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Other: **WPI, EPODOC**

(54) Title of the Invention: **Optical analysis of fluid volumes under centrifugation**
Abstract Title: **Optical analysis of fluid volumes with centrifugal cuvette**

(57) A cuvette 1 for holding a fluid 24 for analysis has a housing defining an internal volume divided into plural interconnected, radially-extending, circumferentially-separated chambers10-13, each chamber being of an upstream type 11, 13 or a downstream type 10, 12. An inlet 16 communicates with at least one upstream chamber at a radially inward position. An outlet 14, 15 communicates with at least one downstream chamber at a radially inward position. At least one communicating passage 20-23 is provided between each upstream and downstream chamber at a radially outward position thereof. The sample spreads from the inlet 16 to fill the cuvette (figures 2b-2e). A method of use comprises at least partly filling the volume of the cuvette with a fluid sample, spinning the cuvette on an axis, passing optical radiation through the sample while the cuvette is spinning, and measuring an optical property of the sample as a function of time and radial position in the cuvette.

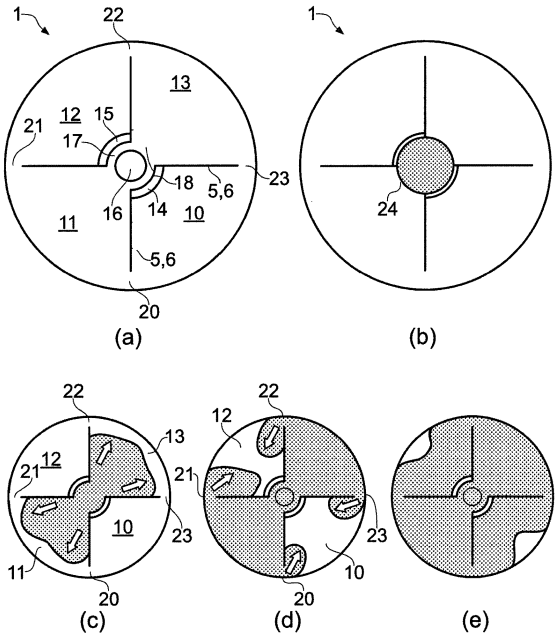

FIG. 2

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

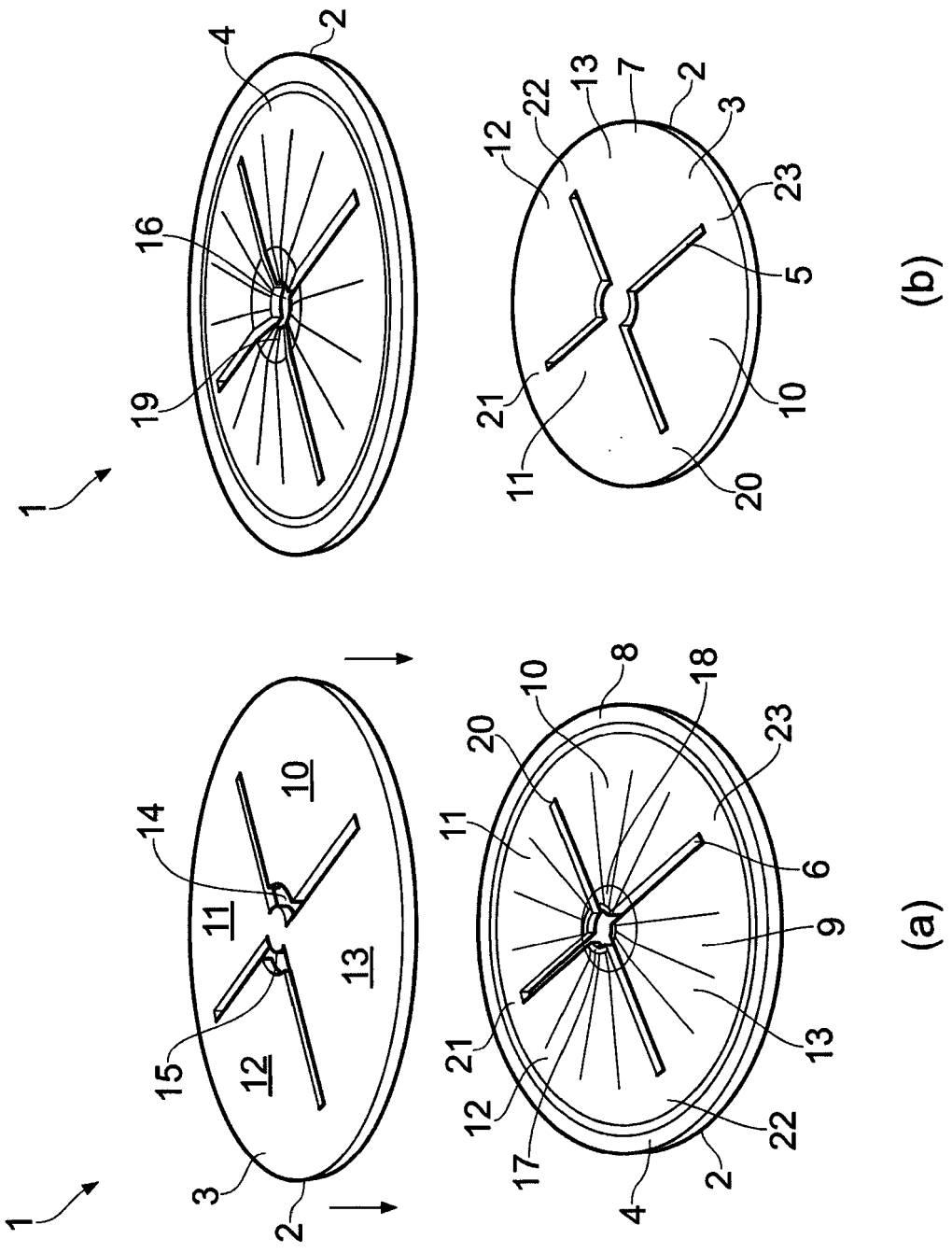
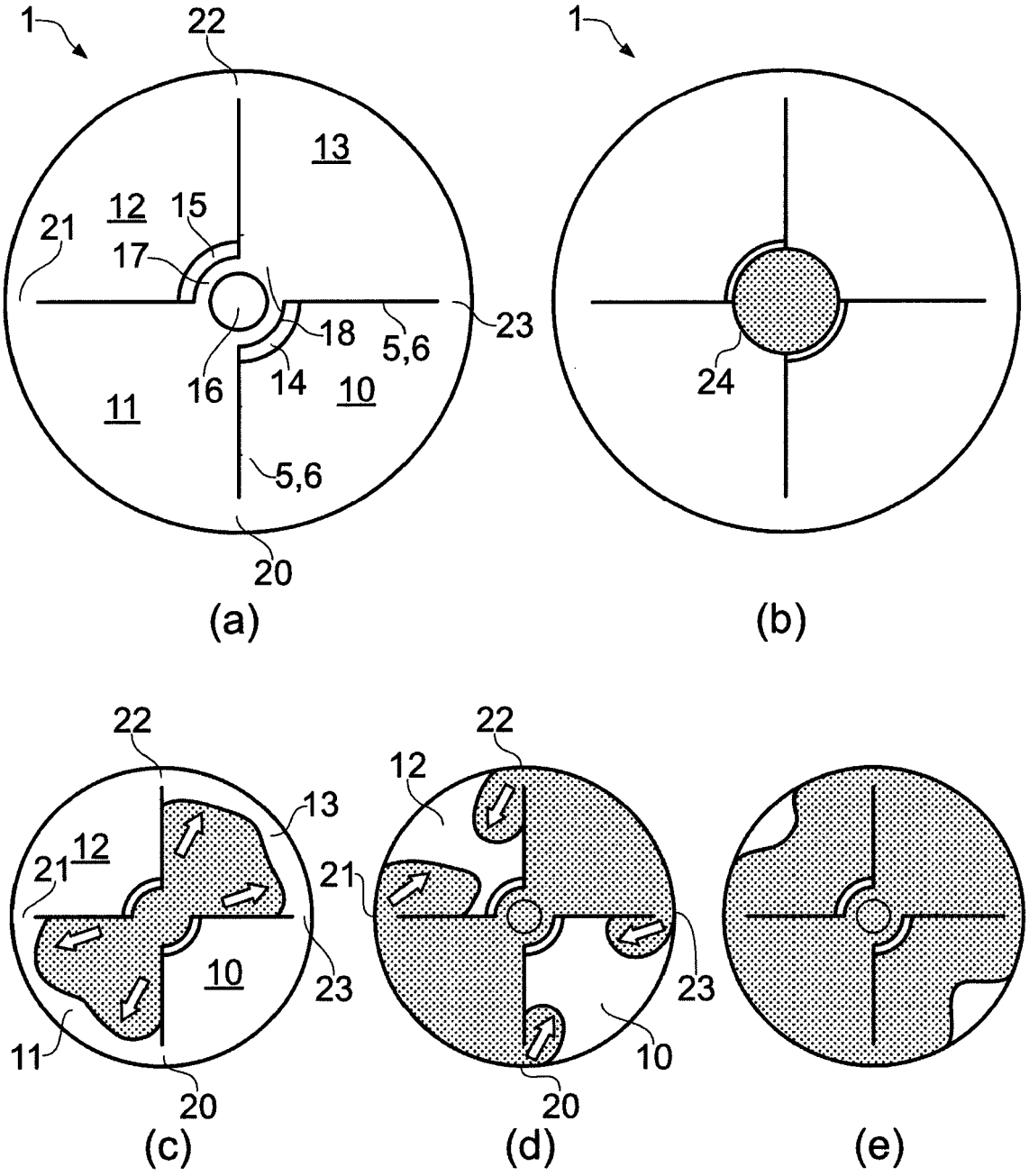

1
4
2
16
19
12
22
13
7
2
3
23
5
21
11
10
20
(b)
1
14
10
11
13
15
12
3
2
20
10
8
18
23
11
6
9
21
12
17
13
22
2
4
(a)

FIG. 1


1
22
13
12
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21
17
18
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5,6
16
14
10
11
5,6
20
(a)
1
24
(b)
22
13
12
21
10
23
11
20
(c)
22
12
21
23
10
20
(d)
(e)

FIG. 2

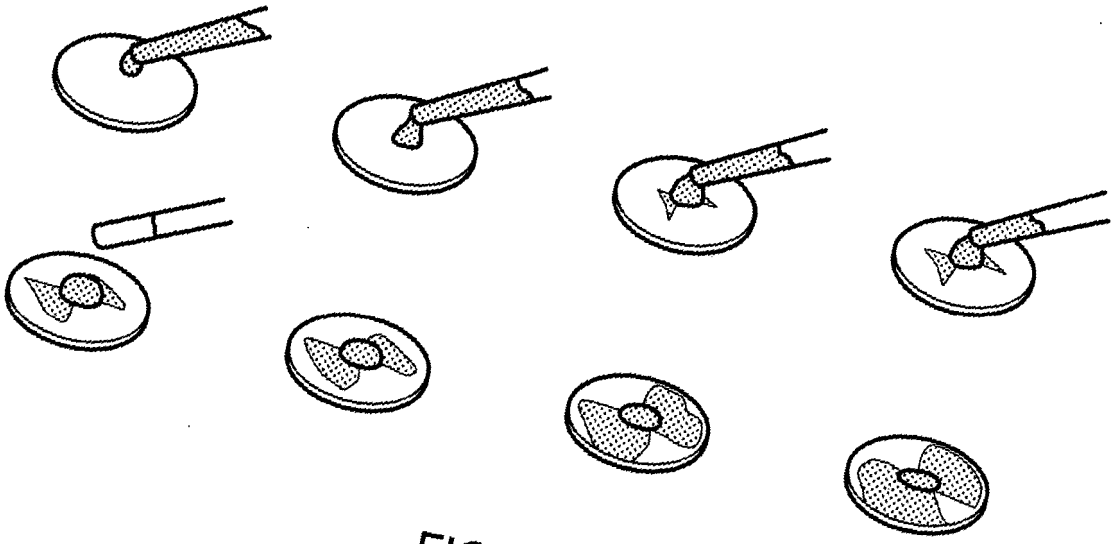


FIG. 3

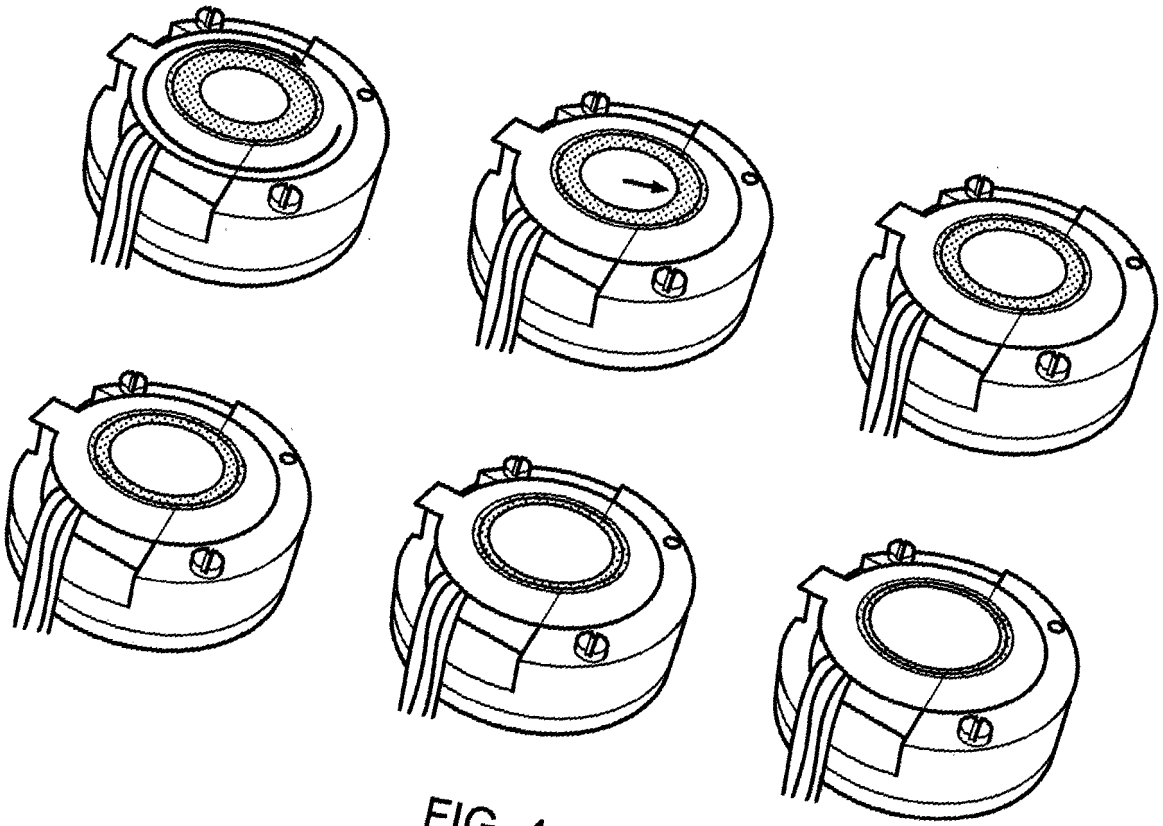
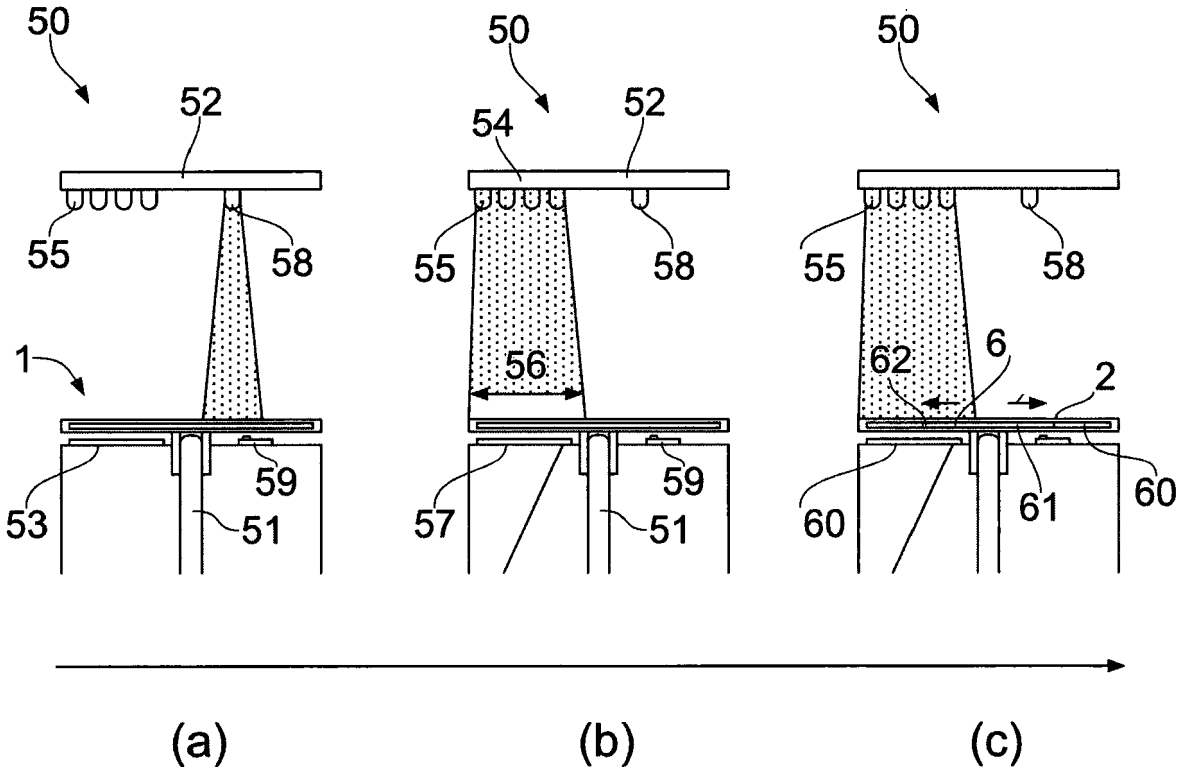


FIG. 4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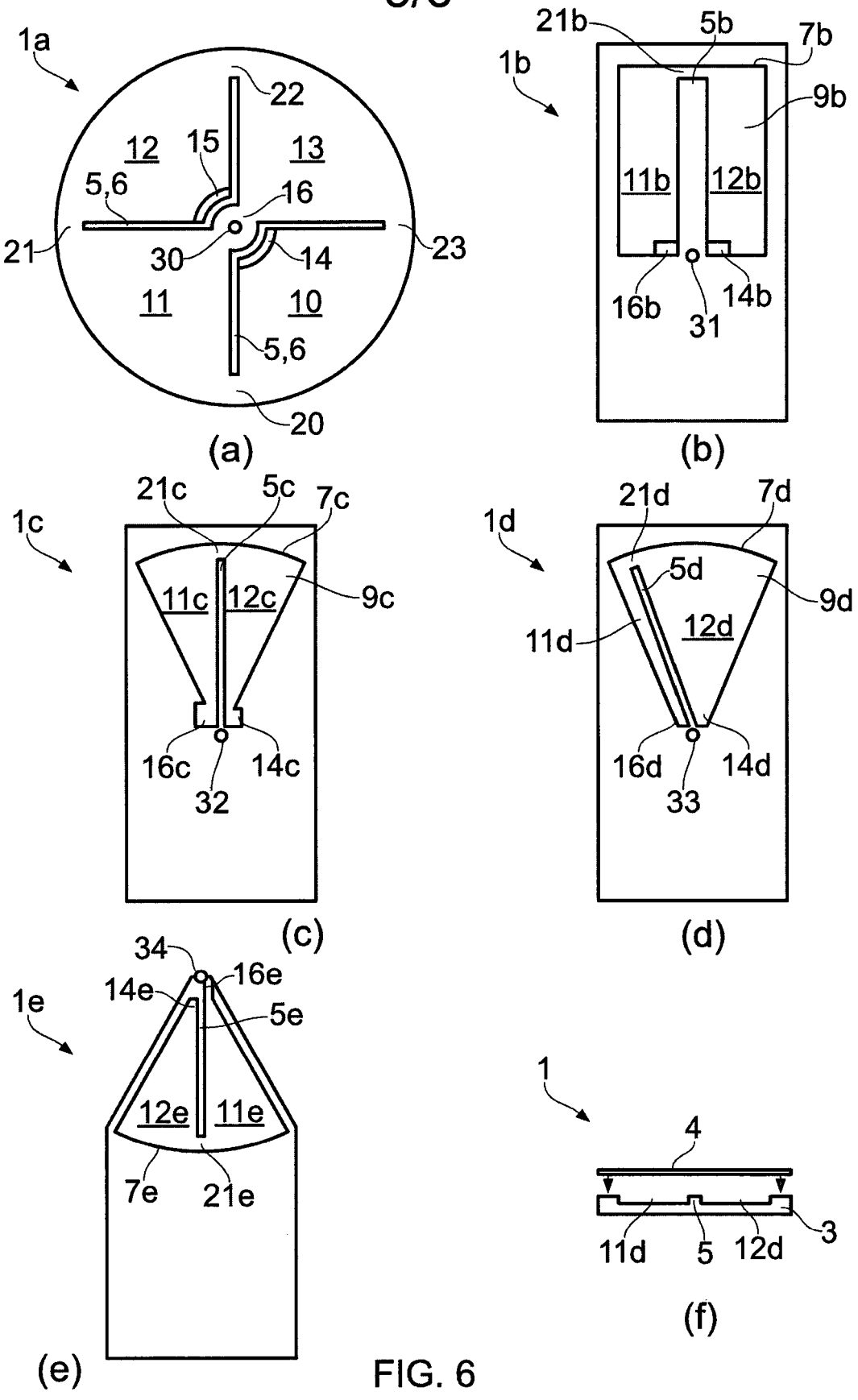
52
55
58
1
53
59
51
50
54
52
55
58
56
57
59
51
50
55
58
62
6
2
60
61
60
(a)
(b)
(c)

FIG. 5


1a
22
12
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16
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14
23
11
10
5,6
20
(a)
21b
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7b
1b
9b
11b
12b
16b
31
14b
(b)
21c
5c
7c
1c
11c
12c
9c
16c
14c
32
(c)
21d
7d
1d
5d
9d
11d
12d
16d
14d
33
(d)
34
16e
14e
1e
5e
12e
11e
7e
21e
(e)
1
4
11d
5
12d
3
(f)

FIG. 6

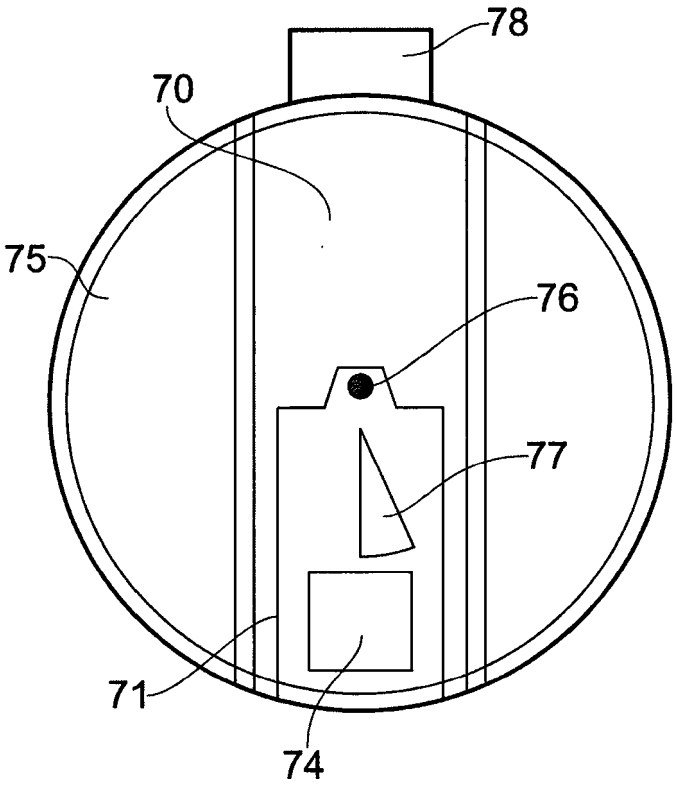

FIG. 7a

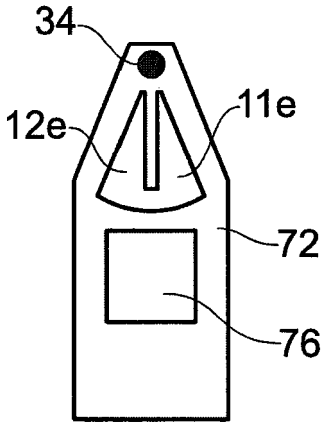

FIG. 7c

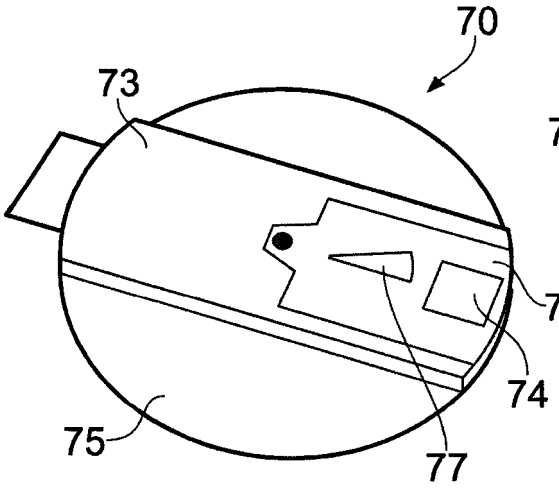

FIG. 7b

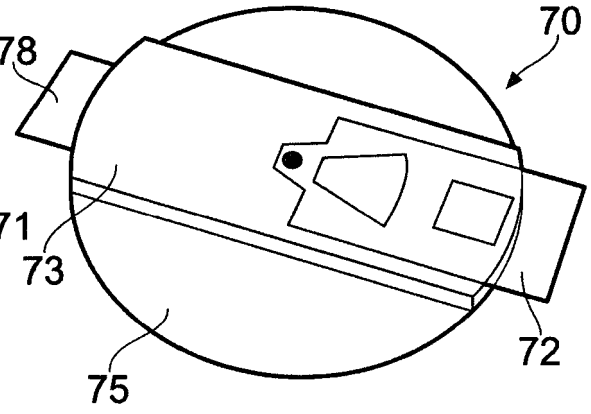

FIG. 7d

OPTICAL ANALYSIS OF FLUID VOLUMES UNDER CENTRIFUGATION

The present invention relates to the optical analysis of fluid samples under centrifugation, such as the analysis of a blood sample to determine haemoglobin, haematocrit and mean corpuscular volume.

Anaemia affects a substantial proportion of the global population and, once diagnosed, it is often curable. Anaemia is endemic in the developing world, and advanced diagnostic systems capable of differentiating types of anaemia and thereby promoting effective treatment have high cost and complexity. Low-cost, point-of-care devices are a desirable alternative, though typically they provide only haemoglobin measurements of less use for providing information on the type or cause of the anaemia. It is desirable to provide a low-cost method and apparatus suitable for the measurement of haemoglobin, haematocrit and mean corpuscular volume which may assist in the diagnosis and differentiation of different common types of anaemia.

It is an object of the present invention to provide a method and apparatus providing improvements in such measurement techniques.

According to one aspect, the present invention provides a cuvette comprising:

a housing defining an internal volume divided into a plurality of interconnected, radially-extending, circumferentially-separated chambers relative to an axis for rotation of the cuvette, each chamber being of a first, upstream, type or a second, downstream type;

an inlet communicating with at least one said upstream chamber at a radially inward position of the chamber;

an outlet communicating with at least one said downstream chamber at a radially inward position of the chamber;

at least one communicating passage between each upstream chamber and a downstream chamber at a radially outward position thereof.

The cuvette chambers may be each separated from an adjacent chamber by a radially extending wall. The upstream and downstream chambers may be disposed circumferentially in alternating fashion. Each upstream chamber may communicate with an inlet and each downstream chamber may communicate with an outlet. Each upstream chamber may communicate with a single inlet disposed on a central axis of the cuvette

and each downstream chamber may communicate with a separate outlet disposed radially outward of the inlet. The cuvette may comprise two upstream chambers and two downstream chambers each subtending an angle of approximately 90 degrees around the central axis. The cuvette may comprise an upstream chamber and a downstream chamber disposed alongside one another on one side of the axis for rotation of the cuvette. The cuvette may comprise only a single upstream chamber and a single downstream chamber. The communicating passage may be formed by the termination of the radially extending wall radially inward of an outer circumferential wall defined by the housing. The inlet may comprise an aperture in a front face of the housing and the outlet may comprise an aperture in a back face of the housing. The housing may comprise two discs coaxially mounted together face to face. The cuvette may define a front face and a back face of the housing, at least one of the faces of the housing being transparent to optical radiation. Both front face and back face may be transparent to optical radiation. The inlet, the outlet and the chamber dimensions may be configured such that water and/or blood disposed on the inlet will be drawn by capillary action into the chambers. The cuvette may have a generally rectangular shape in which the axis for rotation is a central axis of the cuvette. The axis for rotation may be disposed at or proximal to one edge of the cuvette.

According to another aspect, the present invention provides a method of determining an optical property of a fluid contained within a volume of a cuvette, comprising the steps of:

at least partly filling the volume of the cuvette with a fluid sample for analysis;

spinning the cuvette on an axis;

passing optical radiation through the fluid sample while the cuvette is spinning;

measuring an optical property of the spinning fluid sample as a function of time and radial position in the cuvette volume.

The measuring step may comprise determining a radial boundary position of the optical property induced by the centrifugal forces on the fluid sample by the spinning of the cuvette. The measuring step may comprise measuring the radial boundary position at least twice during the spinning of the cuvette. The method may include measuring a rate of change of radial boundary position and using the rate of change to determine a mean volume of solids within the fluid sample. The fluid sample may be blood and the mean volume of solids may comprise the mean corpuscular volume. The method may include measuring a rate of change of radial boundary position and using the rate of change to determine a total volume of solids. The fluid sample may be blood and the total volume

of solids comprise the haematocrit. The method may include using the radial boundary position to determine a ratio of one fluid type to another fluid type. The fluid sample may be blood and the method comprises calculating haematocrit of the blood. The optical property may be transmissivity or absorptivity of the fluid sample. The fluid sample may be contained within a circumferentially- and radially-extending volume of the cuvette, and the spinning step may comprise spinning the cuvette on its axis. The step of measuring may comprise simultaneously monitoring the optical property at a plurality of radial positions by detecting optical radiation from the fluid sample at a plurality of radial positions by plural detectors. The method may comprise detecting the optical transmissivity or absorptivity at plural wavelengths of light. The fluid sample may be blood and the method comprises calculating a haemoglobin level based on the measured optical transmissivity or absorptivity at two different wavelengths of light. The haemoglobin level may be calculated at a function of optical transmissivity or absorptivity at one wavelength of light and as a function of haematocrit level determined according to a radial boundary position of the optical property induced by the centrifugal forces on the fluid sample by the spinning of the cuvette measured at a different wavelength of light.

Embodiments of the present invention will now be described by way of example and with reference to the accompanying drawings in which:

Figure 1a shows a perspective underside view of a cuvette with components separated;

Figure 1b shows a perspective topside view of a cuvette with components separated;

Figure 2 shows a schematic plan view of the cuvette of figure 1b during various stages of a filling operation;

Figure 3 shows perspective topside views of the cuvette of figure 1b during various stages of a filling operation;

Figure 4 shows perspective topside views of the cuvette of figure 3 at various stages during a centrifugation process;

Figure 5 shows a schematic side view of various stages of the centrifugation process of figure 4;

Figures 6a to 6e show schematic plan views of alternative forms of cuvette, and figure 6f shows a schematic cross-sectional view illustrating a method of assembly of the cuvettes of figures 6b to 6e;

Figures 7a and 7b show, respectively, a plan view and a perspective top view of a cuvette holder suitable for use with a centrifuge; figure 7c shows a cuvette suitable for use in the cuvette holder of figures 7a and 7b; and figure 7d shows the cuvette holder of figures 7a and 7b with the cuvette of figure 7c mounted thereon.

Throughout the present specification, the descriptors relating to relative orientation and position, such as "top", "bottom", "up", "down", "lid", "base", "topside", "underside", as well as any adjective and adverb derivatives thereof, are used in the sense of the orientation of the apparatus as presented in the drawings. However, such descriptors are not intended to be in any way limiting to an intended use of the described or claimed invention.

With reference to figures 1a and 1b, there is shown a cuvette 1 having a housing 2 formed from a base 3 and a lid 4. Figure 1a shows the housing components 3, 4 from the underside perspective and figure 1b shows the housing components 3, 4 from the topside perspective. One or other or both of the base 3 and lid 4 define a peripheral circumferential side wall or rim 7, 8 which can engage with the other component when the base and lid are assembled, to form an internal volume 9 of the cuvette. The base 3 and the lid 4 can be assembled together in any suitable manner, such as by glue or by a laminating sheet.

As best seen in figure 1b, the base 3 includes a set of radially extending base ribs 5 and, as best seen in figure 1a, the lid 4 includes a set of radially extending lid ribs 6. The base ribs 5 and the lid ribs 6 are preferably formed such that they co-operate with one another such that when the base 3 and the lid 4 are assembled together as indicated by the arrows, they engage to define four chamber walls within the housing. The four chamber walls 5, 6 divide the internal volume 9 into a set of interconnected chambers 10, 11, 12, 13. The interconnection between the chambers 10-13 can be provided by the ribs 5, 6 terminating at a radially outward position just short of the circumferential side wall 7 and/or 8, to define communicating passages 20, 21, 22, 23. More generally, the ribs 5, 6 may comprise any suitable ridges, walls, channels, struts, tongue-and-groove arrangements, etc and may be disposed on either one or both of the base 3 and the lid 4 in a manner suitable to define the chamber walls.

The base 3 includes a pair of apertures 14, 15 which each occupy a space at a radially inward position within chambers 10, 12. The lid 4 includes a central aperture 16 adjacent to a radially inward end of each of the ribs 5, 6. When the cuvette 1 is assembled, the

apertures 14, 15 provide a communication port out of the chambers 10, 12 and the central aperture 16 provides a communication port into the chambers 11, 13. The central aperture 16 is not in direct communication with chambers 10, 12 because of upstanding walls 17, 18 extending between lid ribs 6 and radially inward of apertures 14, 15.

Thus, it can be seen that the cuvette 1 of figure 1 exemplifies an arrangement having a housing 1 with an internal volume 9 which is divided into a plurality of interconnected, radially-extending and circumferentially separated chambers 10-13. If the central aperture 16 is used as an inlet to the cuvette 1, the chambers 11, 13 can conveniently be referred to as upstream chambers and if the pair of apertures 14, 15 are used as outlets, the chambers 10, 12 can be conveniently referred to as downstream chambers.

The lid 4 preferably includes a depression 19 (e.g. a countersink) in the upper surface surrounding the central aperture 16. This depression 19 acts as a filling reservoir for fluid to flow from, into the central aperture 16 when the central aperture acts as an inlet communicating with the upstream chambers 11, 13.

In a preferred arrangement, the internal volume 9 of the cuvette 1 is less than 100 microlitres and the apertures 14, 15, 16 and chamber 10-13 dimensions are sufficiently small that the cuvette 1 will fill from the reservoir 19 by capillary action and will thereby be self-filling.

The cuvette 1 is preferably constructed entirely from transparent plastic or other suitable material. Some or all of the internal walls may be coated with an anticoagulant, if the cuvette is being used for blood analysis.

Figure 2 illustrates a method of filling the cuvette 1 with a fluid sample such as blood. Figure 2a shows a schematic plan view of the central aperture 16, the chambers 10, 11, 12, 13 separated by the ribs 5, 6 but with communicating passages 20, 21, 22, 23 at radially outward positions at the ends of the ribs, thereby providing communication between adjacent chambers. The apertures 14, 15 respectively provide air release holes for chambers 10 and 12.

With reference to figure 2b, a drop of liquid / suspension, e.g. blood, is placed onto the upper face of the lid 4 within the depression 19 thereby forming a reservoir of blood 24.

The blood is drawn into the internal volume 9 of the cuvette 1, through the aperture 16, by capillary action, so that it starts to fill the upstream chambers 11, 13 as seen in figure 2c. When the upstream chambers 11, 13 are substantially filled, the blood will continue into the downstream chambers 10, 12 via communicating passages 20, 21, 22, 23, as seen in figure 2d. The communicating passages 20-23 occupy radially outward positions of the chambers 10-13. Air is able to escape via the apertures 14, 15 which occupy radially inward positions of downstream chambers 10, 12, and eventually the internal volume 9 of the cuvette 1 is substantially filled as seen in figure 2e.

The internal volume 9 of the cuvette is preferably sized such that one drop of liquid provided in the depression 19 reservoir can completely or substantially fill the internal volume. Once the internal volume 9 has filled, no further blood will be drawn into the internal volume.

The walls defined by the lid 4, base 3, ribs 5, 6 and side wall 8 generally provide a structure which enables the blood to follow a set path through the internal volume 9 so as to substantially fill the cuvette.

Figure 3 shows a series of images taken over 4 seconds as the cuvette 1 fills with blood.

The arrangements described above generally provide a self-filling cuvette designed for centrifuging microliter volumes of blood whilst under continuous optical analysis. The optical response of the blood within the cuvette can be used to determine the mean corpuscular volume, haemoglobin concentration and haematocrit as will described below.

Figure 5 shows a schematic view of the cuvette 1 mounted into a centrifuge apparatus 50 which incorporates an optical analysis system 52, 53. The filled cuvette 1 is mounted on a spindle 51 driven by an electric motor (not shown). An optical illumination module 52 is disposed over the top of the cuvette 1 and a detector module 53 is disposed beneath the cuvette 1. The optical illumination module 52 includes a broad spectrum illumination source 54, such as a white light illumination source. In the arrangement shown, the illumination source 54 comprises an array of LEDs 55. A linear array of LEDs 55 may be deployed. The broad spectrum illumination source 54 is configured to illuminate a radially extending portion of the cuvette 1 indicated in figure 5b at 56, and any suitable illumination source may generally be used which can illuminate such a radial extent of the cuvette 1

in the centrifuge apparatus 50. It may be advantageous to provide the illumination source with a radial intensity profile as flat as possible, though providing a known radially varying intensity profile could be used.

The detector module 53 includes a detector 57 (or detector array 57) sensitive to radiation from the illumination source 54 at various positions under the cuvette 1 so that different levels of transmission / absorption by the blood sample in the cuvette can be detected or determined, as a function of radial position.

To this end, the detector array 57 may be an array of photodiodes. The detector array 57 is able to sample the transmission / absorption at multiple times during the centrifugation of the cuvette. The detector module 53 is thereby configured to measure an optical property of the spinning fluid sample in the cuvette 1 as a function of time and radial position in the cuvette.

In one particular arrangement, the optical illumination source 54 also includes a narrow spectrum illumination source 58, which may be one or more green LEDs. A corresponding detector 59 is disposed in the detector module 53 in a position suitable to detect transmission / absorption of the narrow spectrum illumination from the source 58, by the sample in the cuvette 1. The detector 59 may be a phototransistor. For reasons discussed below, the narrow spectrum illumination source 58 and detector 59 need not necessarily be provided as arrays extending over a radially extending portion of the cuvette 1 but can be positioned relative to any radial position of the cuvette.

A process for operation of the centrifuge apparatus 50 is now described. The cuvette 1 and blood sample within it are mounted on the spindle 51. The centrifuge apparatus 50 may first spin the cuvette 1 at a relatively low speed which is sufficient to remove air bubbles and ensure good distribution of the liquid sample within the chambers 10-13 but insufficient to start separation of the blood components. A suitable relatively low speed may lie within the range 200 to 2000 rpm.

The cuvette 1 is illuminated by the narrow spectrum illumination source 58 (e.g. green LED at 510 nm), as depicted in figure 5a. The intensity of the light transmitted through the cuvette is measured by the detector 59.

The cuvette 1 is illuminated by the broad spectrum illumination source 54 and the centrifuge is accelerated to relatively high speed, e.g. ≥ 5000 rpm, as depicted in figure 5b. Red blood cells will be displaced to outer radial positions within the cuvette as depicted in figure 5c and as shown in the sequential images of figure 4. It can be seen with reference to figures 5b and 5c that while the red blood cells are dispersed throughout the sample (figure 5b) the detector array 57 will detect low levels, and approximately equal levels of absorption of the illuminating light at each radial position. As the centrifugation process separates the heavier components (e.g. red blood cells) to radially outward positions in the cuvette, levels of optical absorption by the sample at radially inward positions will fall over time, and levels of optical absorption by the sample at radially outward positions will remain steady or possibly increase slightly.

By measuring the optical absorption levels both as a function of time and as a function of radial position, it is possible to obtain a measure of both the amount of heavier component that is centrifuged to the radially outward positions and the time taken for those components to reach those radially outward positions. For a blood sample in the cuvette, the amount of heavier component is indicative of the proportion of red blood cells (e.g. total solids) and is thus indicative of haematocrit (Hct), while the time taken for the red blood cells to move to the radially outward positions is indicative of mean corpuscular volume (MCV). Further, the absorption of green light compared to the broad spectrum absorption indicative of red blood cells, or more particularly, the haematocrit determined using the radial position of such absorption after centrifugation, is indicative of the haemoglobin level.

Thus, the haematocrit can be determined according to the radial starting and ending positions of the absorption which is indicative of red blood cells. The detector module 53 is capable of determining a radial boundary position 62 between red blood cells 60 centrifuged outwards and serum 61. Thus, the haematocrit Hct may be calculated as a function of the area ratio of serum to red blood cells e.g. as a function of r_{final}, r_{start}, $r_{cuvette}$, using the equation,

$$Hct = \left(\frac{r_{cuvette}^2 - r_{final}^2}{r_{cuvette}^2 - r_{start}^2}\right),$$

where r_{final} is the last inner radius value measured, r_{start} is the first radius measured and $r_{cuvette}$ is the radius of the cuvette used. This assumes that the walls separating the

chamber are of negligible thickness. Adjustments can be made accordingly in the event that the walls are of non-negligible thickness. The radii r can be calculated from the intensity of the transmitted light such that

$$r = gT$$

where g is a calibration factor and T is the transmitted intensity.

The radii can be determined by detecting: (i) an initial radial boundary position of the interface between red blood cells 60 and any empty space at the most radially inward position of the chambers 10-13, e.g. if the chambers are not completely full, or the most radially inward position of the chambers, if full; and (ii) a radial boundary position 62 of the interface between the red blood cells 60 and the serum 61 after centrifugation.

The radial boundary position can be determined directly or indirectly. For example, the radial boundary position can be determined by reference to the absorption level at each of a plurality of radial positions as measured at those positions by separate elements in a detector array 57, i.e. by detecting which elements in the (spatially-resolvable) array detect high absorption / low transmission and which elements in the array detect low absorption / high transmission. In this case, the radial boundary position may be measured directly, according to when the absorptivity or transmissivity falls or rises to a predetermined threshold.

Alternatively, if the detector module 53 does not provide for multiple spatially resolvable (i.e. separately addressable) detector elements in an array, the radial boundary position may be deduced, i.e. calculated, indirectly based on an absolute total intensity level detected by the detector module over the entire radial extent of the cuvette. If the total intensity level is known (e.g. measured, or assumed) at the start before high speed centrifugation, the radial boundary position of the interface between red blood cells and serum may be deduced by the increasing intensity level received at the detector module 53 as a result of an increasing size of 'window' of serum at 61, above the detector module (figure 5c). The serum 61 blocks substantially less light than the red blood cells 60 and the system may be calibrated with a calibration factor g to provide a relationship between intensity and radial position of the boundary between red blood cells and serum. The starting intensity at the detector module 53 may indicate r_{start} and the final intensity at the

detector module after intensity stops rising during centrifugation may indicate r_{final}. In this arrangement, the radial boundary position is deduced according to the absolute measured level of total absorptivity or transmissivity over the entire radial extent of the detector 57. The detector 57 may therefore comprise a large area detector extending over the entire radius of the cuvette, or at least capturing light from the entire radius of the cuvette.

Thus, the haematocrit Hct may alternatively be calculated from a measured transmission intensity at the end of centrifugation according to the equation:

$Hct = gT_f$

where g is a calibration factor determined by a calibration curve and T_f is the (final) transmission intensity at the end of centrifugation, e.g. when the intensity stops increasing or after a fixed period which is known to be sufficient to effect full separation of the blood components. For accuracy, the above equation may require that the cuvette is completely filled to a predetermined, i.e. known level.

It may also be possible to determine haematocrit Hct by reference to a rate of change of the radial boundary position (e.g. the blood-serum boundary), rather than requiring the final position of the boundary. The rate of change, or "centrifugation response", may provide sufficient information to deduce the final radial boundary position where relevant characteristics of the blood and serum are known in advance or can be assumed. The rate of change can be measured either by measuring directly the position r at suitable intervals, or measuring the transmission intensity T at suitable intervals to deduce r, as discussed above. It may be possible to obtain a relatively accurate estimate of Hct from the centrifugation response alone and not from the final position of the radial boundary position.

In yet another arrangement, the radial boundary position can be determined by a moving (scanning) detector which repeatedly scans in a radial direction during centrifugation to determine the location of the radial boundary position 62 multiple times during the centrifugation process. In this arrangement, the scanning detector should be capable of performing scans at sufficiently fast rate compared to the centrifugation time required to separate red blood cells from serum, that the required temporal resolution is obtained.

The mean corpuscular volume MCV is related to, and may be proportional to, the time taken for the red blood cells to be forced (centrifuged) out.

$$MCV = h(T, t)$$

where h is a function that depends on the transmission intensity T over the course of centrifugation and the time, t, taken. Completion of the centrifugation process for the red blood cells to be separated from the serum can be determined when the radial boundary position stops moving, or when the total intensity measurement stops changing.

The haemoglobin concentration (Hgb) may be calculated from the absorption of the green light. To account for the scattering of the red blood cells, the calculated haematocrit value may be used. The equation to calculate haemoglobin may be of the form

$$Hgb = f(T_s, Hct)$$

where f is a function that depends on the haematocrit Hct and the transmission intensity, T_s before centrifugation or when the cuvette is spun at low speed insufficient to start separation of the blood components.

Various changes can readily be made to the particular design of cuvette shown in figures 1 to 3.

For example, the internal volume 9 need not be divided into four chambers 10-13. The system can work with just two chambers, one being an upstream chamber coupled to the inlet and one being a downstream chamber coupled to the outlet, with one or two fluid communication channels coupling the chambers at, e.g. 0 and 180 degree positions on the circumference of the cuvette. More than four chambers could be used, e.g. dividing the cuvette into any number of circumferentially distributed sectors. An odd number of sectors can be used, e.g. three, since one upstream chamber can communicate with two downstream chambers, or vice versa.

For optimal filling performance (e.g. time and distribution), it may be preferable to distribute the upstream and downstream chambers circumferentially in alternating fashion. In this way, each upstream chamber 11, 13 can feed two adjacent downstream chambers 10, 12 via the communicating passages 20-23. However, other arrangements are possible.

It may be advantageous to provide upstream and downstream chambers that occupy substantially the entire circumference of the cuvette, e.g. with just ribs 5, 6, separating the chambers, since this will maximise the circumferential extent of fluid sample from which optical signals can be obtained during centrifugation. More generally, it may be desirable for the chambers 10-13 to together subtend a substantial portion of the total circumferential angle of the cuvette, e.g. greater than 50% (180 degrees), or greater than 75% (270 degrees), or greater than 90% (324 degrees). However, if appropriate signal magnitude and/or to signal-to-noise ratio can be obtained from an arrangement where the fluid sample does not occupy the full circumferential extent of the cuvette, then such an arrangement can be considered.

Similarly, it may be advantageous to provide upstream and downstream chambers that occupy substantially the entire radial extent of the cuvette (minus the spaces required for inlet and outlet apertures and any axial engagement arrangement for spinning the cuvette), since this may maximise the resolution available for measuring the optical property of the sample as a function of radial position. This can be achieved by the preferred central position of aperture 16 and near central positions of apertures 14, 15. However, smaller chambers can be considered where appropriate.

The embodiments described above show a circular disc shape for the cuvette, since this may provide for optimum balance under centrifugation and for optimum provision of radial and circumferential extent of the sample under analysis for a given volume of sample. However, the cuvette need not be circular. Figure 6 shows some alternative configurations of cuvette.

Figure 6a shows the circular arrangement of cuvette 1a already generally described in connection with figures 1 and 2 for comparison purposes, having a central axis of rotation 30, chambers 10-13, ribs 5, 6 separating the chambers, central aperture 16 forming an inlet and apertures 14, 15 forming outlets, and communicating passages 20-23 at radially outward positions at the ends of the ribs 5, 6 providing communication between adjacent chambers.

Figure 6b shows a rectangular cuvette 1b having a central axis of rotation 31 and an internal volume 9b divided into two interconnected, radially-extending and circumferentially separated chambers 11b, 12b, each of generally rectangular shape. An aperture 16b

serves as an inlet to upstream chamber 11b and an aperture 14b serves as an outlet from downstream chamber 12b. The designation of upstream and downstream chambers, and inlet and outlets, can be reversed. A rib 5b defines a chamber wall separating the upstream chamber 11b and the downstream chamber 12b. The rib 5b, and thus chamber wall, terminates at a radially outward position just short of a circumferential side wall 7b to define a communicating passage 21b between the upstream and downstream chambers 11b, 12b. The method of filling and use of the cuvette 1b is similar to that of cuvette 1, 1a.

For each of the non-circular cuvettes shown in figure 6, the expression "radially-extending" is intended to encompass structure that extends away from the axis of rotation (e.g. the rib 5b and chambers 11b, 12b), and the expressions "radially inward" and "radially outward" respectively refer to positions closer to, or further from, the axis of rotation along a radial line. Similarly, the expressions "circumferential", "circumferentially-separated" and "circumferentially-extending" refer to positions around the axis of rotation. The axis of rotation is orthogonal to the plane of the chambers of the cuvette.

Figure 6c shows a rectangular cuvette 1c having a central axis of rotation 32 and an internal volume 9c divided into two interconnected, radially-extending and circumferentially separated chambers 11c, 12c, each of a generally triangular shape or in the shape of a sector of a circle. An aperture 16c serves as an inlet to upstream chamber 11c and an aperture 14c serves as an outlet from downstream chamber 12c. The designation of upstream and downstream chambers, and inlet and outlets, can be reversed. A rib 5c defines a chamber wall separating the upstream chamber 11c and the downstream chamber 12c. The rib 5c, and thus chamber wall, terminates at a radially outward position just short of a circumferential side wall 7c to define a communicating passage 21c between the upstream and downstream chambers 11c, 12c. The method of filling and use of the cuvette 1c is similar to that of cuvette 1, 1a.

Figure 6d shows a rectangular cuvette 1d having a central axis of rotation 33 and an internal volume 9d divided into two interconnected, radially-extending and circumferentially separated chambers 11d, 12d, each generally in the shape of a sector of a circle. An aperture 16d serves as an inlet to upstream chamber 11d and an aperture 14d serves as an outlet from downstream chamber 12d. The designation of upstream and downstream chambers, and inlet and outlets, can be reversed. A rib 5d defines a chamber wall separating the upstream chamber 11d and the downstream chamber 12d. The rib 5d, and

thus chamber wall, terminates at a radially outward position just short of a circumferential side wall 7d to define a communicating passage 21d between the upstream and downstream chambers 11d, 12d. The cuvette 1d is similar to the cuvette 1c except that the chamber wall 5d divides the internal volume 9d into different volumes of chamber 11d, 12d. Different volumes of chamber 11d, 12d may also be applied to the other cuvettes described herein. The method of filling and use of the cuvette 1d is similar to that of cuvette 1, 1a.

Figure 6e shows a generally rectangular cuvette 1e with a tapering end, and having an axis of rotation 34 which is at, proximal to, or towards, a peripheral edge of the cuvette. An internal volume 9e is divided into two interconnected, radially-extending and circumferentially separated chambers 11e, 12e, each of a generally triangular shape or in the shape of a sector of a circle. An aperture 16e serves as an inlet to upstream chamber 11e and an aperture 14e serves as an outlet from downstream chamber 12e. The designation of upstream and downstream chambers, and inlet and outlets, can be reversed. A rib 5e defines a chamber wall separating the upstream chamber 11e and the downstream chamber 12e. The rib 5e, and thus chamber wall, terminates at a radially outward position just short of a circumferential side wall 7e to define a communicating passage 21e between the upstream and downstream chambers 11e, 12e. The method of filling and use of the cuvette 1e is similar to that of cuvette 1, 1a. The positioning of an aperture (e.g. 16e) serving as an inlet to the upstream chamber (e.g. 11e) on an edge of the cuvette means that edge filling of the cuvette may be possible.

The cuvette 1e is similar to cuvette 1c except that the axis of rotation 34 is at, proximal to, or close to a peripheral edge of the cuvette. The cuvette 1e can be very small and light weight such that the unbalanced nature of the structure around an axis of rotation does not cause a problem under centrifugation. Alternatively, the cuvette 1e could be mounted onto a platform having a central axis at which cuvette axis 34 can be located. An example of such a platform, e.g. serving as a cuvette holder or cuvette mount, will be described in connection with figure 7.

Each of the arrangements of figures 6b to 6e generally exemplify a cuvette with a single upstream chamber and a single downstream chamber disposed alongside one another on one side of a rotation axis of the cuvette.

The axis of rotation for all described embodiments can include any suitable structure for engaging with a mounting spindle of a centrifuge.

Figure 6f shows a cross-sectional view of, e.g., the cuvettes of figures 6b, 6c, 6e showing the rib 5 dividing internal volume 9 into upstream and downstream chambers 11, 12. The cuvette of figure 6d is similar with different position of the rib 5. The cuvettes generally described in this specification can be formed from a suitably profiled base 3 covered with a sealing film 4 serving as a lid which can be placed over the base 3 and adhered thereto as indicated in figure 6f.

In the preferred arrangements, the cuvette is formed from a base 3 and a lid 4 both of which are transparent to optical radiation, so that an illumination source 54 can be disposed on one side of the cuvette 1 and an optical detector 53 can be disposed on the other side of the cuvette. This allows a direct measure of optical absorptivity through the cuvette. However, alternative arrangements can be considered, e.g. where one of the base 3 or the lid 4 provides a reflective internal surface so that both illumination source and detector can be disposed on the same side of the cuvette. The cuvette 1 need not be optically transparent in regions to which the chambers do not extend.

Although the embodiments described above are in connection with measurement of optical absorption or transmission, the cuvette described could also or alternatively be used for measurement of other optical properties, e.g. fluorescence of the sample under illumination with an appropriate excitation beam. Similar measurement of a changing radial distribution of components of a sample under centrifugation can be determined, e.g. by monitoring fluorescence levels or another optical property.

In one exemplary arrangement, the diameter of the disc shaped cuvette of figure 1 is 22 mm, the thickness or height is 1 mm, the chamber diameter is 20 mm, the chamber height is 100 microns, the total internal volume is between 30 and 60 microliters, and the central inlet aperture 16 is 2 mm in diameter. With such an arrangement, a filling time for blood is generally in the range 4 to 8 seconds.

Although the described physical arrangements of cuvette are particularly suitable for use with the described measurement process of monitoring an optical property of a spinning

fluid sample as a function of time and radial position in the cuvette volume, other forms of cuvette could be used with this method.

Although a particularly useful application for the described cuvette and the described method is in the determination of haematocrit, mean corpuscular volume and haemoglobin in blood, it will be recognised that the apparatus and method can be used for optically determining properties of other samples, other than blood.

In general, a radial boundary position can be determined for any distribution of at least two matter types in the chambers where the at least two matter types are distinguishable by their different optical properties and the action of centrifugation results in a redistribution of the matter types as a function of radial position from a centrifugation axis resulting in a change in optical properties as a function of radial position.

Figure 7a shows a cuvette holder 70 suitable for mounting a cuvette 1e as described in connection with figure 6e. Various modifications may be made to the cuvette holder to make it suitable for holding other cuvettes, such as those described in connection with figures 6b to 6d.

Cuvette holder 70 comprises a disc 75 of suitable material with a recess 71 shaped to receive a cuvette 72 (figure 7c). The recess 71 may be defined within a platform 73 bonded or otherwise attached onto the top of the disc 75. A raised block 74 is formed within the recess to provide an engagement structure which engages with a correspondingly shaped recess 76 in the cuvette 72 (figure 7c). A central axis of rotation 34 of the cuvette 72 engages with an axis of rotation 76 of the cuvette holder 70 and the engagement structures exemplified by raised block 74 and recess 76 ensure that the cuvette can be held tightly within the holder 70 during a centrifugation process. The cuvette holder 70 may also include an analysis window 77 in the disc 75 to improve optical transmission through the cuvette and cuvette holder when the cuvette 72 is mounted on the holder 70. The holder 70 may include a counterweight portion 78 which serves to balance the cuvette 72 when mounted on the cuvette holder 70. The counterweight could form part of the disc 75 or platform 73, or both. The cuvette holder 70 may be mounted to a centrifuge spindle.

More generally, the cuvette holder may be configured to hold any required shape of cuvette, and may comprise any suitable engagement mechanism for retaining a cuvette in

place during centrifugation, and may include a counterweight arrangement for balancing the holder when a cuvette is retained therein.

Other embodiments are intentionally within the scope of the accompanying claims.

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CLAIMS

1. A cuvette comprising:

a housing defining an internal volume divided into a plurality of interconnected, radially-extending, circumferentially-separated chambers relative to an axis for rotation of the cuvette, each chamber being of a first, upstream, type or a second, downstream type;

an inlet communicating with at least one said upstream chamber at a radially inward position of the chamber;

an outlet communicating with at least one said downstream chamber at a radially inward position of the chamber;

at least one communicating passage between each upstream chamber and a downstream chamber at a radially outward position thereof.

2. The cuvette of claim 1 in which the chambers are each separated from an adjacent chamber by a radially extending wall.

3. The cuvette of claim 1 in which the upstream and downstream chambers are disposed circumferentially in alternating fashion.

4. The cuvette of claim 1 in which each upstream chamber communicates with an inlet and each downstream chamber communicates with an outlet.

5. The cuvette of claim 1 in which each upstream chamber communicates with a single inlet disposed on a central axis of the cuvette and each downstream chamber communicates with a separate outlet disposed radially outward of the inlet.

6. The cuvette of claim 1 comprising two upstream chambers and two downstream chambers each subtending an angle of approximately 90 degrees around the central axis.

7. The cuvette of claim 1 comprising an upstream chamber and a downstream chamber disposed alongside one another on one side of the axis for rotation of the cuvette.

8. The cuvette of claim 2 in which the communicating passage is formed by the termination of the radially extending wall radially inward of an outer circumferential wall defined by the housing.

9. The cuvette of claim 1 in which the inlet comprises an aperture in a front face of the housing and the outlet comprises an aperture in a back face of the housing.

10. The cuvette of claim 1 in which the housing comprises two discs coaxially mounted together face to face.

11. The cuvette of claim 1 defining a front face and a back face of the housing, at least one of the faces of the housing being transparent to optical radiation.

12. The cuvette of claim 1 in which both front face and back face are transparent to optical radiation.

13. The cuvette of claim 1 in which the inlet, the outlet and the chamber dimensions are configured such that water and/or blood disposed on the inlet will be drawn by capillary action into the chambers.

14. The cuvette of claim 1 having a generally rectangular shape in which the axis for rotation is a central axis of the cuvette.

15. The cuvette of claim 1 in which the axis for rotation is disposed at or proximal to one edge of the cuvette.

16. A method of determining an optical property of a fluid contained within a volume of a cuvette, comprising the steps of:

at least partly filling the volume of the cuvette with a fluid sample for analysis;

spinning the cuvette on an axis;

passing optical radiation through the fluid sample while the cuvette is spinning;

measuring an optical property of the spinning fluid sample as a function of time and radial position in the cuvette volume.

17. The method of claim 16 in which the measuring step comprises determining a radial boundary position of the optical property induced by the centrifugal forces on the fluid sample by the spinning of the cuvette.

18. The method of claim 17 in which the measuring step comprising measuring the radial boundary position at least twice during the spinning of the cuvette.

19. The method of claim 18 further including measuring a rate of change of radial boundary position and using the rate of change to determine a mean volume of solids within the fluid sample.

20. The method of claim 19 in which the fluid sample is blood and the mean volume of solids comprises the mean corpuscular volume.

21. The method of claim 18 further including measuring a rate of change of radial boundary position and using the rate of change to determine a total volume of solids.

22. The method of claim 19 in which the fluid sample is blood and the total volume of solids comprises the haematocrit.

23. The method of claim 17 further including using the radial boundary position to determine a ratio of one fluid type to another fluid type.

24. The method of claim 23 in which the fluid sample is blood and the method comprises calculating haematocrit of the blood.

25. The method of claim 1 in which the optical property is transmissivity or absorptivity of the fluid sample.

26. The method of claim 16 in which the fluid sample is contained within a circumferentially- and radially-extending volume of the cuvette, and the spinning step comprises spinning the cuvette on its axis.

27. The method of claim 16 in which the step of measuring comprises simultaneously monitoring the optical property at a plurality of radial positions by detecting optical radiation from the fluid sample at a plurality of radial positions by plural detectors.

28. The method of claim 25 further comprising detecting the optical transmissivity or absorptivity at plural wavelengths of light.

29. The method of claim 28 in which the fluid sample is blood and the method comprises calculating a haemoglobin level based on the measured optical transmissivity or absorptivity at two different wavelengths of light.

30. The method of claim 29 in which the haemoglobin level is calculated at a function of optical transmissivity or absorptivity at one wavelength of light and as a function of haematocrit level determined according to a radial boundary position of the optical property induced by the centrifugal forces on the fluid sample by the spinning of the cuvette measured at a different wavelength of light.

Application No:	GB1407537.8	**Examiner:**	Simon Colcombe
Claims searched:	1-15	**Date of search:**	20 October 2014

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1 at least	US2002/098528 A1 (GORDON) See Figures 2A-C and related description, for example
A	-	EP1985366 A2 (SAMSUNG)
A	-	WO2006/093978 A2 (UNIVERSITY OF CALIFORNIA)
A	-	US4509856 A (LEE)

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

B01L; G01N

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC

International Classification:

Subclass	Subgroup	Valid From
G01N	0021/07	01/01/2006

Application No:	GB1407537.8	**Examiner:**	Simon Colcombe
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Patents Act 1977
Further Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	16, 17, 20, 23, 25 at least	WO2006/011393 A1 (MATSUSHITA ELECTRIC) Whole document; Claims 1-3 for example
X	16, 17, 20, 23, 25 at least	US2002/098528 A1 (GORDON) Whole document; Figure 4 and related description; claim 3 for example
X	16, 17, 20, 23, 25 at least	WO97/36163 A1 (ZYNOCYTE) Whole document
X	16, 17, 20, 23, 25 at least	WO96/09548 A1 (UNIVERSITY OF GLASGOW) Whole document
X	16, 17, 20, 23, 25 at least	WO93/17792 A2 (MICRO DIAGNOSTICS) Whole document
X	16, 17, 20, 23, 25 at least	WO92/06379 A1 (IDEMITSU PETROCHEMICAL) Whole document

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

B01L; G01N

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC

International Classification:

Subclass	Subgroup	Valid From
G01N	0021/07	01/01/2006