

### Motile cell analysis

5 The present invention relates to systems and methods for analysing motile cells such as spermatozoa.

Existing fertility treatments range from lifestyle changes and medicinal interventions to assisted reproductive technology (ART) procedures such as in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). At all levels of fertility treatment, it is often necessary to  
10 analyse spermatozoa (i.e. motile sperm cells). The analysis may be used to assess sperm health, to assess the effectiveness of treatment, or to identify the best candidate cells for an ART procedure.

Conventionally, spermatozoa are analysed manually by an expert reviewing microscopy images of a semen sample to assess qualitatively the morphology and motility of the cells. This analysis  
15 is more difficult when specific spermatozoa are being selected for use in an ART procedure because fluorescent dyes that may aid quality assessment cannot be used.

In fact it has generally proven difficult to assess reliably the morphology and motility of a large  
20 range of motile cells. An improved approach may be desired.

According to a first aspect of the present invention there is provided a computer-implemented method for analysing a sample comprising a motile cell comprising at least a head portion, the method comprising:

25 receiving a time series of images of the sample, the images of the sample having a first spatial resolution;

producing a time series of images of the cell by identifying a section of each image of the sample which contains the cell;

30 applying a first machine-learning algorithm to said images of the cell to generate an enhanced image of the cell having a second spatial resolution that is numerically smaller than the first spatial resolution;

applying a second machine-learning algorithm to said enhanced image of the cell to determine one or more morphological parameters of the cell;

35 applying a motion tracing algorithm to the time series of images of the sample to determine a motion trace of the head portion of the cell; and

using the one or more morphological parameters of the cell and the motion trace of the head portion of the cell to determine a quality metric for the cell.

- 2 -

According to a second aspect of the present invention there is provided a system for analysing a sample comprising a motile cell comprising at least a head portion, the system comprising:

an input interface arranged to receiving image data comprising a time series of images

5 of the sample, the images of the sample having a first spatial resolution; and

a processing apparatus arranged to:

produce a time series of images of the cell by identifying a section of each image of the sample which contains the cell;

apply a first machine-learning algorithm to said images of the cell to generate an

10 enhanced image of the cell having a second spatial resolution that is numerically smaller than the first spatial resolution;

apply a second machine-learning algorithm to said enhanced image of the cell to determine one or more morphological parameters of the cell;

apply a motion tracing algorithm to the time series of images of the sample to

15 determine a motion trace of the head portion of the cell; and

use the one or more morphological parameters of the cell and the motion trace of the head portion of the cell to determine a quality metric for the cell.

Thus, it will be appreciated by those skilled in the art, that embodiments of the present invention

20 can facilitate convenient quality analysis of motile cells. Combining a motion trace of the head portion of the cell with one or more morphological parameters of the cell can produce more accurate and reliable results than previous qualitative analysis techniques. Moreover, because the motion trace is determined using a separate algorithm to those used to determine the one or more morphological parameters of the cell, each of these algorithms can be optimised

25 separately (e.g. with suitable training), improving the accuracy and reliability of the resulting quality metric.

Because embodiments of the invention use the first machine-learning algorithm to generate an enhanced image with a smaller spatial resolution (i.e. a smaller minimum resolvable feature

30 size) than the input data (i.e. the images of the sample), accurate morphology information can be obtained without needing to directly image each cell with a very small spatial resolution. Imaging equipment used to capture the images of the sample may thus be made less expensive and/or smaller, and/or a wider field of view of the sample may be imaged at the same time (i.e. allowing for the analysis to cover more of the sample). Moreover, the quantity of data needing to

35 be handled in many parts of the analysis may be reduced, with corresponding reductions in necessary processing and/or data transfer resources. Some parts of the analysis (e.g. the

- 3 -

motion tracing algorithm) may even be better optimised when applied to relatively low-resolution image data (e.g. where each cell may be easily approximated as a point-like object).

The present method may be suitable for analysing a variety of motile cells having head portions. In some embodiments the motile cell also comprises a tail portion (e.g. a flagellum or cilium). In some embodiments the motile cell may comprise a midpiece portion between the head portion and the tail portion. Motile cells suitable for analysis with methods disclosed herein include prokaryotic cells such as bacteria and eukaryotic cells such as spermatozoa. In a set of embodiments the motile cell is a human spermatozoon. In other words, some embodiments of the invention may be used to analyse a sample comprising one or more human spermatozoa.

In a set of embodiments, the sample comprises a plurality of motile cells, each comprising at least a head portion (and optionally comprising a tail portion and/or a midpiece portion). Some embodiments may involve producing a time series of images of each cell (i.e. a plurality of time series corresponding to the plurality of cells), by identifying a sections of each image of the sample which contain each cell. Some embodiments may involve applying the first machine-learning algorithm to each of the plurality of time series of images of the cells to generate an enhanced image of each cell having a second spatial resolution that is numerically smaller than the first spatial resolution. Some embodiments may involve applying the second machine-learning algorithm to each enhanced image to determine one or more morphological parameters of each cell. Some embodiments may involve applying the motion tracing algorithm to the time series to determine a motion trace of the head portion of each cell. Some embodiments may involve for each cell, using the one or more morphological parameters of the cell and the motion trace of the cell to determine a quality metric for the cell (i.e. to determine a quality metric for each of the plurality of motile cells in the sample).

The motion tracing algorithm may comprise any suitable motion tracing algorithm known in the art *per se*. However, in a set of embodiments, the motion tracing algorithm is based on statistical analysis of intensity fluctuations in the time series of images of the sample (i.e. fluctuations caused at least partially by cell movement). In a set of embodiments the motion tracing algorithm comprises the Multiple Signal Classification Algorithm (MUSICAL). The motion tracing algorithm may comprise:

selecting from the time series of images a plurality of windows, each window comprising a respective stack of spatially-coincident sections of the images;

for each window:

decomposing the window into eigenimages and corresponding values;

- 4 -

calculating, for each of a plurality of test points in the window, a respective first value as a first function of the eigenimages;

calculating, for each of the plurality of test points in the window, a respective second value as a second function of the eigenimages; and

5 determining, from the first and second values, a respective indicator value for each test point in the window, representative of a likelihood of the cell being present at a location corresponding to the test point; and

combining the indicator values to produce an output image comprising a motion trace of the cell through the time series of images.

10

The light emitted from the sample (i.e. from a cell in the sample) has an intensity pattern that depends not only upon the physical distribution of particles within the sample, but also upon factors that may change over time including the movement of the cell. As a result of the movement, the different images in the time series contain different intensity patterns. However, 15 the intensity pattern also varies due to noise (e.g. shot noise, quantization noise, dark current noise). However, with suitable processing, the temporally-varying intensity patterns can be used to determine the motion trace of the cell.

20

The motion tracing algorithm may be at least partially parallelised. In a set of embodiments the motion tracing algorithm comprises performing at least one of the decomposing, calculating or determining steps for a first window of the plurality of windows by a first processor at the same time as performing at least one of the decomposing, calculating or determining steps for a second window of the plurality of windows by a second processor.

25

The time series of images can be described as a “stack” of images. Each window preferably spans the full depth (i.e. time axis) of the stack. The plurality of windows preferably collectively includes all of the data in the image stack (i.e. every part of the image stack is included in at least one window).

30

The respective spatially-coincident sections of the plurality of windows may have the same shape and/or size (i.e. in the image plane). In some embodiments the sections are all squares. They may all be equal-sized squares. Thus a window may comprise a spatially-aligned square section of each image. Because all the image sections of any window are spatially coincident (i.e. have the same position within each image of the stack), each window may correspond to a 35 different respective area of the sample region over time. The plurality of windows may overlap. In some embodiments, the windows are selected to each have a size of at least half of a point spread function of an imaging apparatus that captured the stack of images, and preferably the

- 5 -

windows each have a size of between one and two times the point spread function of the imaging apparatus that captured the stack of images. In some embodiments the windows are rectangles having sides each at least three pixels in length, and preferably at least five pixels in length. This may avoid artefacts associated with discretization errors.

5

In a set of embodiments, each image in the stack of images comprises a plurality of pixels, and a respective window is selected centred on each of the pixels of an image of the stack of images. In other words, the plurality of windows may comprise as many windows as there are pixels in each image of the stack of images. In some embodiments, some of the windows may extend beyond the edge of the image data (e.g. windows centred on an edge pixel). In such

10

embodiments empty portions of these windows may be filled with a predetermined pixel value, or an average (e.g. mean) of the image data pixels in that window, or a floor value of the image data pixels in that window.

15

The eigenimages into which each window is decomposed may represent particular patterns of intensity found in the stack of images, with the corresponding values indicating the relative prominence of each pattern in the stack. For example, a large value may indicate that the pattern of the corresponding eigenimage is a prominent pattern in the image stack (i.e. one which is more likely to be due to inherent structure in the sample than due to random noise).

20

Decomposing one or more of the windows into eigenimages and corresponding values may comprise a singular value decomposition (SVD) process. Additionally or alternatively, decomposing one or more of the windows into eigenimages and corresponding values may comprise an eigenvalue decomposition (ED) process. The corresponding values may be

25

singular values or eigenvalues.

30

In some sets of embodiments, each first value represents a contribution to its respective test point of one or more signal eigenimages (i.e. those which represent patterns that are prominent in the image stack). Each second value may represent a contribution to its respective test point of one or more noise eigenimages (i.e. those patterns that are not prominent in the image stack). Accordingly, in some embodiments the first and/or second function includes a projection operation.

35

In some embodiments, the first value consists of a contribution to the test point of a single eigenimage and/or the second value consists of a contribution to the test point of a single eigenimage. In such embodiments, determining the respective indicator value for each test point may comprise combining a plurality of first values and/or combining a plurality of second values.

- 6 -

Alternatively, in some embodiments, each first value and/or each second value comprises a combination of contributions to the test point of a plurality of eigenimages. In other words, the method may comprise combining contributions from eigenimages in a calculating step or in the determining step.

5

In some embodiments it may be useful to apply different weights to the contributions from different eigenimages. Accordingly, some embodiments comprise applying a first weight function and/or a second weight function to eigenimages of the window. The weight functions may be applied in a calculating step or the determining step. In embodiments where each first value and/or each second value comprises a combination of contributions to the test point of a plurality of eigenimages (i.e. where the combining happens in the calculating step), calculating each first value may include applying a first weight function which weights eigenvalue contributions to the first value (e.g. the first value comprises a weighted sum of eigenvalue contributions defined by the first weight function). Similarly, calculating each second value may include a second weight function which weights eigenvalue contributions to the second value (e.g. the second value comprises a weighted sum of eigenvalue contributions defined by the second weight function).

Alternatively, in embodiments where the first value consists of a contribution to the test point of a single eigenimage and/or the second value consists of a contribution to the test point of a single eigenimage (e.g. where the combining happens in the determining step), determining the respective indicator value for a test point may comprise combining a plurality of first values using a first weight function (e.g. as a weighted sum defined by the first weight function) and/or combining a plurality of second values using a second weight function (e.g. as a weighted sum defined by the second weight function).

In some embodiments, calculating the first and/or second value may comprise first projecting a point spread function (PSF) of the respective test point onto several eigenimages, and then calculating a weighted sum of the projection results according to the corresponding first and/or second weight function. Alternatively, calculating the first and/or second value may comprise first applying a weight to a plurality of eigenimages, projecting a PSF of the respective test point onto the weighted eigenimages and then simply summing the result without further weighting.

The first and/or second weight functions may be a function of one or more properties of the eigenimages or one or more properties related to the eigenimages. In a set of embodiments, the first and/or second weight function is a function of the corresponding values of the eigenimages.

- 7 -

In other words, the weight applied to the contribution of an eigenimage may be determined based on the value corresponding to that eigenimage.

5 Weighting contributions from eigenimages may include assigning a weight of zero to one or more eigenimages, i.e. excluding that eigenimage from contributing to the first/second value. Weighting contributions from eigenimages may include assigning a weight of one to one or more eigenimages, i.e. selecting fully that eigenimage to contribute to the first/second value. In other words, the first and/or second weight function may be equal to one or zero for one or more eigenimages (e.g. for ranges of corresponding values).

10 In some embodiments, the first weight function selects fully (i.e. applies a weight of one) contributions from a first set of eigenimages that satisfy a first condition or a set of first conditions, and excludes (i.e. applies a weight of zero) other eigenimages. The second weight function may select fully contributions from a second set of eigenimages that satisfy a second condition or a set of second conditions, and exclude other eigenimages. The first and second condition(s) may be different. The first and second sets may be non-overlapping (i.e. with no eigenimages appearing in both sets). For instance, the first and second weight functions may effectively split the eigenimages into two sets, with one set used to calculate the first value(s) for each test point and the other set used to calculate the second value(s) for each test point. In  
15 some embodiments one or more of the eigenimages is not included in either set.  
20

The first and/or second conditions may comprise a minimum or maximum threshold to which a property of the eigenimage or a property related to the eigenimage (e.g. its corresponding value) is compared. For instance, the first weight function may select all eigenimages with a  
25 corresponding value above a first threshold value, and/or the second weight function may select all eigenimages with a corresponding value below a second threshold value (i.e. the first and/or weight second functions may comprise step functions with a value of zero on one side of the first/second threshold and a value of one on the other side of the first/second threshold). In other words, in a set of embodiments, calculating the first value for each test point may  
30 comprise calculating a first contribution to the test point of the eigenimages of the window that have a corresponding value greater than a first threshold value. Additionally or alternatively, calculating the second value for each test point may comprise calculating a second contribution to the test point of the eigenimages of the window that have a corresponding value less than a second threshold value.

35 The first and second thresholds may be equal, e.g. such that the first and second weight functions are step functions that are inverted versions of each other. In such embodiments the

- 8 -

eigenimages are effectively divided between the first and second sets about a common threshold value. In such embodiments any eigenimages of a window that have a corresponding value equal to the common threshold value (i.e. not greater than or less than) may be included with the first set (or, alternatively, in other embodiments, may be included with the second set).

5 In other words, in a set of embodiments, calculating the first value for each test point may comprise calculating a first contribution to the test point of the eigenimages of the window that have a corresponding value greater than a common threshold value. Additionally or alternatively, calculating the second value for each test point may comprise calculating a second contribution to the test point of the eigenimages of the window that have a

10 corresponding value less than a common threshold value. Eigenimages of the window that have a corresponding singular value equal to the common threshold value may be used for calculating the first value, the second value, neither value or both values.

The first and/or second weight functions may be discontinuous or continuous. The first and/or

15 second weight functions may include linear expressions, polynomial expressions, exponential expressions and/or logarithm expressions. The weight functions may have different parameters and/or forms for different input ranges (e.g. different ranges of corresponding values). The first and/or second weight function may be defined partially or entirely by a look-up table. For instance, the first and/or second weight functions may be defined by a list of values or ranges of

20 values and corresponding weights (e.g. in a look-up table). The weight functions may be defined graphically by a user (e.g. by manually drawing a weighting curve using a user interface such as a touch screen).

In some embodiments, calculating the first and second values for a window comprises

25 multiplying a test matrix, comprising the PSFs for the test points in that window, with the eigenvalues of that window to produce a projection matrix.

The first and second values may then be calculated by summing elements of the projection matrix according to first and second weight functions as discussed above. This process allows

30 all of the first and second values for a given window to be calculated using a single set of matrix manipulations, which may be more efficient than individual calculations for each test point. For instance, in embodiments where the first weight function is a step function defined by a first value threshold, the first values may be calculated by summing those elements of the projection matrix that have corresponding values greater than the first value threshold. In embodiments

35 where the second weight function is a step function defined by a second value threshold, the second values may be calculated by summing those elements of the projection matrix that have corresponding values less than the second singular value threshold.

5 In embodiments where the when the first and second weight functions are both step functions and the first and second value thresholds are equal, it will be appreciated that the projection matrix is effectively divided into two sections corresponding respectively to the first and second values.

10 The indicator values may represent the likelihood of a cell being present at the test-point location (i.e. the likelihood that the cell has passed through the test point as it moves) in any suitable way. They are not necessarily equal to actual probability values (e.g. between zero and one), although they may scale linearly with statistical likelihood.

15 As mentioned above, in some examples, determining the respective indicator value for each test point may comprise combining a plurality of first values for each test point and/or combining a plurality of first values for each test point. The plurality of first and/or second values may be combined using first and/or second weight functions as described above.

20 Determining the respective indicator value for a test point may comprise dividing the first value or the combination of first values for the test point by the second value or the combination of second values for the test point. The result of this division may be raised to the power of another value, referred to herein as a contrast index, to increase or decrease a contrast level in the output image. The contrast index may be predetermined, e.g. chosen for a particular imaging apparatus that captured the image data. The contrast index may be selected by a user. In a set of embodiments the contrast index is between one and five and is preferably approximately two.

25 The first and/or second weight functions (e.g. first and/or second threshold values) may be predetermined (i.e. fixed before the method is performed and possibly even before any image data is captured). For example, the first and/or second weight function (e.g. first and/or second threshold values) may be determined based at least in part on one or more characteristics of imaging apparatus used to produce the image data (e.g. a sample holder, imaging optics and/or an image sensor). The first and/or second weight function (e.g. first and/or second threshold values) may be determined based at least in part on one or more known properties of the sample. The first and/or second weight function (e.g. first and/or second threshold values) may be the same as or derived from a previously-used function (e.g. first and/or second threshold values that previously returned good imaging results).

30

35

- 10 -

Using predetermined functions may allow the calculating steps to be carried out for a window as soon as that window has been decomposed (i.e. without any need to wait for other windows to be decomposed). This may reduce the number and/or volume of data transfers required and may improve the overall efficiency of the system. For instance, a single processor may be arranged to execute all of the decomposing, calculating and determining steps for a single window without needing to transfer data to or from other elements in between these steps. Alternatively, in some embodiments where the first and/or second weight functions are predetermined (i.e. not dependent on the outcome of the decomposing steps), one or more of the calculating or determining steps for one or more windows may be performed at the same time as one or more other windows are still being decomposed. This may allow for more optimal resource use, because processors that would otherwise be idle can be used to start the calculating or determining steps whilst the decomposing step is still being performed by other processors.

In some sets of embodiments, the first and/or second weight function (e.g. first and/or second threshold values) is determined based at least partially on the image data. For example, the method may comprise determining the first and/or second weight function (e.g. first and/or second threshold values) based on eigenimages and/or corresponding values of one or more of the windows. One or more of the processors may be arranged to determine the first and/or second weight function based at least partially on the image data.

Additionally or alternatively, in some embodiments the first and/or second weight function (e.g. first and/or second threshold values) is determined based at least partially on a user input. In other words, the method may comprise receiving an input from a user and determining the first and/or second weight function (e.g. first and/or second threshold values) based at least partially on said input. In some embodiments, the method may comprise providing to a user a representation of one or more of the corresponding values (e.g. a distribution of the values), to allow a user to interpret and select the appropriate first and/or second weight function (e.g. a suitable first and/or second threshold values). The image processing system may comprise a user interface for receiving an input from a user (e.g. for receiving information that determines or leads to the determination of the first and/or second weight function) and/or for providing an output to a user (e.g. a representation of one or more of the corresponding values). The user interface may comprise an output device such as a display and/or an input device such as a keyboard and/or mouse.

In some sets of embodiments, the method comprises decomposing all of the windows before any of the calculating and/or determining steps is carried out. In some such embodiments, the

- 11 -

method may comprise decomposing at least a subset, and potentially all, of the plurality of windows at the same time (i.e. in parallel). However, as explained above, in some embodiments it may be efficient (e.g. to maximise utilisation of a fixed computing resource) to carry out one or more of the calculating and/or determining steps for one or more windows at the same time as one or more other windows are still being decomposed.

Each window may be decomposed into a full set of eigenimages and corresponding values. However, in some embodiments, a window is decomposed into only a partial set of eigenimages (e.g. eigenimages which have a minimum and/or maximum corresponding value). In such embodiments, the partial set of eigenimages may be used to calculate the first values for that window. In some such embodiments, the second values for that window may then be calculated from the partial set of eigenimages without explicitly computing any further eigenimages. For instance, as mentioned above, the first values may represent contributions to their respective test point of signal eigenimages and the second values may represent contributions to their respective test point of noise eigenimages. In such cases the signal and noise eigenimages may be orthogonal, and this orthogonality allows the second values to be calculated without explicitly decomposing the window into noise eigenimages.

The motion tracing algorithm may utilise a point spread function corresponding to the head portion of the cell. The point spread function may be determined from reference data (e.g. computational models), determined by measuring the cell(s) directly before applying the motion tracing algorithm, or determined by measuring one or more optically similar analogues. For instance, the motion tracing algorithm may include a point spread function calculated from measurements of beads with optical properties similar to the head portion of the motile cell. The beads may be suspended in a medium with optical properties similar to a medium in which the motile cells are suspended (e.g. the non-sperm component of semen). Additionally or alternatively, the motion tracing algorithm may utilise a point spread function calculated from measurements of a test sample comprising cells (similar to or of the same type as the motile cells being analysed) that have been fixed in place with a fixing agent. Calculating the first value may comprise projecting a point spread function (PSF) of the respective test point onto one or more eigenimages of the window. Calculating the second value may comprise projecting a PSF of the respective test point onto one or more eigenimages of the window.

In set of embodiments, the second machine-learning algorithm is arranged to determine one or more morphological parameters from the following group: head portion height, head portion width, head portion length. In embodiments where the motile cell comprises a tail portion, the one or more morphological parameters determined by the second machine-learning algorithm

- 12 -

may include one or more of: tail portion height, tail portion width, tail portion length, a ratio of a head portion or tail portion dimension to another head portion or tail portion dimension (e.g. a ratio of head portion width to head portion length). In embodiments where the motile cell comprises a midpiece portion between the head portion and the tail portion, the one or more morphological parameters determined by the second machine-learning algorithm may include a midpiece portion height, a midpiece portion width or a midpiece portion length, or a ratio of a head portion, tail portion or midpiece portion dimension to another head portion, tail portion or midpiece portion dimension. In some embodiments, the one or more morphological parameters may include one or more indirect indicators of the morphology of the cell(s) or part of the cell(s) (e.g. head portion, midpiece portion or tail portion). Possible indirect indicators include optical thickness, surface area, eccentricity, dry mass.

The first machine-learning algorithm (that uses a time series of images of a cell to generate an enhanced image of said cell) may be trained using any appropriate supervised or unsupervised learning technique. In a set of embodiments the first machine-learning algorithm is trained using training data comprising a set of numerically high-spatial-resolution training images and a corresponding set of numerically low-spatial-resolution training images (i.e. high and low spatial resolution images of the same subjects). The method may comprise training the first machine learning algorithm by inputting the set of high-spatial-resolution training images and iteratively optimising one or more parameters (e.g. neural network weightings) of the machine-learning algorithm based on a comparison between enhanced images generated by the first machine-learning algorithm and the low-spatial-resolution training images. The sets of training images may be images of motile cells. Preferably, the sets of training images used to train the first machine-learning model are images of the same type of motile cell that the method is used to analyse. In some embodiments, the first machine learning algorithm comprises a neural network. The first machine learning algorithm may comprise a deep learning neural network (i.e. a neural network comprising more than three layers).

The second machine-learning algorithm (that uses an enhanced image of a cell to determine one or more morphological parameters of the cell) may be trained using any appropriate supervised or unsupervised learning technique. In a set of embodiments the second machine-learning algorithm is trained using training data comprising a set of training images and a corresponding set of training morphological parameters. The method may comprise training the second machine-learning algorithm by inputting each of the set of training images and iteratively optimising one or more parameters (e.g. neural network weightings) of the second machine-learning algorithm based on a comparison between morphological parameters generated by the second machine-learning algorithm and the training morphological parameters. The set of

- 13 -

training images may comprise images of motile cells. Preferably, the set of training images used to train the second machine-learning model are images of the same type of motile cell that the method is used to analyse. The set of training images may include images captured using fluorescence microscopy and optionally images of the same cells captured using corresponding label free microscopy. In some embodiments, the second machine learning algorithm comprises a neural network. The second machine learning algorithm may comprise a deep learning neural network (i.e. a neural network comprising more than three layers).

The quality metric may comprise a numeric value (e.g. where a larger number indicates a higher-quality cell), a letter grade, a rank, or a linguistic label (e.g. fuzzy or subjective). When the method is used to analyse one or more spermatozoa, the quality metric may indicate a fertilization potential, health status, or a suitability for use in a fertilisation procedure according to WHO criteria, or any other suitable criteria known in the art *per se*.

Determining the quality metric may comprise applying a third machine-learning algorithm to the one or more morphological parameters of the cell and the motion trace of the head portion of the cell. The third machine learning algorithm may use the motion trace directly or it may use data derived from the motion trace, such as speed, helicity, regularity of motion, and/or gradient change in motion pattern. The third machine-learning algorithm may be trained using any appropriate supervised, unsupervised or self-supervised learning technique, although in some embodiments separate training may not be needed. In a set of embodiments the third machine-learning algorithm is trained using training data comprising a set of training morphological parameters and training motion traces for a motile cell, along with a corresponding set of training quality metrics. The training data may comprise quality metrics determined by clinical analysis. The method may comprise training the third machine-learning algorithm by inputting each of the set of training morphological parameters and training motion traces and iteratively optimising one or more parameters (e.g. neural network weightings) of the third machine-learning algorithm based on a comparison between quality metrics generated by the third machine-learning algorithm and the training quality metrics. Preferably, the training data used to train the third machine-learning model uses the same type of motile cell that the method is used to analyse. In some embodiments, the third machine learning algorithm comprises a neural network. The third machine learning algorithm may comprise a deep learning neural network (i.e. a neural network comprising more than three layers).

The time series of images of the sample may be retrieved from a data archive (e.g. a database of suitable time series). For instance, the sample may be analysed at a completely separate time and possibly with completely separate equipment to that used to capture the image of the

- 14 -

sample. However, in a set of embodiments the method comprises capturing the time series of images of the sample. In some embodiments the system may comprise an imaging apparatus for capturing a time series of images of the sample. The time series of images of the sample may be captured using microscopy techniques such as bright-field microscopy, dark field  
5 microscopy or phase contrast microscopy apparatus. In some embodiments the time series of images of the sample is captured using tomography or holography, or quantitative phase microscopy.

The time series may comprise a minimum number of images based on an exposure time, an  
10 average speed of the motile cell(s), expected motion patterns, and/or parameters of the motion tracing algorithm. Some images of the sample (e.g. at the beginning of a capturing session) may be captured and used for optimising imaging and/or analysis parameters (e.g. window size). Some or all of these images may not form part of the time series used for analysis.

15 The system for analysing the sample may comprise a imaging apparatus (e.g. microscopy apparatus) arranged to capture the time series of images of the sample. The imaging apparatus may comprise an image sensor (e.g. a charge coupled device), one or more optical components (an objective lens) and a light source. The imaging apparatus may include a light source. The type of light source used may depend on the type of imaging being performed. For instance,  
20 bright-field microscopy may utilise a low coherence light source such as an LED or halogen lamp. In other embodiments, a high coherence light source may be used such as a laser. In some embodiments a pseudo thermal light source is used.

Preferably the sample is imaged using label-free microscopy. In a set of embodiments the  
25 sample is imaged using low-light dose microscopy (e.g. involving light energy doses of  $200 \text{ Jcm}^{-2}$  or less,  $100 \text{ Jcm}^{-2}$  or less or  $50 \text{ Jcm}^{-2}$  or less). Using a label-and/or low light-dose free imaging technique to produce the images of the sample (i.e. avoiding contaminating the sample with labels such as fluorescent dyes or high doses of light) may mitigate biological reactions that can reduce the accuracy of determined motility and morphology information and/or prevent the  
30 cell(s) from being used after analysis is complete. For instance, labelling spermatozoa with fluorescent markers may render them unsuitable for subsequent use in an assisted reproductive technology (ART) procedure.

The quality metric may be a useful result in and of itself. For instance, it may be useful to  
35 compare quality metrics of different cells or samples or the same cell or sample at different times. In embodiments comprising the analysis of a plurality of cells (i.e. of or making up a cell population), the quality metrics of said cells may be used to determine one or more properties of

- 15 -

the cell population. For instance, the quality metrics of a plurality of sperm cells may be used to determine one or more properties such as: cell concentration, ratio of living to dead cells, cell motility, progressive motility. Quality metric(s) may be a useful guide for identifying one or more cells satisfying a certain quality criterion, or for selecting specific cells with the highest quality.

5

In a set of embodiments, the method comprises outputting a visual indication the quality metric (or, in relevant embodiments, an indication of a plurality of quality metrics of different cells). For instance, the system for analysing the sample may comprise an electronic display, and said display may be arranged to output a visual indication of the quality metric. The visual indication may comprise a colour of a colour model (e.g. comprising different RGB values for different quality scores) and/or an intensity level. In some embodiments, the quality metric of a given cell may be associated visually with an image and/or motion trace of said cell. For instance, a visual indication of the quality metric may be displayed adjacent or overlaid on one of the time-series of images (e.g. the most-recent image).

15

The time series of images used to analyse the sample may include a sub-set of or all available images of the sample (e.g. all images captured in a given imaging session of the sample). The analysis may be performed only after all imaging of the sample has been completed. For instance, a sample may be imaged and then put into storage or discarded once imaging is complete, with analysis following immediately or at a later time. However, it may be useful to continue to image the sample during and/or after the analysis, for instance to be able to associate the determined quality metric with a particular cell in real time. In other words, the analysis may be performed at the same time as further images are captured of the sample.

20

In a set of embodiments, the method comprises receiving one or more further images of the sample (e.g. by capturing one or more further images of the sample). The cell for which the quality metric is determined may be identified in said one or more further images. In other words, the cell may be tracked through the one or more further images. A future trajectory of the cell may be determined from earlier images in the time series to assist tracking in later images. This may allow a user to associate the quality metric with the relevant cell in subsequent images. In some embodiments, a visual indication of the quality metric may be displayed adjacent or overlaid on a further image. For instance, a visual indication of the quality metric is overlaid on a real-time or near-real time image of the cell or of the sample. This may facilitate real-time or near-real time analysis of the sample. For instance, a user may use a visual indication to identify in real time high quality and low quality cells. This may facilitate the manual extraction of high and low quality cells, e.g. for use in an in vitro fertilisation (IVF) procedure.

30

35

- 16 -

In some embodiments, the sample may be continually re-analysed using further images (e.g. newly captured images). For example, one or more further images may be used to refine the quality metric of a cell (e.g. by adjusting a previously determined quality metric, or through a complete re-analysis using a time-series of images ending with the most recent further image).  
5 In other words, the analysis may be performed in a rolling manner as new images are received.

The sample to be analysed may be held in any suitable holder known in the art *per se*, such as a petri-dish or a sample well manufactured from PDMS, or any other form of sample holder (e.g. semen sample holder) suitable for imaging. In a set of embodiments, the system for analysing  
10 the sample comprises a sample holder. The sample holder may comprise one or more structures arranged to facilitate preparation of the sample (i.e. to improve imaging quality and/or consistency). The sample holder may comprise one or more structures arranged to facilitate handling of the sample once it has been imaged (e.g. to facilitate sorting and/or selection of cells based on the determined quality metric(s)). For instance, the sample holder may comprise  
15 one or more microfluidic channels or ducts, one or more markers or one or more grooves. The sample holder may comprise a plurality of structures having different dimensions (e.g. ducts of different diameters). The structure(s) may work passively, e.g. using on capillary effects or gravity, or may be operated actively, e.g. by applying specific flow rates and/or fluid dynamic profiles.

20 In some embodiments, the sample holder may comprise one or more structures or markings which facilitate analysis. For example the sample holder may comprise one or more fiducial markers or grids with a known dimension. Such markers may help to identify the positions and/or sizes of cells in the sample.

25 In some embodiments, the sample holder may be arranged to hold multiple samples. This may facilitate analysis of multiple samples in quick succession and/or may facilitate a process carried out as a result of the analysis. For instance, in embodiments where the sample comprises spermatozoa, the sample holder may be arranged to additionally hold one or more egg cells.  
30 This may facilitate a fertilisation process carried out on the basis of the determined quality metric(s).

According to a further aspect of the present invention there is provided computer software that, when executed by suitable computing apparatus, causes said computing apparatus to execute  
35 a method disclosed herein. The computing apparatus may comprise a memory storing said software. The computing apparatus may comprise a processor arranged to execute said software.

As explained above, analysing a sample with the method disclosed herein may allow an operator to identify high quality cells in the sample and to extract manually one or more of these for use in a subsequent process (e.g. an IVF procedure).

5

In some embodiments this cell selection may be partially or fully automated. The present invention extends to a method of selecting one or more motile cells from a sample comprising a plurality of motile cells comprising at least a head portion, the method comprising:

using the method disclosed herein to determine a quality metric for each cell of the plurality of cells; and  
automatically selecting one or more cells from the sample based on the quality metric(s) of said cells.

10

Correspondingly, the present invention extends to a system for selecting one or more motile cells from a sample comprising a plurality of motile cells comprising at least a head portion, the system comprising:

15

the system for analysing a sample as disclosed herein arranged to determine a quality metric for each cell of the plurality of cells; and

20

a cell selection apparatus arranged to extract one or more cells from the sample based on the quality metric(s) of said cells.

Selecting the one or more cells may comprise physically removing the cell(s) from the sample (e.g. from the sample holder holding the sample). Alternatively, selecting the one or more cells may comprise moving the one or more cell to a specific area of a sample holder for subsequent extraction. The one or more cells may be selected using tweezers such as micromechanical tweezers or non-contact tweezers (e.g. optical, acoustic, electrical, magnetic, or fluid (for example vortex) tweezers).

25

Features of any aspect or embodiment described herein may, wherever appropriate, be applied to any other aspect or embodiment described herein. For instance, the processing apparatus of the second aspect of the invention may be arranged to perform any of the features of the computer implemented method described above. Where reference is made to different embodiments, it should be understood that these are not necessarily distinct but may overlap.

30

One or more non-limiting examples will now be described, by way of example only, and with reference to the accompanying figures in which:

35

- 18 -

Figure 1 is a schematic diagram of a system according to an embodiment of the invention;

Figure 2 is a time-series of images of a semen sample analysed using the system;

Figure 3 is a flow diagram illustrating operation of the system;

5 Figure 4 illustrates the identification of cells in the sample;

Figure 5 is a time series of images of a first cell in the sample;

Figure 6 shows a precision motion trace for the first cell;

Figure 7 shows an enhanced image of the first cell used for determining morphological parameters of the cell;

10 Figure 8 is a schematic diagram of the processing device of the system; and

Figures 9-14 are schematic diagrams that illustrate steps of an motion tracing algorithm used by the system.

Figure 1 illustrates a system 2 for analysing a semen sample 4 containing human spermatozoa.

15 Figure 9 illustrates the structure of a spermatozoa 900. The spermatozoa 900 comprises a head portion 902, a midpiece portion 904 and a tail portion 906. The system 2 may also be used to analyse samples containing other similar types of cells which also feature head portions.

The system 2 comprises a sample holder 6, an illumination device 8, an objective lens 10, a beam splitter 12, a camera 14, a processing device 16, a display 18 and a cell selection apparatus 20.

25 The sample holder 6 comprises a polydimethylsiloxane (PDMS) well 22 which holds the sample 4. The volume of the sample 4 and the dimensions of the well 22 are such that the sample thickness is around 100-150  $\mu\text{m}$ .

The processing device 16 comprises an input interface 24 via which image data is received from the camera 14, and an output interface 26 via which analysis results are output to the display 18.

30 The illumination device 8, the objective lens 10 and the camera 14 form a label-free bright-field microscope imaging system. Other microscopy methods may alternatively be used to image the sample 4 such as dark field microscopy or phase contrast microscopy.

35 The illumination device 8 illuminates the sample 4 with low-dose white light, and the objective lens 10 captures resulting light from the sample 4 and directs it (via the beam splitter 12 to the camera 14. An electrical sensor (e.g. a CCD) of the camera 14 captures an initial time-series of

- 19 -

images 100 (illustrated in Figure 2) of the sample 4 at a sub-second acquisition rate. The time-series 100 is shown in Figure 2 with only three images 100, 102, 104 for ease of illustration. It will be appreciated that, in practice, a much larger set of images may be used.

5 The images captured using the illumination device 8, the objective lens 10 and the camera 14 have a wide field-of-view including most of the PDMS well 22. The imaging system has a low numerical aperture (NA) of less than 0.25. The images have a spatial resolution of roughly 1  $\mu\text{m}$  to 2  $\mu\text{m}$ .

10 Each image of the sample 102, 104, 106 in the time series 100 features a plurality of spermatozoa 50A, 50B, 50C. Only three cells are illustrated in Figure 2 for ease of illustration. Because the images captured by the camera 14 have a wide field of view and low spatial resolution, the cells 50A, 50B, 50C are resolved at relatively low detail.

15 The images 100 captured by the camera 14 are processed by the processing device 16 to determine a quality metric for each sperm cell in the sample 4. This processing will now be explained in more detail with additional reference to the flow diagram 100 of Figure 3 and to Figures 4-7.

20 In a first step 202, image data comprising the initial time-series of images of the sample 100 captured by the camera 14 is received by the processing device 16 via the input interface 24.

In a second step 204, the processing device 16 identifies sections in each of the images of the sample 102, 104, 106 which contain the different cells 50A, 50B, 50C. The sections 102A, 102B, 102C of the first image 102 in the time series 100 are labelled in Figure 4. The segmentation engine thus effectively produces time series of images of each cell. Figure 5 shows a time series 150A of images of the first cell 50A made up of sections 102A, 104A, 106A.

25

The processing device 16 now uses the time series of images of the cells in two parallel processing branches: a motion and kinematics branch 210 and a morphology analysis branch 208.

30

In the motion and kinematics branch 206, in step 210, the processing device 16 tracks each spermatozoon through the time series of images of the sample 100 (i.e. the processing device 16 keeps track of the positions of all of the spermatozoa). The processing device 16 tracks the head portion of each cell. In step 212, the processing device 16 uses a motion tracing algorithm

35

- 20 -

to determine a precision motion trace of the head portion of each spermatozoon (i.e. a precise record of how the head portion of each cell has moved through the time series 100).

The motion tracing algorithm captures motion patterns that are smaller than the spermatozoon itself and smaller than the spatial resolution of the images of the sample 100. Figure 6 shows a precision motion trace 300A for the first cell 50A. As described in more detail later, the motion tracing algorithm uses the Multiple Signal Classification Algorithm (MUSICAL) technique described for instance in *Agarwal, K., Macháň, R. Multiple signal classification algorithm for super-resolution fluorescence microscopy. Nat Commun 7, 13752 (2016).*

In step 214, the processing device 16 predicts a future trajectory of each sperm cell based on the tracking. This step may use raw tracking data from step 210 along with the precision motion trace from step 212.

Steps 210, 212 and 214 are performed for each cell 50A, 50B, 50C to produce a motion trace of each cell 50A, 50B, 50C.

In the morphology branch 208, in step 216, the processing device 16 applies a first machine-learning algorithm to the images of each cell to generate an enhanced image of each cell 50A, 50B, 50C. Each enhanced image has a second spatial resolution that is smaller than the first spatial resolution. An enhanced image 400A of the first cell 50A is illustrated in Figure 7.

In step 218, the processing device applies a second machine-learning algorithm to the enhanced image of each cell to determine morphological parameters of the cell including head length, head width, midpiece length, midpiece width and tail length.

Steps 216 and 218 are performed for each cell 50A, 50B, 50C to produce morphological parameters for each cell 50A, 50B, 50C.

In step 220 the processing device 16 applies a third machine-learning algorithm to motion traces from the tracking branch 206 and corresponding morphological parameters from the morphology analysis branch 210 to produce a quality grade for each cell.

Whilst the processing device 16 processes steps 206-220 for a given set of images, the camera 14 continues to capture new images of the sample 4. The new images are continually received by the processing device 16 in step 221. The processing device 16 repeats steps 204 and 206

- 21 -

for each new image frame, keeping track of the movement of each sperm cell whilst their quality grades are being calculated from previous images.

5 In step 222, the processing device 16 retrieves the latest position information for each sperm cell and associates this with the quality grade determined in step 220.

10 In step 224, the processing device 16 outputs via the display 18 a real-time (or near real-time) view of the sample 4. The view shows the latest image of the sample 4 captured by the camera 14 with a colour overlay indicating the quality grade determined for each sperm cell. For instance, a sperm cell given a high quality grade may be overlaid with a green tint, and a sperm with a low quality grade may be overlaid with a red tint.

15 In step 226 the processing device 16 controls the cell selection apparatus 20 (e.g. optical tweezers) to select automatically the highest quality cells in the sample 4 based on the determined quality metrics. The selected cells may then be used in an in vitro fertilisation (IVF) process.

20 The operation of the motion tracing algorithm will now be described in more detail with reference to Figures 8-14.

Figure 8 illustrates components of the processing device 16 used for the motion tracing algorithm in more detail. The processing device 16 comprises the input interface 24, data storage 806 (e.g. comprising volatile and/or non-volatile memories), a main processor 808, a plurality of auxiliary processors 810 and a physical user interface 812 (e.g. comprising a display screen and a keyboard). The main processor 808 operates as a scheduler and a job distributor and is also responsible for handling inputs from and outputs to the user interface 812. The data storage 806 is used for storing image data but may also provide software memory on which software for execution by the processors 808, 810 is stored. The processor device 16 may be implemented in a single location (e.g. as a single computing appliance), or it may be physically distributed. The main processor 808 is connected to each of the auxiliary processors 810 via a respective first communication channel 814. Each of the auxiliary processors 810 is connected to the data storage 806 via a respective second communication channel 816. The second communication channels 816 support a higher maximum rate of data transfer than the first communication channels 814.

35 In a first step, illustrated in Figure 9, the time series 100 (i.e. an image stack) is received by the input interface 24 and stored in the data storage 806. The main processor 808 establishes a

- 22 -

plurality of windows  $w_i$  over the image stack, with each window  $w_i$  defining a respective set of spatially-coincident image sections that contains a respective section of each image in the stack. All the image sections of a given window have the same dimensions and the same position within each image of the stack. Each window  $w_i$  is centred on a different pixel  $i = (x_i, y_i)$  in the X-Y plane. The windows  $w_i$  are square, and all the same size, having dimensions of  $n \times n$  pixels. A window may overlap one or more other windows.

In the next step, the main processor 808 (acting as a scheduler) allocates a different respective window to each auxiliary processor 810 by informing the auxiliary processor 810 of the coordinates of the window in the image stack 100 (e.g. its centre coordinate,  $i$ , assuming the dimension  $n$  is already known to the auxiliary processors 810). The auxiliary processors 810 retrieve the image data of their allocated window from the data storage 806. Alternatively, the main processor 208 may retrieve the image data of the allocated window itself and pass it to the respective auxiliary processor 810. The main processor 808 may also, in some examples, retrieve image data and perform image processing operations itself (e.g. alongside the auxiliary processors 810).

As illustrated in Figure 10, each auxiliary processor 810 performs singular value decomposition (SVD) of the data in its allocated window to produce an eigenimage matrix  $U_i$  and a singular value matrix  $S_i$ . Each eigenimage matrix  $U_i$  consists of  $n^2$  eigenimages  $u_i$  of size  $1 \times n^2$ , and the singular value matrix  $S_i$  is a diagonal matrix containing  $n^2$  associated singular values  $\sigma_i$  for that window (an array containing only the diagonal elements of the matrix  $S_i$  may alternatively be used). Because there are a plurality of auxiliary processors 810, a plurality of windows are decomposed at the same time (i.e. in parallel).

If there are more windows than available auxiliary processors 810, the additional windows are queued to be decomposed by the next available processor 810. The auxiliary processors 810 continue to be allocated windows to decompose until an eigenimage matrix  $U_i$  and a singular value matrix  $S_i$  has been produced for all of the windows. These matrices are stored in the data storage 806.

The main processor 808 then displays all of the singular values  $\sigma_i$  to a user via the user interface 812. The user reviews the singular values  $\sigma_i$  and selects suitable first and second weight functions to apply to the eigenvalues. In this example, the first and second weight functions are both step functions defined by a common threshold singular value  $\sigma_0$  chosen by the user. The user may select the threshold using heuristic techniques (e.g. based on a

- 23 -

threshold that previously produced a high quality final image). The first weight function selects all eigenimages  $u_i$  with a corresponding singular value  $\sigma_i$  greater than or equal to the threshold  $\sigma_0$ . The second weight function selects all eigenimages  $u_i$  with a corresponding singular value  $\sigma_i$  less than the threshold  $\sigma_0$ . In other examples, the main processor 208 may instead select a suitable common threshold  $\sigma_0$  itself without user input, or a fixed (e.g. hard-coded) predetermined threshold  $\sigma_0$  may be used. Other weight functions may also be used.

As explained below, the threshold  $\sigma_0$  will be used to separate the eigenimages  $u_i$  into those corresponding to signals from cells in the sample and those associated with noise. Those eigenimages  $u_i$  with an associated singular value  $\sigma_i$  that is greater than the threshold  $\sigma_0$  are categorised as representing cell signals and those with a singular value  $\sigma_i$  that less than the threshold  $\sigma_0$  are categorised as representing noise.

In the next step, illustrated in Figure 11, the processors 810 are used to establish first and second values for a series of test points  $r_{test}$  in each window. The first and second values represent the relative signal and noise contributions of each of the eigenimages to the different test points  $r_{test}$ . The test points represent respective pixels in the output image. There are therefore  $N^2$  test points per input image pixel, where  $N$  is an expansion factor for the output image.

To determine the values, the processor device 24 projects a point spread function (PSF) of each test point onto each eigenimage of each window. To do so the processors 2=810 multiply each of the eigenimage matrices  $U_i$  with a test matrix  $G$  that represents the PSF for each of the test points  $r_{test}$  within the window:

$$P_i = U_i G$$

There are  $N^2$  test points per original pixel,  $n^2$  pixels per window, and  $n^2$  eigenimages per window, so the test matrix  $G$  has dimensions of  $n^2 \times n^2 N^2$ . The matrix multiplication for at least some of the windows, and potentially for each window, is performed on a different respective auxiliary processor 810, so that the matrix  $P_i$  for multiple windows is determined simultaneously (i.e. in parallel). If there are more windows than there are auxiliary processors 810, the additional windows are queued for processing when a processor 810 becomes available.

The resulting matrices  $P_i$  are squared element-wise to produce  $P_i^2$ . The matrix  $P_i^2$  is then split into two groups of rows according to the threshold  $\sigma_0$ . The first group of rows corresponds to

- 24 -

eigenvalues that have a singular value that is equal to or above the threshold and the second group of rows corresponds to eigenvalues that have a singular value that is below the threshold. The first group of rows are summed to produce a signal contribution matrix  $d_{sig}$  and the second group of rows are summed to produce a noise contribution matrix  $d_{noise}$ . The signal contribution matrix  $d_{sig}$  contains the first values for each test point (i.e. the contributions to each test point of the eigenimages of the window associated with signals in the sample). The noise contribution matrix  $d_{noise}$  contains the second values for each test point (i.e. the contributions to each test point of the eigenimages of the window associated with noise).

Next, as shown in Figure 12, the signal contribution matrix  $d_{sig}$  is divided by the noise contribution matrix  $d_{noise}$  (i.e. the first values are divided by the second values) and the result is raised to a contrast parameter  $\alpha$  to calculate an indicator value matrix  $f$  for all of the test points in the window. The contrast parameter  $\alpha$  may be selected by a user or may be predetermined. Each indicator value in the matrix  $f$  represents a likelihood of a cell being present in the sample at a position corresponding to the respective test point. They have a very large number at a likely cell location and a small number otherwise. They do not equal true statistical likelihood (e.g. they are not scaled between zero and one), but they may correlate with statistical likelihood. The elements of the  $1 \times n^2 N^2$  indicator function matrix  $f$  are then rearranged to produce a square  $nN \times nN$  window 900.

The auxiliary processors 810 return the windows 900 to the data storage 806. Finally, as illustrated in Figure 14, the main processor 808 combines all of the windows 900 to produce the final motion trace image 1000 of width  $NX$  and height  $NY$ , by aligning the windows spatially and combining (e.g. adding or mean averaging) values in the overlapping portions.

While the invention has been described in detail in connection with only a limited number of embodiments, it should be readily understood that the invention is not limited to such disclosed embodiments. Rather, the invention can be modified to incorporate any number of variations, alterations, substitutions or equivalent arrangements not heretofore described, but which are commensurate with the scope of the invention. Additionally, while various embodiments of the invention have been described, it is to be understood that aspects of the invention may include only some of the described embodiments. Accordingly, the invention is not to be seen as limited by the foregoing description, but is only limited by the scope of the appended claims.

Claims

1. A system for analysing a sample comprising a motile cell comprising at least a head portion, the system comprising:
- 5 an input interface arranged to receive image data comprising a time series of images of the sample, the images of the sample having a first spatial resolution; and
- a processing apparatus arranged to:
- produce a time series of images of the cell by identifying a section of each image of the sample which contains the cell;
- 10 apply a first machine-learning algorithm to said images of the cell to generate an enhanced image of the cell having a second spatial resolution that is numerically smaller than the first spatial resolution;
- apply a second machine-learning algorithm to said enhanced image of the cell to determine one or more morphological parameters of the cell;
- 15 apply a motion tracing algorithm to the time series of images of the sample to determine a motion trace of the head portion of the cell; and
- use the one or more morphological parameters of the cell and the motion trace of the head portion of the cell to determine a quality metric for the cell.
- 20 2. The system of claim 1, wherein the sample comprises a plurality of motile cells, each comprising at least a head portion and the processing apparatus is arranged to:
- produce a time series of images of each cell by identifying a sections of each image of the sample which contain each cell;
- apply the first machine-learning algorithm to each of the plurality of time series of images of the cells to generate an enhanced image of each cell having a second spatial resolution that is numerically smaller than the first spatial resolution;
- 25 apply the second machine-learning algorithm to each enhanced image to determine one or more morphological parameters of each cell;
- apply the motion tracing algorithm to the time series to determine a motion trace of the head portion of each cell; and
- 30 for each cell, use the one or more morphological parameters of the cell and the motion trace of the cell to determine a quality metric for the cell
3. The system of claim 1 or 2, wherein the motion tracing algorithm is based on statistical analysis of intensity fluctuations in the time series of images of the sample.
- 35 4. The system of claim 3, wherein the motion tracing algorithm comprises:

- 26 -

selecting from the time series of images a plurality of windows, each window comprising a respective stack of spatially-coincident sections of the images;

for each window:

decomposing the window into eigenimages and corresponding values;

5                calculating, for each of a plurality of test points in the window, a respective first value as a first function of the eigenimages;

calculating, for each of the plurality of test points in the window, a respective second value as a second function of the eigenimages; and

10              determining, from the first and second values, a respective indicator value for each test point in the window, representative of a likelihood of the cell being present at a location corresponding to the test point; and

combining the indicator values to produce an output image comprising a motion trace of the cell through the time series of images.

15              5.        The system of any preceding claim wherein the motion tracing algorithm uses a point spread function corresponding to the head portion of the cell.

20              6.        The system of any preceding claim, wherein the second machine-learning algorithm is arranged to determine one or more morphological parameters from the following group: head portion height, head portion width, head portion length.

7.        The system of any preceding claim, wherein the motile cell comprises a tail portion and optionally a midpiece portion between the head portion and the tail portion.

25              8.        The system of claim 7, wherein the one or more morphological parameters determined by the second machine-learning algorithm includes one or more of: tail portion height, tail portion width, tail portion length, a ratio of a head portion or tail portion dimension to another head portion or tail portion dimension; a midpiece portion height, a midpiece portion width or a midpiece portion length, or a ratio of a head portion, tail portion or midpiece portion dimension to another head portion, tail portion or midpiece portion dimension.

30              9.        The system of any preceding claim, wherein the one or more morphological parameters include one or more indirect indicators of morphology of the cell or part of the cell such as optical thickness, surface area, eccentricity or dry mass.

35

- 27 -

10. The system of any preceding claim, wherein the first machine-learning algorithm is trained using training data comprising a set of numerically high-spatial-resolution training images and a corresponding set of numerically low-spatial-resolution training images.
- 5 11. The system of any preceding claim, wherein the second machine-learning algorithm is trained using training data comprising a set of training images of motile cells including images captured using fluorescence microscopy and a corresponding set of training morphological parameters.
- 10 12. The system of any preceding claim, wherein determining the quality metric comprises applying a third machine-learning algorithm to the one or more morphological parameters of the cell and the motion trace of the head portion of the cell.
- 15 13. The system of claim 12, wherein the third machine-learning algorithm is trained using training data comprising a set of training morphological parameters and training motion traces for a motile cell, along with a corresponding set of training quality metrics determined by clinical analysis.
- 20 14. The system of any preceding claim, comprising a microscopy imaging apparatus for capturing the time series of images of the sample.
15. The system of any preceding claim, wherein the microscopy imaging apparatus is a label-free microscopy imaging apparatus.
- 25 16. The system of any preceding claim, comprising an electronic display arranged to output a visual indication of the quality metric.
- 30 17. The system of claim 16, wherein the quality metric of a given cell is associated visually with an image and/or motion trace of said cell.
18. The system of any preceding claim, wherein the input interface is arranged to receive one or more further images of the sample and the processing apparatus is arranged to use said one or more further images to refine the quality metric of a cell.
- 35 19. The system of any preceding claim, comprising a sample holder comprising one or more structures arranged to facilitate preparation of the sample.

- 28 -

20. The system of any preceding claim, comprising a sample holder comprising one or more structures arranged to facilitate handling of the sample once it has been imaged.
21. The system of any preceding claim, comprising a sample holder comprising one or more fiducial markers or grids with a known dimension.
22. The system of any preceding claim, arranged to analyse a sample comprising one or more human spermatozoa.
23. A computer-implemented method for analysing a sample comprising a motile cell comprising at least a head portion, the method comprising:
- receiving a time series of images of the sample, the images of the sample having a first spatial resolution;
  - producing a time series of images of the cell by identifying a section of each image of the sample which contains the cell;
  - applying a first machine-learning algorithm to said images of the cell to generate an enhanced image of the cell having a second spatial resolution that is numerically smaller than the first spatial resolution;
  - applying a second machine-learning algorithm to said enhanced image of the cell to determine one or more morphological parameters of the cell;
  - applying a motion tracing algorithm to the time series of images of the sample to determine a motion trace of the head portion of the cell; and
  - using the one or more morphological parameters of the cell and the motion trace of the head portion of the cell to determine a quality metric for the cell.
24. Computer software that, when executed by suitable computing apparatus, causes said computing apparatus to execute the method of claim 23.
25. A method of selecting one or more motile cells from a sample comprising a plurality of motile cells comprising at least a head portion, the method comprising:
- using the method of claim 23 to determine a quality metric for each cell of the plurality of cells; and
  - automatically selecting one or more cells from the sample based on the quality metric(s) of said cells.
26. A system for selecting one or more motile cells from a sample comprising a plurality of motile cells comprising at least a head portion, the system comprising:

- 29 -

the system of any of claims 1-22, arranged to determine a quality metric for each cell of the plurality of cells; and

a cell selection apparatus arranged to extract one or more cells from the sample based on the quality metric(s) of said cells.