracted from the OPD, if the refractive index **142** is known or estimated, as will be detailed below with reference to Fig. 2. In certain cases, a topographic height map **140** may be obtained by using an atomic force microscope to directly measure the physical thickness of dehydrated, fixated, un-labeled sperm cells.

Based on at least the OPD or dry mass topographic maps (dry mass or dry mass surface density can be used interchangeably herein), in step **160** the following physical parameters may be obtained by using signal/image processing techniques, such as threshold, masks, moments and other known techniques:

Physical parameters:

- The head area;
- The total volume of the cell head (the 2D integral of the height over the area of the cell);
- The width and length of the head area;
- The relative area (or volume) of the acrosome and nucleus within the cell head;
- The total dry mass of the cell head;
- The dry mass of different compartments (such as acrosome and nucleus) within the cell head;
- The volume of vacuoles within the cell head, and their relative volume;
- The centroid and weighted centroid of each head region is obtained and the distance between them is determined;
- Mean OPD of each head region is determined;
- Variance or standard deviation of each head region is determined;

- Mean anterior –posterior difference = is the difference between the mean OPD values of the anterior and posterior halves of the sperm head. If there is no nucleus or no acrosome (or a very small acrosome) this value will be close to zero.
- Analysis of 2D shape of the sperm head:
  - Form factor: (4*pi*Area excluding holes)/(Perimeter*Perimeter)
  - Roundness: (4*Area including holes)/pi*(Maximum diameter*Maximum diameter)
  - Aspect ratio: (Major axis)/(Minor axis)
  - Effective diameter: (Area including holes/pi)*2
  - Circular degree: pi*(Major axis)/4*(Area excluding holes)
  - Circularity ratio: (4*pi*Area including holes)/(Perimeter*Perimeter)
  - Thin degree: (Maximum diameter)/(Pattern width)
  - Compact aspect ratio: $((4/pi)*(Area\ including\ holes)))^{1/2}/(Major\ axis)$
  - Elongation: (Perimeter*Perimeter)/(Area including holes)
  - Roughness: (perimeter*perimeter)/(4*pi*(area excluding holes))
  - Degree of circularity: $2*(pi*(Area\ including\ hole))^{1/2}/Perimeter$.

In addition, all other parameters required by WHO for assessing sperm morphology can be determined from the IPM images, including, *inter alia*:

- Midpiece width;
- Midpiece length;
- Tail length;
- Presence of cytoplasmic droplets;
- Tail form;
- Head form.

According to the invention, a training database is created by imaging a cell with IPM and then defining “potent” and “impotent” (or normal and abnormal) cells based on clinical decision. One example for a method for clinically defining the potency for fertilization may include measuring all of the above parameters for sperm cells from many different male donors, and for specific sperm cells that are selected for fertilization by a clinician, recording if the fertilization was successful or not. Alternatively, an expert can define whether a cell is normal or abnormal based on clinical criteria, or on criteria defined by the WHO. Therefore, for each sperm imaged with IPM, a label “potent” or “impotent” (or normal and abnormal) will be attached to it. Then using statistical, multiparameter methods (such as ANOVA, clustering or any other method) a score that combines the statistically relevant parameters from the above list will be assigned to each configuration of measured parameters (or only to a selection of them).

Thus, in **step 170**, a training parameter database is built to obtain the physical parameters and/or their spatial, spectral or polarization distribution within a sperm cell for normal and abnormal cells. The method of the present invention comprises evaluation of the images according to the morphological features of a cell and comparison of the outcome of the evaluation with a database of normal (e.g. potent) and abnormal (e.g. non-potent) cells. The computerized system of the present invention, as described herein below, can be used in sperm evaluation and selection for assistive reproduction techniques, such as IVF or ICSI. The database of normal and abnormal cells is built, in order to select the parameters that are used to provide a fertility quality score for the sperm cell.

As shown in **step 180**, the evaluation output may be a quantitative score that describes the potential of a given sperm to successfully fertilize an egg and produce a healthy offspring (potency). A "sperm quality score" that combines several of the physical parameters is created to evaluate sperm cells. For each new cell that is imaged using an interferometric phase microscope, a score is provided based on comparison of the measured parameters to those of the database. The comparison between the measured images to the database provides a score for the cell that reflects its potency for fertilization. This allows automatically informed screening of cells prior to selection for fertilization or for automatic diagnosis.

In some embodiments of the invention, the quality score is calculated based on the spatial distribution of a measured parameter (for example dry mass) within the cell. This spatial distribution can be calculated by the center of mass, the centroid of the cell, the skewness of the mass profile along a dimension of a cell or other parameters. Therefore, the spatial distribution of the measured parameters is a parameter for quality increasing the quality score of the sperm cell.

In yet other embodiments, the evaluation output is a quantitative score that describes chromosomal aberrations within the nucleus of the cell, such as Down's syndrome.

In yet another embodiment, the evaluation output is a quantitative score that describes DNA fragmentation within the cell.

It should be noted that, for example, the dry mass of the nucleus may be related to the amount of DNA within the nucleus. Variations in the amount of the nucleus weight indicate chromosomal aberrations or DNA fragmentation. It is known that fragmented DNA has a low chance of fertilization, thus by providing a "low score" to a sperm with abnormal dry mass (below or above a certain threshold), the technique of the present invention prevents selection of such cells for assisted reproduction. In addition, selection of cells without chromosomal aberrations reduces the occurrence of male caused genetic disorders in the offsprings, such as Down's syndrome, or death of the fetus. Another parameter that may be included in the score is the dry mass of the acrosome as well as its location and volume. As cells with a low acrosomal volume are also known to have DNA fragmentation, this additional information may be combined within the score. The polarization state or the birefringence distribution with a cell can also be used as a parameter.

In another embodiment of the invention, the evaluation output is a quantitative measure that relates to the sex of the sperm cell.

Reference is made to **Figs. 1B-1D** and tables 1 - 4, showing experimental data for exemplary OPD maps and measured parameters for sperm cells imaged.

In Fig. 1B, an OPD map of two sperm cells #1 and #2 is presented and used, for example, to determine the head width, head length, head area, head volume, Acrosome area and its fraction, Acrosome volume and its fraction, dry mass of the head, the acrosome and the nucleus. The quantitative results are shown in Tables 1-3.

Table 1:

Cell #	Head Width (um)	Head Length(um)	Head area(um^2)	Volume (n=1.35) (um^3)
1	2.9	5.1	11.62	32.52
2	3.3	4.7	12.18	38.98

Table 2:

Cell #	Acrosome	Acrosome	Fractional area	Fractional

	area(um^2)	Volume (n=1.35) (um^3)	%	volume %
1	4.40	1.32	38%	4%
2	5.70	1.37	47%	4%

Table 3:

Cell #	Dry mass of head (pgrams)	Dry mass of acrosome (pgrams)	Dry mass of nucleus
1	6.8	1.3	5.5
2	7.2	1.1	6.1

All dry mass calculations used the following formula:

$$drymass\ [\mu g] = (area\ [\mu m^2]) \times \frac{mean\ OPD\ over\ area\ [\mu m]}{1.8 \times 10^5 \left[\frac{\mu m^3}{\mu g}\right]}$$

The acrosome is identified in the area where the OPD per pixel is less then 300nm (or another threshold value) for live cells in medium with a refractive index of 1.33. Therefore, the relative area of the pixels with an OPD lower than 300nm (after filtering out the edges of the cells) may be calculated.

Fig. 1C exemplifies a sperm cell #3 with Vacuoles, the volume of vacuoles is calculated based on the radius inferred from the 2D image (assuming a sphere shape).

The values of the different physical parameters are shown in Table 4.

Table 4:

Cell #	Head area Volume (um^3)	Acrosome Volume (um^3)	Vacuoles Volume (um^3)
3	14.62	2.40	0.22

Based on all the volume measurements, a volume threshold for "potent" or "impotent" cell can be determined.

Additional analysis based on the topographic maps 120-150 may also include the following.

Determination of anterior and posterior sides of the cell's head (head alignment):

In order to determine which side of each head region is the anterior side and which is the posterior side, the mask is compared to the original mask that was produced by applying the threshold or edge detection to the OPD map (specifically, the mask is obtained by applying a morphological opening filter using for example disk shaped structuring element with radius between 18-21 pixels, thus eliminating the pixels in the previously acquired "mask" that represent the tails of the sperm cells, as well as various other pixels that represent random noise or small artifacts and debris). By comparing matching regions from both masks and determining their respective centroid, the inventors can determine which side of the head region is which. This is due to the significant shift of the centroid from the center of the head in the current mask towards the tail still present in the original mask. Based on the elliptical shape of the head, the inventors can then determine precisely where the anterior and posterior sides of the head are.

Determination of mean anterior-posterior difference:

The difference between the mean OPD values of the posterior and anterior halves of the head is calculated by taking the mean of the OPD value of the posterior half and subtracting from it the mean OPD value of the anterior half. This value acts as a basic indicator of acrosome presence as well as nucleus presence.

Isolation of acrosome and nucleus:

Using a threshold defined, for example, as the average value of , for example 5, pixels with the highest OPD value in the head multiplied by, for example 0.6, the nucleus is determined and isolated from the rest of the head. Holes in the two different regions of the head that are comprised of 50 pixels or less are removed in order to facilitate proper isolation and measurement of nucleus and acrosome. In order to isolate the acrosome from the periphery of the head morphological opening is used with a disc shaped structuring element whose radius is determined by subtracting the width of the head without the periphery from the total width of the head and dividing by 2. The acrosome is then isolated by selecting the largest suitable region remaining on the anterior half of the head.

Fig. 1D exemplifies how the OPD map is used to determine by algorithm the borders 122 and 124 of the acrosome and nucleus respectively.

As mentioned above, the topographic height map **140** is used in order to obtain some of the physical parameters which are then used to generate the quality score of the sperm cell. The height map is the physical thickness of each point in the sperm cell, and can be calculated using the OPD map and a corresponding refractive indices map. Below, there are described various methods for acquiring the refractive index map and building a database of characteristic refractive index map to be used with the OPD data in order to give the physical thickness/height needed for calculation of several physical parameters.

Reference is made to **Fig. 2** showing, in accordance with an embodiment of the present invention, a method **200** of quantitative analysis of unlabeled biological cell/organism, such as a sperm cell, by utilizing the topographic height map (i.e., the physical thickness). The method may be used for effective and improved selection of a label-free sperm cell for intra cytoplasmic sperm injection (ICSI).

The method concerns quantitative IPM-based analysis of label-free sperm cells, taking into account the physical thickness of the sperm in all of its points. The method includes generating a characteristic refractive index map (e.g. refractive index distribution along the sperm cell) determined on labeled sperm cell(s) (e.g. training set), and use of the characteristic refractive index map for analysis of label-free sperm cells. More specifically, the characteristic refractive index map may then used to calculate the real physical thickness of each point of any label-free sperm cell by only using a measured phase map of the label-free sperm cell (e.g. by acquiring an interferometric image of the label-free sperm cell). For determining the characteristic refractive index map, generally at least one labeled sperm cell can be used, or a training set of a plurality of labeled sperm cells can be used and data indicative of the plurality of the cells is processed, e.g. averaged.

The refractive index map, and consequently the characteristic refractive index map, may be acquired by utilizing different methods, as will be detailed below.

A first non-limiting example of a method for obtaining the refractive index map is illustrated in **steps 210** and **220** of **Fig. 2**. In the first **step 210**, a labeled sperm cell is immersed in a medium M1 having a known refractive index n_1 and imaged (e.g. using an interferometric system) to obtain phase data corresponding to optical thickness map. The complex wave front of the phase interference is recorded and an optical thickness map is acquired. The optical thickness is a function of the real physical thickness and the refractive index. The equation which describes this relation in this specific scenario is:

$\varphi_1(x,y)=2\pi/\lambda\cdot h(x,y)\cdot(n(x,y)-n_1)$;

where $\varphi_1(x,y)$ is the optical thickness recorded; λ is the light wavelength, h(x,y) is the physical thickness of the sperm cell and n(x,y) is the distribution of the sperm cell refractive indices in every point in the 2-D image.

As appreciated, if the labeled sperm cell is irradiated with light of the same light property(ies) (e.g. a single wavelength, and/or the same polarization state), this equation has two unknowns h(x,y) and n(x,y). In order to find the values of the unknowns, the labeled sperm cell is immersed in a second medium M2 (second **step 220**) having a refractive index n_2 and imaged to capture the optical thickness map (phase map) $\varphi_2(x,y)$, where:

$\varphi_2(x,y)=2\pi/\lambda\cdot h(x,y)\cdot(n(x,y)-n_2)$.

Then, the unknowns h(x,y) and n(x,y) can be found for each point (x, y) in the imaged sperm.

Alternatively and equally effective, instead of immersing the labeled sperm cell in two mediums having two known refractive indices, a second method for obtaining the refractive index map may include immersing the labeled sperm cell in one medium with a known refractive index n_0, and irradiating the labeled sperm cell twice, each time with light of different light properties, such as a different wavelengths or different polarizations, provided that the sample refractive index has a large/strong dependency in this specific light property. In this example, use of different wavelengths is considered. As appreciated, the unknowns are still h(x,y) and n(x,y), which can be found from the following set of equations:

$\varphi_1(x,y)=2\pi/\lambda_1\cdot h(x,y)\cdot(n(x,y,\lambda_1)-n_0)$;

$\varphi_2(x,y)=2\pi/\lambda_2\cdot h(x,y)\cdot(n(x,y,\lambda_2)-n_0)$.

When using the second method of changing the wavelength of the light, the two wavelengths may be in the close UV, where the dependency of n(x,y) and λ is strong. Alternatively, adding a dye to the medium may enhance the measurement so that n_0 will be strongly dependent on the wavelength.

A third non-limiting example of a method for obtaining the refractive index map is to utilize imaging of the labeled sperm cell by IPM with light of known optical properties, e.g. wavelength λ_0, while the cell is immersed in a medium having a known refractive index n_0, and obtaining data about the physical thickness h(x,y) using a system that can measure the physical thickness of the cell. Measuring physical thickness can be done by several known systems, such as Atomic Force Microscopy (AFM), Confocal Microscopy or Reflection interferometry. In this method, the only unknown in the equation $\varphi(x,y)=2\pi/\lambda_0 \cdot h(x,y) \cdot (n(x,y) - n_0)$ is the refractive index map n(x,y) (since h(x,y) is measured by another method), so it can be easily calculated.

In **step 230**, data indicative of the refractive index map of the labeled sperm cell is recorded as a characteristic refractive index map, and stored in a storage utility (database) to be accessed and used when measuring on label-free sperm samples. It is assumed that the refractive indices of the different organelles in the sperm cell have constant values in every sperm cell. **Steps 210** and **220** are performed using a labeled sperm cell in order to define the transverse locations of the different organelles in the cell and correlate between the location of the different organelles and the distribution of the calculated refractive indices n(x,y), such that each refractive index n(x,y) in the map/distribution corresponds to a specific organelle in the sperm cell.

In **step 240**, an unlabeled (label-free) sperm cell is measured. To this end, the unlabeled sperm cell may be immersed in a medium *M* of a known refractive index *n* (e.g. n1), is imaged with IPM, and an optical phase map φ(x,y) is measured. Similar to the above equations, the acquired optical phase map φ(x,y) is related to the real physical thickness h(x,y) of the sperm cell and the refractive index map n(x,y), as follows:

$$\varphi(x,y)=2\pi/\lambda \cdot h(x,y) \cdot (n(x,y) - n).$$

The refractive index map, n(x,y), is previously determined as described above and stored in the memory. The only one unknown in the equation is h(x,y), which thus can be calculated for every location (x1,y1) of a specific organelle, according to the n(x1,y1) value corresponding to the specific organelle, as was found. In other words, the thickness/height of every organelle in the label-free sperm cell can be calculated.

The so-obtained physical thickness distribution / map can then be used to obtain measured 3D morphology of the label-free sperm cell.

This thickness map data can for example be used to assess the sperm cell and its suitability for different clinical procedures, such as ICSI procedure.

Further, as shown in **Fig. 2**, additional quantitative analysis and image processing may be performed on the obtained phase image of the sperm cell. In **step 250** (optional), dimensions in the XY plane (transverse locations) in an acquired image of an organelle in the sperm cell can be combined with previously known morphological data, e.g. literature data, about the organelle in order to generate a three-dimensional computerized model of the organelle. This computerized 3D model of the organelle may be compared to the measured 3D morphological shape of the organelle that was obtained by the IPM measurements as described above, i.e. the physical thickness map of the specific organelle. This comparison may teach about the functionality of the sperm cell during an examination for IVF and ICSI procedures.

For example, the sperm cell neck may be examined. It is known that the neck of a sperm cell is cylindrical. By calculating the width W of the neck in XY plane in an acquired IPM image (for simplicity, if it is supposed that the sperm is aligned along the x axis with length X, then the neck width W is along the y axis), this width W is practically the transverse diameter of the cylindrical neck along the y axis. Supposing a perfect cylinder, then the physical depth along z axis should also be equal to W. A computerized cylindrical form of a diameter W and a length X can be constructed using specialized software. On the other side, the real 3D shape of the neck can be also formed by using the measured thickness map H(x,y). The computerized cylindrical form (3D model) and the measured 3D shape of the neck can be correlated and compared, using fitting software, in order to determine the healthiness of the sperm cell.

According to the invention, another diagnostic technique for the label-free sperm cell is based on creation of increased image contrast from sperm organelles, e.g. vacuoles, without labeling. Since different organelles have different refractive indices, by immersing the sperm cell in a medium of appropriate refractive index, it is possible to enhance the contrast for a specific organelle during imaging with IPM which would otherwise not provide desired image contrast. As a non-limiting example, it is known that the refractive index of the vacuoles is significantly lower than the refractive index of the other cellular contents. If the sperm cell is immersed in a medium having a refractive index higher than the refractive index of the vacuoles (plus the cellular material located above and below the vacuoles), the contrast in the vacuole location will be inverted and enhanced. In order to control contrast and induce distinctive contrast from the vacuole location, the sperm cell is immersed in a medium with a higher refractive index as compared to that of vacuole location. Thus, generally, the medium for immersing the sample therein for IPM measurements should preferably have a refractive index higher than at least that of the cell components having relatively low refractive index.

Referring to **Fig. 3**, there is provided an embodiment of a non limiting example of a device used for inducing contrast from various organelles in cells in general, and in sperm cells in particular, while the cells are being imaged. A flow chamber **10** includes an inlet container **20**, an outlet container **40** and a micro-channel **30** between them. Two switchable membrane gates **24** and **44**, separate between the inlet container **20** and the micro-channel **30** and between the micro-channel **30** and the outlet container **40**, respectively. Plurality of sperm cells is entered into the inlet container **20** through an inlet **22**. At a time, one sperm cell flows into the micro-channel **30** and the switchable gate **24** closes such that the sperm cell is trapped and no other sperm cell exists in the micro-channel. The switchable gate **44** is also closed at this time to prevent the sperm cell from exiting the micro-channel **30**. When in the micro-channel **30**, the sperm cell is imaged by any method or means desired, specifically by IPM. The switchable membrane gates **24** and **44** are permissible to medium flow but not to cells. The medium through the whole flow chamber can thus be altered and substituted (i.e. the refractive index of the medium is changed) in order to enhance contrast for specific organelles in the cell, according to the description above. Alternatively, the medium may be changed only inside the micro-channel **30**, through a flow circuit connected only to it (not shown); in this case, the switchable gates **24** and **44** are preferably not permissible to any flow through them.

The same sperm cell may be imaged several times inside the micro-channel **30**, each time while surrounded by a different medium enhancing a different organelle of the sperm cell. If the sperm cell is label-free, it can be tested for ICSI eligibility. The flow chamber **10** may be used to image the sperm cell for real time 3D morphology evaluation as described earlier. After finishing with imaging, the switchable membrane gate **44** is opened (while the switchable membrane gate **24** is closed), and the sperm cell moves into the outlet container **40**. Based on the real-time analysis of the sperm cell, it may be selected to flow through the exit **46**, if it is chosen for ICSI or any relevant procedure, or otherwise flow through the exit **42** as not suitable for the procedure.

Fig. 4 illustrates a general, non-limiting, example of a system **50** utilizing the present invention. The system **50** includes a computerized system **70** configured according to the invention for executing the steps of methods 100 and 200, e.g. processing and analyzing phase image data of a biological organism and determining data indicative of the morphology of the organism. The phase image data may be input into system **70** from a phase imaging system, such as Interferometric Phase Microscopy system (IPM) **60** or from a storage device where such image data has been previously stored. Optionally, the system **50** may also include the flow chamber **10** depicted in **Fig. 2**. Alternatively, the system **50** may be connected to the flow chamber **10** or any other platform designed for accommodating biological samples and specifically sperm cells.

The IPM setup **60** is well known in the art and therefore need not be described in details. Usually, an IPM system **60** includes a conventional microscope **62** and an interferometry device **64** connected to the microscope. A camera **66** may be integral with or added to the IPM system. One example of an IPM system suitable to be used in the invention has been developed by the co-inventor of the present application and is described in PCT patent application number WO 2013/140396, titled "Portable interferometric device". This system includes a conventional inverted microscope and a portable interferometric module connected between the output of the microscope and a conventional digital camera.

The computerized system **70**, while connected to the IPM system **60** or storage device **82** as the case may be, receives the measured image data for performing signal processing, image digital analysis and quantitative analysis on the measured data according to the invention. In addition, the computerized system **70** may control the operation of the IPM system **60** and/or the optionally connected flow chamber **10** (via an appropriate controller utility, which is not specifically shown) as will be further detailed below. Generally, the computerized system **70** includes a communication utility **72** being in data communication (e.g. via wires or wireless signal transmission) with the IPM system **60** and/or storage device **82** for receiving measured data (phase image data, dry mass data, birefringence data and data about the refractive index of the medium in which the sperm cells are immersed) relating to label-free biological organism; a data processor and analyzer **76** (e.g., a CPU) that analyzes the measured data as described above. It should be noted that system **70** is also configured for accessing memory (internal or external) to obtain the characteristic refractive index map (previously prepared and stored as described above). The system **70** may also be configured to control the measurements (e.g., according to predefined parameters) performed by the IPM system **60** and/or the flow chamber **10**. The system **70** also typically includes a memory utility (module) **74** which saves, *inter alia*, the received measured data and the results of analysis of the data, and may for example also store the topographic maps (OPD, dry mass, height, birefringence), and characteristic refractive index map obtained according to the method described above. The computerized system **70** may be a conventional computer including a conventional image and signal processing software, or it may be a specialized system running a specific firmware designed to execute the measurements, processing, and analysis according to the invention. The computerized system **70** may be constituted by an application program interface (API) installable on any suitable computer device and being capable of processing received image data, using the topographic maps data and the characteristic refractive index map data, which is either stored in the device or obtained by the API via accessing the remote storage device via a communication network. Thus, generally, the computerized system **70** may be associated with a separate device or may be integrated with either the IPM **60** or the flow chamber **10**.

The analyzer unit (module) **76** may be programmed to directly extract information of the measured data received from the IPM system (e.g. the digital camera), process the extracted information data and generate an output data corresponding to differential phase contrast (DIC) and dark field images of the sperm cell, based on the IPM images, completely digitally, without additional equipment. As described above, DIC is the basis of sperm organelle morphology examination (MSOME), which cannot be or it is hard to be implemented in most fertility clinics due to the cost of equipment. The generation of the DIC and/or the dark field images may be done by software which is implemented in the computerized system **70**.

The computerized system **70** may optionally include or be connected to a display / monitor **80**, to present relevant and useful data, including the images and the data processed by the system, to a user (e.g., a clinician) during an examination/selection procedure.

If a flow chamber **10** is connected to the computerized system **70**, the computerized system **70** may control all the operations of the opening and closure of the switchable membrane gates **24** and **44** as well as the flow of the fluid/liquid inside the chamber and the substitution of the different fluids/liquids (controlling the flow circuit) for enhancing the contrast of specific organelles. If no flow chamber **10** is used, then the sperm sample may be accommodated on a microscope slide in a conventional way.

Every operation of the system **50** may be performed on-line in real time, such as during a selection procedure using the flow chamber **10**, or offline.

CLAIMS:

1. A method for use in sperm analysis, the method comprising processing measured data comprising at least interferometric phase data of a label-free sperm cell, said processing of the measured data comprising determining topographic optical phase delay (OPD) map of the label-free sperm cell, determining at least one physical parameter of the label-free sperm cell, and generating data indicative of sperm quality for the label-free sperm cell.

2. The method of claim 1, wherein the processing of the measured data further comprises generating a topographic dry mass map of the label-free sperm cell.

3. The method of claim 1 or 2, wherein the processing of the measured data further comprises generating a topographic height map of the label-free sperm cell.

4. The method of claim 3, wherein the generating of the topographic height map comprises utilizing a refractive index map being characteristic of sperm cells.

5. The method of any of the preceding claims, wherein the measured data comprises a birefringence image data, and the processing of the measured data comprises generating a topographic birefringence map of the label-free sperm cell.

6. The method of any of the preceding claims, wherein said at least one physical parameter comprises at least one of the following: head area; total volume of the head; width and length of the head; relative area or volume of the acrosome and nucleus within the head; total dry mass of the head; dry mass of the acrosome and nucleus; volume of vacuoles within the head, and their relative volume; centroid and weighted centroid of each head region and the distance between them; Mean OPD of each head region; variance or standard deviation of each head region; Mean anterior – posterior difference; midpiece width; midpiece length; tail length; presence of cytoplasmic droplets; tail form and head form.

7. The method of any of the preceding claims, wherein said data indicative of sperm quality for the label-free sperm cell comprises sperm fertility quality data.

8. The method of any of the preceding claims, wherein said data indicative of sperm quality for the label-free sperm cell comprises a quantitative quality score.

9. The method of any of the preceding claims, comprising processing of the measured data of a plurality of label-free sperm cells, and building a training data set comprising said at least one physical parameter for normal and abnormal sperm cells.

10. The method of claim 9, wherein said training data set comprises data indicative of at least one of spatial, spectral and polarization state and birefringence distribution within a sperm cell.

11. The method of claim 9 or 10, comprising determining said data indicative of sperm quality being indicative of a plurality of physical parameters by comparing measured physical parameters with physical parameters of said training data set.

12. The method of any of the preceding claims, wherein said sperm quality is indicative of at least one of chromosomal aberrations within a nucleus of the cell, DNA fragmentation within the cell and sex of the sperm cell.

13. The method of any one of claims 4 to 12, comprising obtaining said refractive index map by processing image data of at least one labeled sperm cell.

14. The method of claim 13, wherein said processing of the image data indicative of the at least one labeled sperm cell comprises calculating a sperm refractive index map corresponding to different points in the labeled sperm cell, and generating and storing data indicative of a characteristic sperm refractive index map of said different points in the labeled sperm cell to thereby determine properties of a label-free sperm cell.

15. The method of claim 14, wherein said properties of a label-free sperm cell comprise morphological parameters of the label-free sperm cell.

16. The method of claim 14 or 15, wherein said different points in the labeled sperm cell correspond to at least one of the following cellular compartments of the labeled sperm cell: vacuoles, nucleus, acrosome, head, neck region and tail.

17. The method of any one of claims 13 to 16, comprising imaging the at least one labeled sperm cell and obtaining the image data.

18. The method of claim 17, comprising imaging the labeled sperm cell with interferometric phase microscopy (IPM), while sequentially immersing said at least one labeled sperm cell in a first medium having a first refractive index and in a second medium having a second different refractive index, said image data comprising a first and second image data indicative of first and second phase map data, respectively.

19. The method of claim 17, comprising imaging the labeled sperm cell with interferometric phase microscopy (IPM), while immersing said at least one labeled sperm cell in a medium with a known refractive index and sequentially illuminating said at least one labeled sperm cell with light having first and second different light properties, said image data comprising first and second image data indicative of first and second phase map data, respectively.

20. The method of claim 19, wherein the light property comprises at least one of wavelength and polarization.

21. The method of claim 17, comprising imaging the labeled sperm cell with interferometric phase microscopy (IPM), while immersing said at least one labeled sperm cell in a medium with a known refractive index, said image data comprises a first image data indicative of a phase map data and a second image data indicative of physical thickness of said different points in the labeled sperm cell.

22. The method of any one of the claims 13 to 21, comprising receiving and processing of the image data indicative of a plurality of labeled sperm cells, building a training data set comprising a plurality of sperm refractive index maps corresponding to the plurality of labeled sperm cells, and determining a characteristic sperm refractive index map from said plurality of the sperm refractive index maps.

23. The method of claim 22, comprising determining said characteristic sperm refractive index map by averaging said plurality of sperm refractive index maps.

24. The method of any one of claims 2 to 23, comprising analyzing said topographical height map data and determining 3D morphology of the label-free sperm cell based on physical thickness distribution.

25. The method of claim 24, comprising evaluating the measured 3D morphology of the label-free sperm cell by correlating between it and theoretical data indicative of a computerized 3D morphology model of the label-free sperm cell.

26. The method of claim 25, wherein the computerized 3D morphology is based on at least one characteristic dimension in an image of the label-free sperm cell.

27. The method of claim 26, wherein said measured data of the label-free sperm cell comprises IPM data.

28. A method for use in sperm analysis, the method comprising, processing image data indicative of at least one labeled sperm cell, calculating a sperm refractive index map corresponding to different points in the labeled sperm cell, generating and storing data indicative of a characteristic sperm refractive index map of said different points in the labeled sperm cell to thereby determine properties of a label-free sperm cell.

29. The method of claim 28, wherein said characteristic sperm refractive index map is obtained by carrying out the method of any one of claims 18 to23.

30. A method for use in analysis of a biological organism, the method comprising imaging at least one labeled biological organism and obtaining image data, processing the image data and calculating a refractive index map corresponding to different points in the labeled biological organism, and generating and storing data indicative of a characteristic refractive index map and of said different points in the labeled biological organism to be used for determining properties of a label-free biological organism.

31. A computerized system for use in sperm analysis, the system comprising: a communication utility for receiving interferometric measured data of a sperm cell; and a processor utility configured and operable for processing said measured data and determining at least one physical parameter of said sperm cell, said processing comprising determining topographic optical phase delay (OPD) map of the label-free sperm cell, determining at least one physical parameter of the label-free sperm cell, and generating data indicative of sperm quality for the label-free sperm cell.

32. The computerized system of claim 31, wherein said data indicative of sperm quality for the label-free sperm cell comprises sperm fertility quality data.

33. The computerized system of claim 31 or 32, wherein said data indicative of sperm quality for the label-free sperm cell comprises a quantitative quality score.

34. The computerized system of any of claims 31 to 33, wherein said processor utility is further configured and operable for utilizing the measured data and a characteristic refractive index map of a sperm cell, stored in a memory, calculating a physical thickness distribution of different points in said measured label-free sperm cell, and generating topographic height map for the label-free sperm cell.

35. The computerized system of any of claims 31 to 34, being configured to control a flow chamber configured and operable to trap said sperm cell and to allow imaging thereof.

36. The computerized system of claim 35, wherein said processor utility is configured and operable to control at least one of operation of said flow chamber, flow of the fluid inside the chamber, and substitution of different fluids for enhancing contrast of said sperm cell.

37. The computerized system of any one of claims 31 to 36, comprising a memory utility configured and operable to store at least one of said measured data, results of analysis of the measured data, and characteristic refractive index map of said measured data.

38. A device for use in analysis and selection of cells, comprising a flow chamber comprising a micro-channel configured and operable to allow applying analysis and imaging to a cell flowing in a medium thereinside, and two switchable gates at both sides of the micro-channel configured and operable to trap a single cell at a time inside said micro-channel.

39. The device of claim 38, further comprising a flow circuit configured and operable to substitute the medium inside said micro-channel while the cell thereinside, thereby enabling enhancing image contrast for specific organelles in the cell.

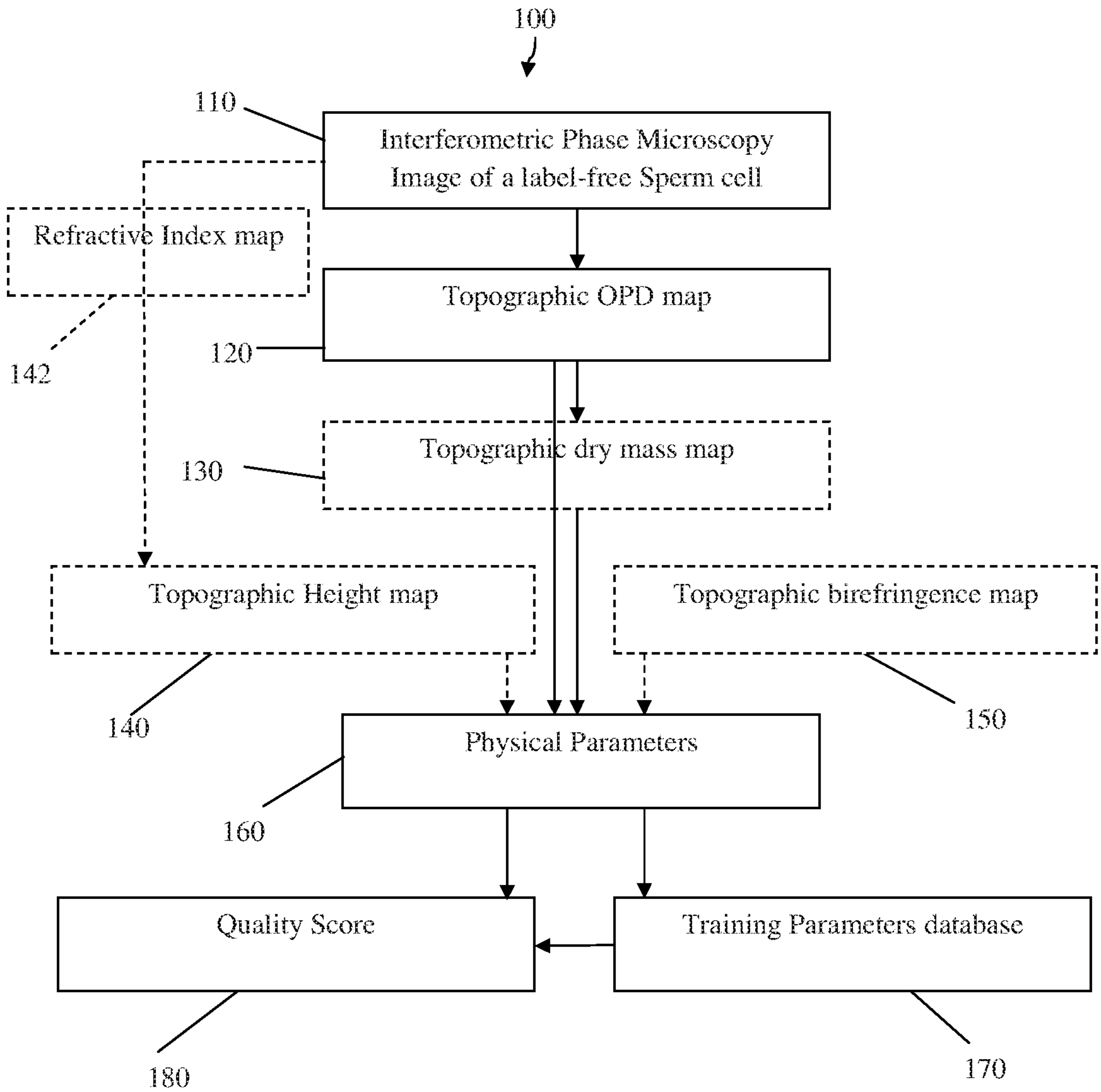
100
110
Interferometric Phase Microscopy Image of a label-free Sperm cell
Refractive Index map
142
Topographic OPD map
120
Topographic dry mass map
130
Topographic Height map
Topographic birefringence map
140
150
Physical Parameters
160
Quality Score
Training Parameters database
180
170

Fig 1A

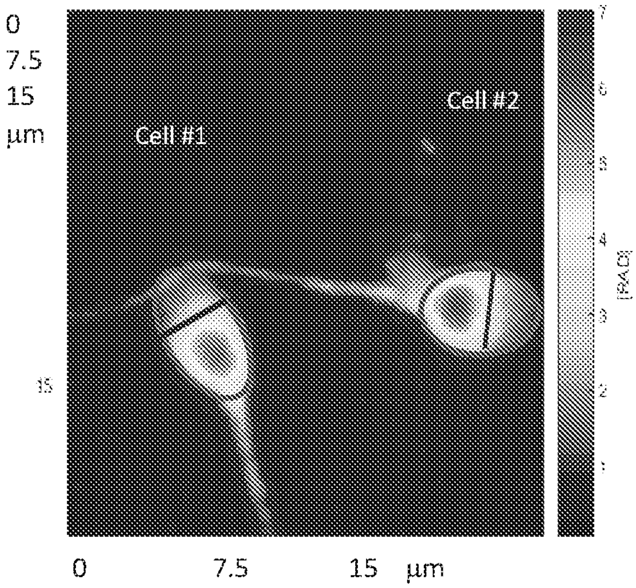

Fig. 1B

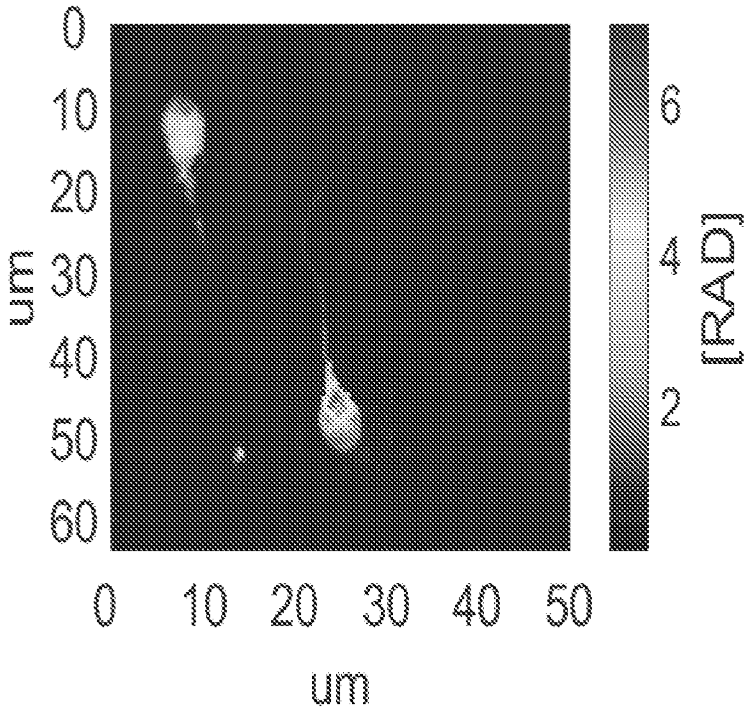

Fig. 1C

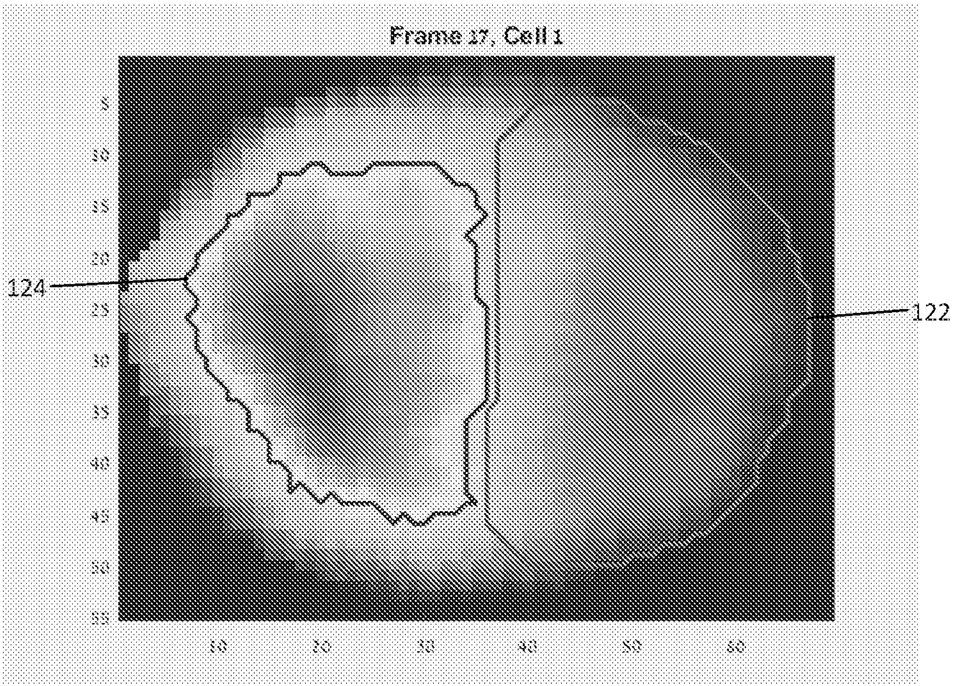
Frame 17, Cell 1
5
10
15
20
25
30
35
40
45
50
55
10
20
30
40
50
60
124
122

Fig. 1D

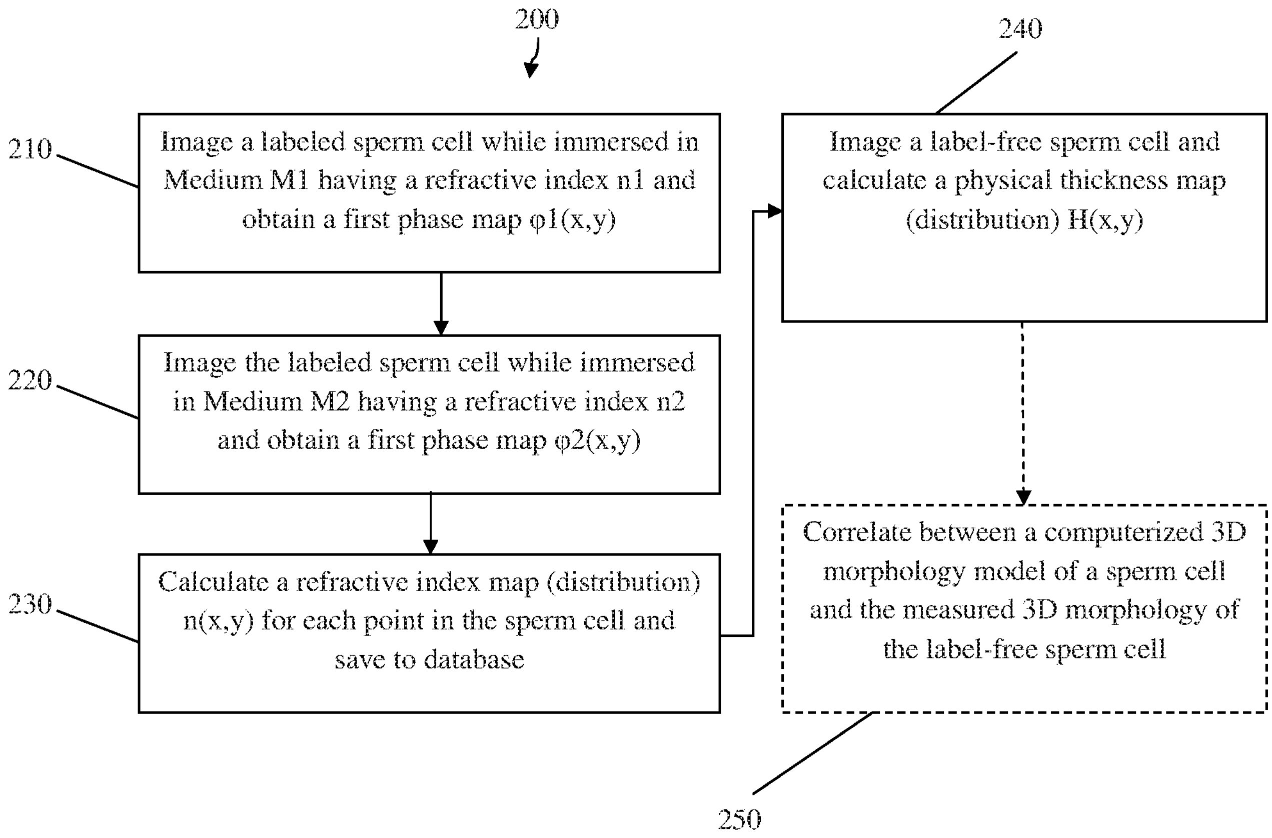
200
240
210
Image a labeled sperm cell while immersed in Medium M1 having a refractive index n1 and obtain a first phase map φ1(x,y)
Image a label-free sperm cell and calculate a physical thickness map (distribution) H(x,y)
220
Image the labeled sperm cell while immersed in Medium M2 having a refractive index n2 and obtain a first phase map φ2(x,y)
230
Calculate a refractive index map (distribution) n(x,y) for each point in the sperm cell and save to database
Correlate between a computerized 3D morphology model of a sperm cell and the measured 3D morphology of the label-free sperm cell
250

Fig 2

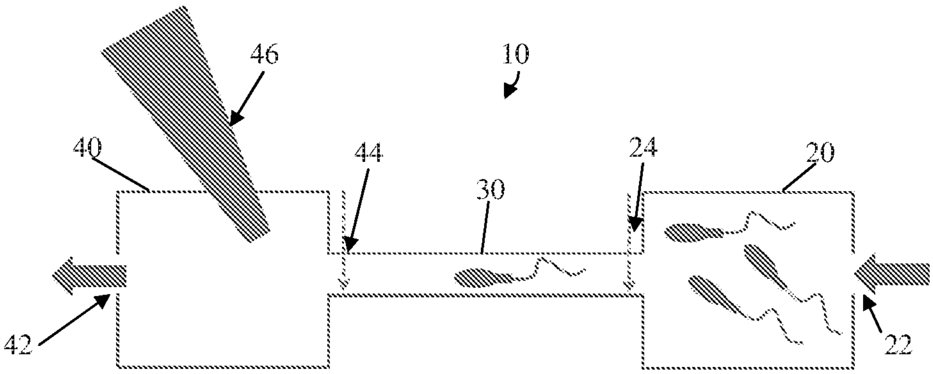
46
10
40
44
24
20
30
42
22

Fig. 3

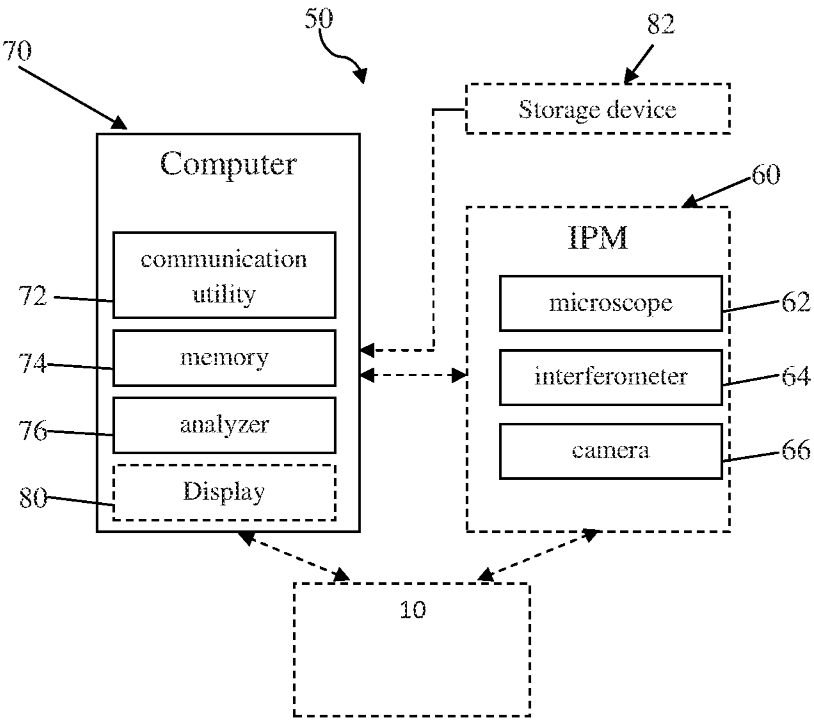
50
82
70
Storage device
Computer
60
IPM
communication utility
72
microscope
62
74
memory
interferometer
64
76
analyzer
camera
66
80
Display
10

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2016/050478

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2016.01) G01N 33/487, G01N 21/00, G01N 15/02, G01N 33/48, G01N 21/88

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)
IPC (2016.01) G01N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Digital Holographic Microscopy in Human Sperm Imaging." Journal of Assisted Reproduction and Genetics 28.8 (2011): 725–729. Crha, Igor et al. 28 Aug 2011 (2011/08/28) the whole document	1-30
X	US 8842901 B2 OZCAN AYDOGAN, ; ERLINGER ANTHONY F, ; SU TING-WEI, ; THE REGENTS OF THE UNIVERSITY OF CALIFORNIA 23 Sep 2014 (2014/09/23) the whole document	1-37
X	JP 2005521425 A FLUIDIGM CORPORATION 21 Jul 2005 (2005/07/21) the whole document	38,39
A	"4D tracking of clinical seminal samples for quantitative characterization of motility parameters," Biomed. Opt. Express 5, 690-700 (2014); Di Caprio, Giuseppe et al 11 Feb 2014 (2014/02/11) the whole document	1-39

☐ 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
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 Aug 2016	25 Aug 2016

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	KASHEVAROV Alexandra AlexandraKa@justice.gov.il Telephone No. 972-2-5657825

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PCT/IL2016/050478

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PCT/IL2016/050478

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PCT/IL2016/050478

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B. FIELDS SEARCHED:

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

Databases consulted: Google Patents, Google Scholar, FamPat database
Search terms used: EN_DE:(quality microscop*) AND EN_DE:(live label-free sperm selection) AND EN_CL:(at least one physical parameter)

((SPERM S ANALYSIS)/CLMS/DESC/ODES/OBJ AND (REFRACTIVE S INDEX)/CLMS/DESC/ODES AND (BIREFRINGENCE)/DESC/ODES AND (QUALITY)/DESC/ODES/CLMS AND (PHYSICAL S PARAMETER+)/CLMS/DESC/ODES)

EN_DE:aberration* AND EN_DE:icsi AND EN_DE:("sperm image"~5)

icsi sperm semen quality image topograph*

en?q=icsi&q=sperm&q=gate&q=quality&q=micro&q=flow&q=interfer&q=imaging&scholar

phase contrast microscopy sperm ICSI quality device image processing