



(51) International Patent Classification:

G01N33/50 (2006.01) G01N21/00 (2006.01)
G01N33/68 (2006.01)

(21) International Application Number:

PCT/GB20 13/000003

(22) International Filing Date:

4 January 2013 (04.01.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1200178.0 6 January 2012 (06.01.2012) GB

(71) Applicant: **CYTOSYSTEMS LIMITED** [GB/GB];
Cruickshank Building, Craibstone, Aberdeen AB21 9TR
(GB).

(72) Inventors: **GALLOWAY, David**; Cytosystems Limited,
Cruickshank Building, Craibstone, Aberdeen, AB21 9TR,
GB (GB). **MAYNARD, Daniel Mark**; Orion Automation
Ltd, 2 High Street, Alconbury Weston, Huntingdon, Cam-
bridgeshire PE28 4JP (GB).

(74) Agent: **O'NEILL, Michelle**; Harrison IP Limited, 1st
Floor, Box Tree House, Northminster Business Park,
Northfield Lane Y026 6QU (GB).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

[Continued on nextpage]

(54) Title: DIAGNOSTIC METHOD AND SYSTEM

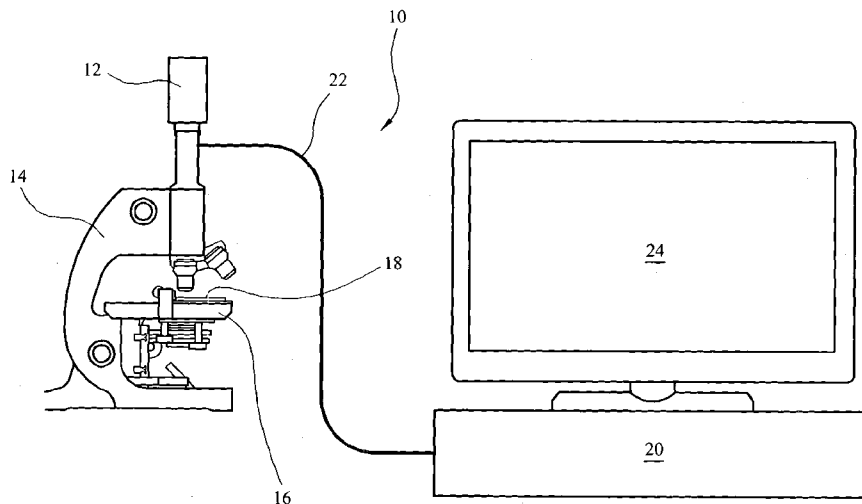


FIG. 1

(57) Abstract: The present invention relates to a method of diagnosis which identifies one or more individual cells and comprises determining at least one of a cell dimension and a cell area, and determining at least one of dark/light cell contrast characteristics, cell area characteristics, cell colour characteristics, cell roughness characteristics, distances between cell nuclei and cell convexity. A computer readable medium, a computer apparatus and a diagnostic system is also provided.



- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

Diagnostic method and systemField of the Invention

The present invention relates to diagnostic methods and systems
5 and particularly to diagnostic methods and systems for
analysing abnormal cells and more particularly to analysing
abnormal cells for the diagnosis of cancers.

Background to the invention

10 The diagnostic method of taking cells from living organs,
processing the cells using, for example, a colour dye, and then
observing the cells under a microscope for abnormalities, has
been used for many years. One such example is the "Pap" smear
test, which is widely used to screen for cervical cancer in
15 women.

This known diagnostic method is carried out by skilled
operators. However, in being a human activity and due to the
relatively large number of cells and cell characteristics which
20 are required to be viewed, it is inevitable that there is a
degree of subjectivity in determining abnormal cells.
Furthermore, the requirement of a skilled operator means that
it is a relatively expensive procedure and inhibits a wider
application where there is a skills shortage.

25 Such diagnostic methods using routine cytology are carried out
to investigate a variety of different conditions such as, for
example, cervical cancer with the "Pap test", cancer of the
oesophagus and diseases of the urinary tract.

30 Cancer of the bladder is currently the fourth most common
cancer in men and the seventh most common cancer in women. The
condition is currently investigated by cystoscopy to observe
signs of malignancy. It is estimated that up to 3.6 million

cystoscopies for TCC (Transitional Cell Carcinoma of the bladder) are carried out each year in Europe and the USA. However, cystoscopy is a relatively expensive procedure to administer and it is also an intrusive procedure and can be
5 uncomfortable for the patient, who requires a local or general anaesthetic. Moreover, post-treatment bladder cancer patients often have to undergo a large number of surveillance cystoscopies for life.

10 The above-mentioned disadvantages are also relevant and equally applicable to other diagnostic methods and the diagnosis of other diseases, particularly other cancers.

It is an object of the present invention to at least
15 substantially mitigate these disadvantages. It is also an object of the present invention to provide a cost effective and standardised method and system for analysing abnormal cells and more particularly to the analysis of abnormal cells for the diagnosis of cancers.

20

Statements of Invention

According to a first aspect of the present invention there is provided a method of diagnosis comprising:

observing one or more cell samples; identifying one or more
25 individual cells; determining one or more characteristics of the, or each, individual cell; comparing the, or each, determined characteristic with a respective determined or predetermined characteristic; and recording the correlation of the, or each, determined characteristic and the respective
30 predetermined characteristic; wherein identifying the one or more individual cells comprises determining at least one of a cell dimension and a cell area, and wherein determining the one or more characteristics comprises determining at least one of the dark/light cell contrast characteristics, cell area

characteristics, cell colour characteristics, cell roughness characteristics, distances between cell nuclei and cell convexity .

5 The method advantageously further comprises providing image processing means; receiving an image of the one or more cell samples; identifying one or more individual cells on the received image; determining one or more characteristics of the, or each, individual cell from the captured image;

10 comparing the, or each, determined characteristic with a respective determined or predetermined characteristic; and recording the correlation of the, or each, determined characteristic and the respective predetermined characteristic; wherein identifying the one or more individual cells comprises

15 the image processing means determining at least one of a cell dimension and a cell area, and wherein determining the one or more characteristics comprises the image processing means determining at least one of the dark/light cell contrast characteristics, cell area characteristics, cell colour

20 characteristics, cell roughness characteristics, distances between cell nuclei and cell convexity.

The method preferably comprises the steps of:

- 25 (i) isolating cells from the sample to provide a cell sample;
- (ii) contacting said cell sample with an antibody capable of binding a minichromosome maintenance (MCM) polypeptide (s); and
- (iii) determining the binding of said antibody to the cell
- 30 sample.

The MCM is selected from the group consisting of MCM 2, 3, 4, 5, 6 and 7. The MCM may be a combination of two or more different MCMs, for example, two different MCMs selected from

the group consisting of MCM 2, 3, 4, 5, 6 and 7. For example the MCM may include MCM2 and one other MCM selected from MCM 3, 4, 5, 6 and 7. By way of further example the MCM may include MCM5 and one other MCM selected from MCM 2, 3, 4, 6 and 7. In
5 a preferred method of the invention, the MCM is selected from the group consisting of MCM 2, 3, 5 and 7. In a further preferred method of the invention, the MCM is selected from the group consisting of MCM 2, 5 and 7.

10 The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen, whether natural or partly or wholly synthetically produced. The term also covers any
15 polypeptide or protein having a binding domain which is, or is homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes (e. g., IgG, IgE, IgM, IgD and IgA) and
20 their isotypic subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies (i.e. protein fragments). Antibodies may be polyclonal or monoclonal .

25 As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of
30 antibodies, humanised antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic.

Antibodies which are specific for a MCM may be obtained using techniques which are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit) with the protein or a fragment thereof or a cell or virus which expresses the protein or fragment.

"Specific" is generally used to refer to the situation in which one member of a specific binding pair will not show any significant binding to molecules other than its specific binding partner(s), e. g., has less than about 30%, preferably 20%, 10%, or 1% cross-reactivity with any other molecule.

The antibody may be a monoclonal antibody having an antigen binding domain specific for MCM. Monoclonal antibodies specific for MCM are known in the art, for example, anti-MCM2 antibody may be obtained from the Cancer Cell Unit, Hutchison/MRC Research Centre, Hills Road, Cambridge CB2 0XZ.

The specific antibody may be labelled with a detectable label, for example a radiolabel such as Iodine or ^{99}Tc , which may be attached to the antibody using conventional chemistry known in the art of antibody imaging. Such labelling allows those cells that are bound to the antibody to be detected/visualised. Labels also include enzyme labels such as horseradish peroxidase or alkaline phosphatase. Labels further include chemical moieties such as biotin which may be detected via binding to a specific cognate detectable moiety, e.g. labelled avidin.

The reactivities of an antibody on normal and test samples may be determined by any appropriate means. Other labels include fluorochromes, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red.

Suitable chromogenic dyes include diamine-benzidine. Other labels include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Alkaline phosphatase or horseradish peroxidase are generally employed.

15

The method advantageously further comprises summing the number of individual cells which have determined characteristics which correlate with the predetermined characteristics.

20

The method advantageously further comprises scanning a tissue or cytology sample to provide the image of the one or more cell samples.

The scanning preferably comprises raster scanning.

25

The method advantageously further comprises staining (i.e. dying) a said tissue or cytology sample prior to scanning.

30

The step of identifying the individual cells advantageously comprises identifying respective nuclei on the image.

The nuclei may be identified by determining dark areas of the image.

The method advantageously further comprises determining the intensity of the contrast of the dark/light image areas of the said cell.

5 The method advantageously further comprises searching for coloured regions of the cell on the image.

The step of determining the cell dimension advantageously comprises the image processing means determining one or more of
10 the cell contour area, cell circularity, cell axis ratio, cell length and cell width.

According to a second aspect of the present invention, there is provided a computer readable medium carrying a computer program
15 configured to carry out the method of the first aspect of the present invention.

According to a third aspect of the present invention, there is provided a computer apparatus comprising:
20 a memory storing processor readable instructions; and
a processor configured to read and execute instructions stored in said program memory;
wherein said processor readable instructions comprise instructions controlling the processor to carry out the method
25 according to the first aspect of the present invention.

According to a fourth aspect of the present invention there is provided a diagnostic system comprising:
a sample slide mount for receiving a slide having cell samples
30 disposed thereon;
image capture means operable to capture an image of the cell samples; and
a computer apparatus according to the third aspect of the present invention.

The image capture means preferably comprises magnifying means. The magnifying means is preferably a microscope.

5 The system may further comprise a motor operable to move the sample slide mount relative to the image capture means in a controlled manner. The motor preferably comprises a faster scanner to move the sample slide mount relative to the image capture means in a controlled manner.

10

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", means "including but not limited to", and is not intended to (and does not) exclude
15 other additives, components, integers or steps.

Throughout the description and the claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite
20 article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical
25 moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

30 The present invention will now be described by way of example only with reference to drawings, in which:

Figure 1 is a schematic drawing showing a diagnostic system according to one aspect of the present invention; and

Figure 2 is a flow diagram showing a diagnostic method according to another aspect of the present invention.

5 Referring to Figure 1, a diagnostic system 10, according to the present invention, comprises image capture means, such as a still or video camera 12. The camera 12 is mounted on magnifying means, such as a microscope 14. The microscope 14 has a sample slide mount 16, for receiving a slide (e.g. a
10 cytology slide) 18 having sample cells disposed thereon. The slide mount 16 is moveable relative to the camera 12 in a controlled manner in order to provide a raster scanning function .

15 The diagnostic system 10 further comprises a computer processor 20, which is electrically connected to the camera 12 and microscope 14 by a communication link 22, such that the images captured by the camera are transmittable to the processor 20 and the computer processor can control focusing of the camera
20 and scanning movements of the slide mount.

The system further comprises a computer VDU 24, for displaying images and data.

25 Prior to undertaking an analysis, using the diagnostic method of the present invention, the cell samples are advantageously processed. For example, human bladder cells are processed with minichromosome maintenance proteins (MCM). Processing bladder cells with MCM provides a colour change in the nuclei of the
30 cells which is indicative of cell turnover rate which may be an indication for cancer. It will be appreciated that other stains (i.e. dyes) and processes may be used in relation to other types of cells and diagnostic methods within the working of the present invention.

Although, the diagnostic method of the present invention will be described herein with reference to the diagnosis of bladder cancer, it will be appreciated that the method is equally applicable to the analysis of other cells, such as, for example, prostate, lung, cervical and colo-rectal cells and, moreover, that the method is equally applicable to automated slide (e.g. cytology slide or histology slide) analysis in other areas of diagnosis or monitoring.

10

Referring also to Figure 2, the diagnostic method is undertaken through a computer program stored in computer processor 20.

The diagnostic method is started by carrying out a slide positioning step 202, in which a slide 18, on which cell samples are disposed, is positioned on the slide mount 16 in a first field location. Typically, the cell samples cover a sample area of approximately 10mm to approximately 20 mm in diameter on the slide (e.g. 13mm to 20mm) and a field is a portion of the sample area. There is therefore a plurality of fields within each sample area.

A scanning step 204 is then undertaken in which the positioned first field is focused 206 through the lenses of the microscope 14. Mechanical focusing of the first field through the microscope is achieved using an edge-based algorithm. If sample cells are not detected, within the first field, focusing is not carried out and the slide is indexed to the next positioned field 208. The scanning step 204 is then repeated for the next positioned field. However, if sample cells are detected, the focused first field is scanned and the image is recorded using the camera 12 and stored in memory within the computer processor. The scanning step 204 involves faster scanning the sample cells.

The method then comprises a cell identification step 210 in which the individual sample cells are identified on the slide 18. This is carried out using image processing to locate areas of the image which match specific predetermined characteristics indicative of the presence of a cell of interest, such as, for example, for bladder cells the predetermined characteristics may be: initial location and dimensions of cytoplasm outline 212 which correlate with predetermined criteria; location and dimensions of dark areas (e.g. brown) 218 which correlate with predetermined criteria to indicate the presence of a nucleus; location and dimensions of cytoplasm using light/dark contrast analysis 220, which correlate with predetermined criteria; and analysis of colour matching 222 to correlate with predetermined criteria.

For bladder cells the criteria for the location and dimensions of the cytoplasm outline 212 are a length greater than a length in the range of approximately 5 to 15 μm (preferably 9 μm), a width greater than a width in the range of approximately 5 to 15 μm (preferably 8 μm) and an area greater than an area in the range of approximately 5-250 μm^2 (preferably 5 μm^2). The criteria for the location and dimensions of dark areas (e.g. brown) 218 includes a contour area of greater than an area in the range of approximately 5 - 250 μm^2 (preferably 25 μm^2) and less than an area in the range of approximately 1000 μm^2 to 5000 μm^2 (preferably 2500 μm^2) and a minimum circularity in the range of approximately 0.01 to 0.1 (preferably 0.05). The cells are checked 220 and are deemed to be acceptable if the cell area is greater than an area in the range of approximately 100 to 1000 μm^2 (preferably 500 μm^2) and has a dark cytoplasm area greater than 5 μm^2 (preferably 40 μm^2) and less than 500 μm^2 (preferably 250 μm^2) and a cell contrast greater than a specific

value in the range of 10 to 60 (preferably 35). The colour matching analysis 222 requires a search for dark areas and determining whether the colour of the dark area falls within the range of the values of hue, saturation and intensity of a predetermined area. Thus, the threshold may be 0-120 and the predetermined area may be an area greater than an area in the range of 5 - 250 μm^2 (e.g. 25 μm^2). For the avoidance of doubt, where ranges are provided, the criteria may be set at any value within that range. Clearly, the value used will be consistent throughout the process.

When analysing bladder cells during the initial step of determining the location and dimensions of cytoplasm outlines 212, if anything is detected which has a cell length of less than the defined criteria for cell length (e.g. less than a length in the range of approximately 5 to 15 μm , preferably 9 μm) and a cell width of less than the defined criteria for cell width (e.g. less than a width in the range of approximately 5 to 15 μm , preferably 8 μm) it is not considered relevant and instead considered to be debris and not counted 214. Similarly, if anything is detected which has a cell area less than the predetermined value (e.g. less than an area in the range of 5 - 250 μm^2 , e.g. 5 μm^2) it is not considered relevant and instead considered to be debris and not counted 216.

The individual analysis starts with an analysis of the nucleus.

The analysis of the nucleus begins with a check for a distinct nucleus whereby the image of the selected cell is analysed to determine the nucleus. This initially involves testing the dark areas for a fit into a defined area (bounding box) 224. If the length of the cell nucleus bounding box is greater than a length in the range of 5 to 15 μm (preferably 12 μm) then it is

determined that there is a cluster of nuclei and the cluster is split into individual centres 226 prior to looping through identifiable dark areas 228, whereas if the cell nucleus bounding box 224 is lower than the defined value, no splitting step 226 is necessary prior to lopping through dark areas 228. Looping though dark areas may be indicative of the location of a nucleus within the cytoplasm of the selected cell image. Then the area inside the dark area contour is divided by the area of the smallest rectangle that can be fit around the same contour 230 to provide a ratio. Suitably, the minimum ratio is a ratio in the range of 0.2 to 0.6 (preferably 0.4). The degree of dark/light contrast of the dark area relative to the cytoplasm is then determined 232 whereby a minimum level of contrast is required to ascertain a distinct nucleus. The minimal level of contrast may be set at a value in the range of 10 to 60 (preferably 35).

If the criterion of the steps 224 to 232 is within the relevant thresholds then the nucleus is considered to be distinct which is indicative of the presence of the cell and the cell is added to a Field Total Cell Count (FTCC) 234 which is recorded in memory 236.

For each distinct nucleus, the method further comprises a step of determining the nucleus intensity 238. If the intensity is less than a defined intensity in the range of 60 to 150 (preferably 120), the method further comprises a colour matching step 242. If the intensity is greater than the defined intensity, the cell is considered not to be relevant for the cell count which is recorded accordingly 240.

The colour matching step 242 comprises the signal processing determining the colours of the nucleus of cells which match a

predetermined range of colour indicative of the presence of disease .

If the colour matching step **242** does not result in the
5 detection of any distinct colours or if distinct colours are
detected but the detected colours do not match the
predetermined colour (i.e. no match) then the cell is
considered not to be relevant for the cell count which is
recorded accordingly **244**.

10

If the colour matching step **242** results in a positive outcome
(i.e. colour match detected - for example, for bladder cells
processed with MCM a dark coloured stain (e.g. brown coloured)
in the nucleus is indicative of cell turnover rate which may be
15 indicative of the presence of cancer) then it is added to a
Field Colour Match Total (FCMT) count **246**. The FCMT count **246**
is the sum of the FTCC counts **236** minus the number of high
nucleus intensity cells **240** and minus the number of no colour
match cells **244** .

20

The number of cells having particular stain characteristics can
be significant for diagnoses. Therefore, checking so-called
"raspberry clumps", where a number of cells are in contact with
each other, is important as one or more cells within a clump
25 may be significant for a diagnosis and others may not.

In order to take this into account, the method further
comprises the step of determining whether there are more than a
set number of nuclei present **248**, wherein the set number is in
30 the range of **4** to **8** (preferably **4**) and, if so, calculates: the
distance between nuclei using mean and standard deviation to
nearest neighbour, overall area and axis ratio of cytoplasm **250**
to ascertain whether or not it is indicative of a "raspberry
clump" .

Nuclei touching each other can be difficult to define. However, this has been achieved whereby the image processing identifies the darker centre of each nucleus in the clump. Additionally, or alternatively, the image processing defines the shape of the outline contour of the each nucleus.

If it is determined that there is a "raspberry clump" it is considered a field positive cell and recorded accordingly 272.

10

If it is determined that there are not more than four nuclei present (under step 248) or that there are more than four nuclei present (under step 248) and a "raspberry clump" is also determined not to be present (under step 1250), the method proceeds to cell exclusion steps 252 and 256 which determines non-viable cells and macrophage cells. The cell exclusion step involves the image processing determining the roughness of the cell by analysing the intensity in the gradient of the image of the cell. The analysis may be undertaken on the cytoplasm and/or the nucleus of the cell.

20

If the roughness of the cytoplasm is determined to be greater than a set value in the range of 5 to 25, preferably 15 (under step 252), the cell is considered not to be relevant for the cell count which is recorded accordingly 254.

25

If the roughness of the nucleus is determined to be greater than a set value in the range of 5 to 25, preferably 12 (under step 256), the cell is considered not to be relevant for the cell count which is recorded accordingly 258.

30

The method further comprises a nucleus contour analysis step 260 which determines the roughness of the contour edge of the nucleus. This step involves the image processing determining

the roughness of the nucleus contour edge by analysing the intensity in the gradient of the image of the nucleus. If the contour roughness is determined to be too rough the nucleus is likely to be degenerative.

5

If the intensity in the gradient is determined to be less than a set value in the range of 20 to 40 (preferably 30) then the cell is considered not to be relevant for the cell count which is recorded accordingly 262.

10

If the intensity in the gradient is determined to be greater than the set value in the range of 20 to 40 (preferably 30) then the cell is checked for coloured stain in the cytoplasm 264 .

15

If stain is detected in the cytoplasm of the cell in step 264 then the cell is considered not to be relevant for the cell count which is recorded accordingly 266.

20

If a stain is not detected in the cytoplasm of the cell in step 264 then it proceeds to the small nuclei exclusion step 268.

25

The presence of large nuclei is considered to be more indicative of disease and therefore step 268 eliminates nuclei which are relatively small in size from the cell count. The image processing undertakes step 268 by determining a ratio of the size of the nucleus of the cell relative to the cytoplasm and comparing the ratio to a predetermined ratio. Any nuclei which are determined to be relatively small based on the predetermined ratio (i.e. less than a set value in the range of 0.2 to 0.4, preferably 0.32) are considered not to be relevant for the cell count which is recorded accordingly 270. Any nuclei which are determined to be greater than the

30

predetermined ratio are considered to be field +ve cells and are recorded accordingly 272.

Another optional step which may be undertaken prior to record
5 of the nuclei as field +ve is whereby the image processing measures the nucleus convexity (not shown). A positive, viable nucleus is considered to have a definite edge and be mostly convex. Therefore, a cell not having such a nucleus would be excluded from the cell count.

10

The Field +ve cells which are relevant for the diagnosis are then summed and recorded for the current field of interest 272, whereby the relevant cell count for the field is $FMCM = FCMT(\text{step } 246) - C1(\text{step } 254) - C2(\text{step } 258) - C3(\text{step } 262) - C4(\text{step } 266) - C5(\text{step } 270)$.

15

A Slide Total Cell Count (STCC) 274 is also summed as the analysis of each field of the sample cells disposed on the slide is completed.

20

A Slide Colour Match Total (SCMT) 276 is also summed whereby $SCMT = SCMT(\text{for each analysed field}) + FCMT(\text{step } 246)$.

25

A Slide +ve Total Cell Count (SMCM) 278 is also summed whereby $SMCM = SMCM(\text{for each analysed field}) + FMCM(\text{Step } 2725)$.

30

After completion of the image processing analysis of the first field, the field location is incremented 208 to the second field area of the sample cells, the second field is scanned and the method continues therefrom as described above with reference to the image processing analysis of the first field.

Claims

1. A method of diagnosis comprising:
 - providing a cell sample;
 - contacting said cell sample with an antibody capable of binding a minichromosome maintenance (MCM) polypeptide (s);
 - observing one or more cell samples;
 - identifying one or more individual cells;
 - determining one or more characteristics of the, or each, individual cell;
 - comparing the, or each, determined characteristic with a respective determined or predetermined characteristic; and
 - recording the correlation of the, or each, determined characteristic and the respective predetermined characteristic;wherein identifying the one or more individual cells comprises determining at least one of a cell dimension and a cell area, and wherein determining the one or more characteristics comprises determining at least one of the dark/light cell contrast characteristics, cell area characteristics, cell colour characteristics, cell roughness characteristics, distances between cell nuclei and cell convexity.

2. A method of diagnosis as claimed in claim 1, further comprising:
 - providing image processing means;
 - receiving an image of the one or more cell samples;
 - identifying said one or more individual cells on the received image;
 - determining one or more characteristics of the, or each, individual cell from the captured image;

comparing the, or each, determined characteristic with a respective determined or predetermined characteristic; and recording the correlation of the, or each, determined characteristic and the respective predetermined characteristic ;

wherein identifying the one or more individual cells comprises the image processing means determining at least one of a cell dimension and a cell area, and wherein determining the one or more characteristics comprises the image processing means determining at least one of the dark/light cell contrast characteristics, cell area characteristics, cell colour characteristics, cell roughness characteristics, distances between cell nuclei and cell convexity.

3. A method as claimed in claim 1 or 2, further comprising summing the number of individual cells which have determined characteristics which correlate with the predetermined characteristics.
4. A method as claimed in claim 2 or claim 3, further comprising scanning a tissue sample to provide the image of the one or more cell samples.
5. A method as claimed in claim 4, wherein the scanning comprises raster scanning.
6. A method as claimed in 4 or 5, further comprising dying a said tissue sample prior to scanning.
7. A method as claimed in any of the preceding claims wherein identifying the individual cells comprises identifying respective nuclei on the image.

8. A method as claimed in claim 7, wherein the nuclei are identified by determining dark areas of the image.
9. A method as claimed in any of the preceding claims, further comprising determining the intensity of the contrast of the dark/light image areas of the said cell.
10. A method as claimed in any of the preceding claims, further comprising searching for regions of predetermined colour of the cell on the image.
11. A method as claimed in any of the preceding claims wherein determining the cell dimension comprises determining one or more of the cell contour area, cell circularity, cell axis ratio, cell convexity, cell length and cell width.
12. A computer readable medium carrying a computer program configured to carry out the method of any preceding claim.
13. A computer apparatus comprising:
 - a memory storing processor readable instructions; and
 - a processor configured to read and execute instructions stored in said program memory;wherein said processor readable instructions comprise instructions controlling the processor to carry out the method of any one of claims 1 to 11.
14. A diagnostic system comprising:
 - a sample slide mount for receiving a slide having cell samples disposed thereon;
 - image capture means operable to capture an image of the cell samples; and
 - a computer apparatus as claimed in claim 13.

15. A system as claimed in claim 14 wherein the image capture means comprises magnifying means,
16. A system as claimed in claim 15, wherein the magnifying means is a microscope.
17. A system as claimed in any one of claims 14 to 16 further comprising a motor operable to move the sample slide mount relative to the image capture means in a controlled manner .
18. A system as claimed in claim 17, wherein the motor comprises a raster scanner.
19. A method as substantially described herein with reference to the accompanying drawings .
20. A computer readable medium and a computer apparatus as substantially described herein with reference to the accompanying drawings.
21. A system as substantially described herein with reference to the accompanying drawings.

-1/2-

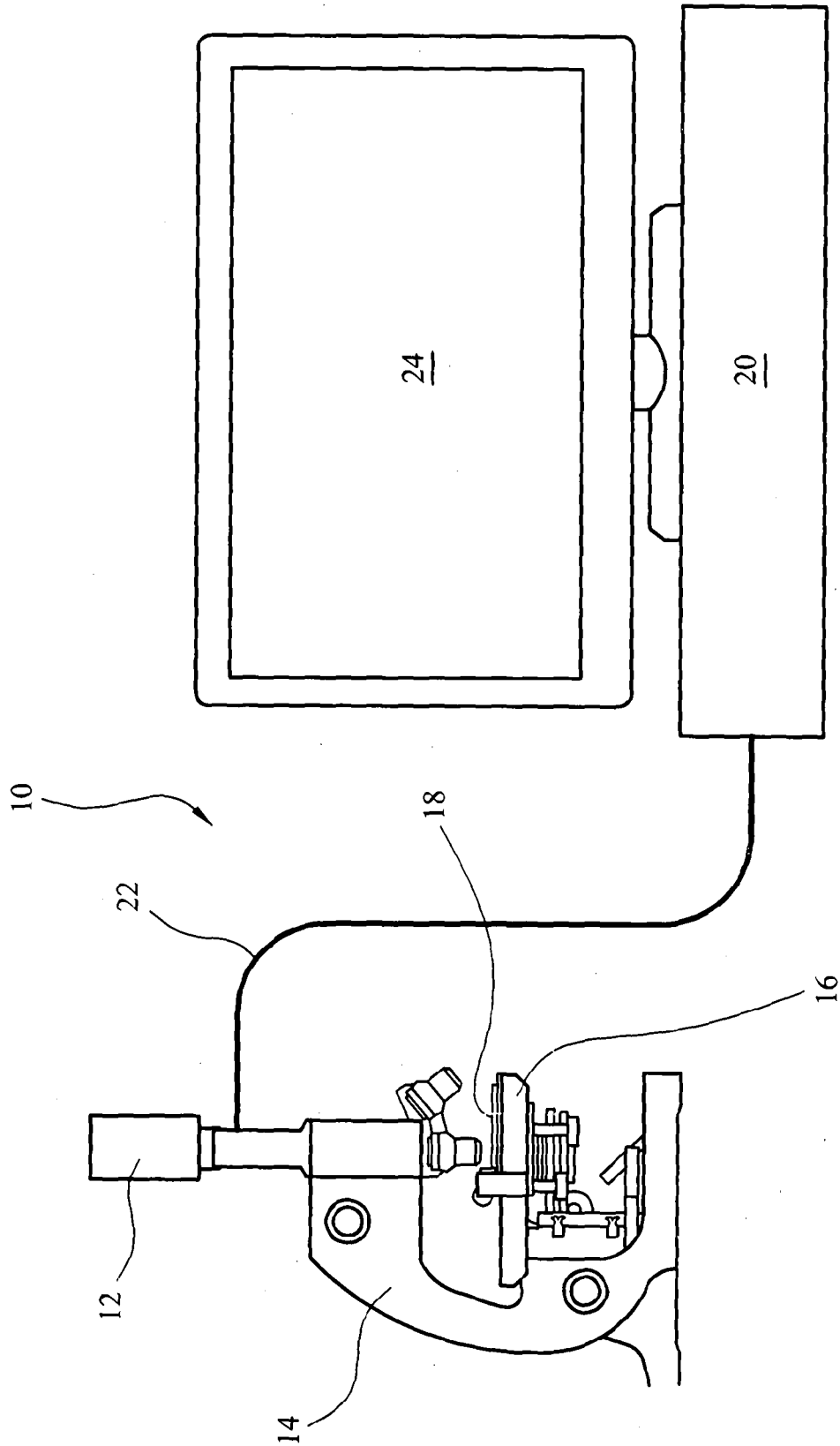
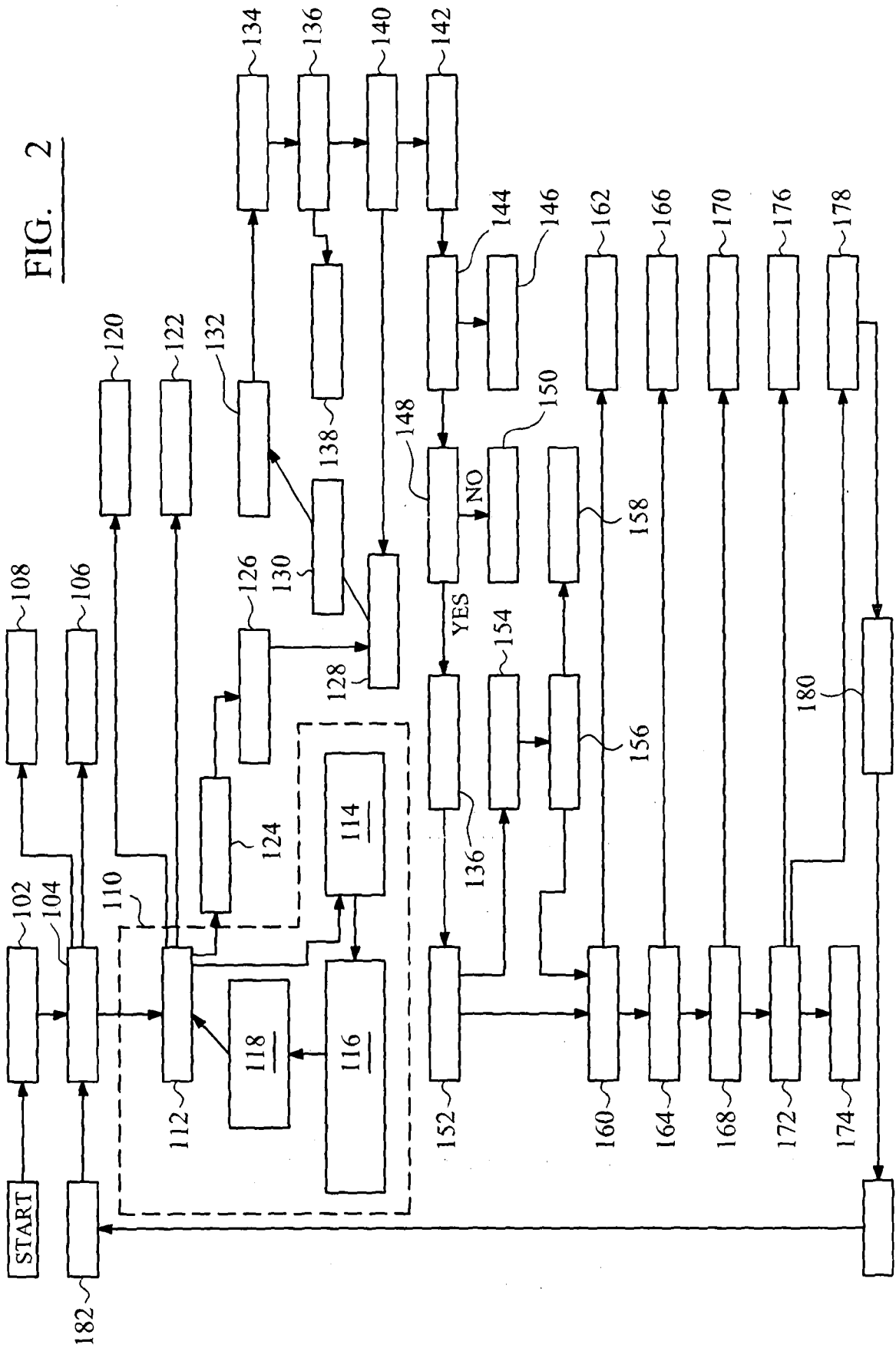


FIG. 1



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/000003

A. CLASSIFICATION OF SUBJECT MATTER
 INV. G01N33/50 G01N33/68 G01N21/00
 ADD.

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)
 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)
 EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Anonymous: "Scorpion Vision Based Cancer Detection System Unveiled", INTERNET ARTICLE, 8 November 2011 (2011-11-08), page 1, XP055057411, Retrieved from the Internet: URL:http://scorpionvision.co.uk/announcements/scorpion-vision-based-cancer-detection-system-unveiled [retrieved on 2013-03-21] the whole document ----- -/--	1-21

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"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</p> <p>"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</p> <p>"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</p> <p>"&" document member of the same patent family</p>
---	---

Date of the actual completion of the international search 8 April 2013	Date of mailing of the international search report 29/04/2013
---	--

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Weber, Peter
--	--

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2013/000003

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>Alessia Donnini ET AL: "Pilot study using an automated digital counting microscope in urine cytology", 6 November 2011 (2011-11-06), page 1, XP055057308, BT Convention Centre, Liverpool, UK Retrieved from the Internet: URL:http://scorpionvision.co.uk/newsletters/Cytology%20Pilot%20System.pdf [retrieved on 2013-03-21] the whole document</p>	1-21
X	<p>Alessia Donnini ET AL: "Pilot study using an automated digital counting microscope in urine cytology", 7th NCRI Cancer Conference, 6 November 2011 (2011-11-06), page 1, XP055057312, BT Convention Centre, Liverpool, UK Retrieved from the Internet: URL:http://www.ncri.org.uk/ncriconference/2011abstracts/abstracts/LB7.html [retrieved on 2013-03-21] the whole document</p>	1-21
X	<p>Paul: "Scorpion Vision for biomedical analyses", Scorpion Vision Web Shop Blog, 24 April 2009 (2009-04-24), page 1, XP055057440, Retrieved from the Internet: URL:http://scorpionvision.wordpress.com/2009/04/24/scorpion-vision-for-biomedical-analyses/ [retrieved on 2013-03-22] the whole document</p>	1-21