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(54) DEVICE AND METHOD FOR THE QUANTIFICATION OF CELLULAR AND NON-CELLULAR BLOOD COMPONENTS

VORRICHTUNG UND VERFAHREN ZUR QUANTIFIZIERUNG ZELLULÄRER UND NICHTZELLULÄRER BLUTBESTANDTEILE

DISPOSITIF ET PROCÉDÉ DE QUANTIFICATION DE COMPOSANTS SANGUINS CELLULAIRES ET NON CELLULAIRES

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Description

[0001] The present invention relates to a device and relative method for the quantification of cell and non-cell components present in a solution containing a blood sample, by means of the concentration and magnetophoretic separation of the components of interest from the rest of the sample and the impedentiometric detection of the quantity of these components. For the purposes of the present description "corpusculated components" refer to the cell components of the blood such as red blood cells, white blood cells and platelets. "Non-corpusculated components" refer instead to those non-cell components, in any case with a volume smaller than or comparable to that of the cells, yet greater than that of the single molecules. These non-corpusculated components can be, for example, crystals of some substances which develop in particular pathological conditions, such as for example the crystals of haemozoin produced by the plasmodium of malaria. More particularly, the present invention relates to a device and relative method which allows and provides for the isolating and concentrating spatially of one or more corpusculated haemocomponents, as well as one or more substances present in the plasma, exploiting the differences between the magnetic properties of said haemocomponents and/or said substances and the magnetic properties of the other haemocomponents or substances not of interest. Once separation and concentration have taken place, the device and the relative method of the present invention provide, therefore, for the quantification, of these corpusculated haemocomponents and/or of these substances present in the plasma to take place by means of the measurement of the variation in impedance between two or more electrodes placed in proximity of the zones of concentration.

[0002] The field of application of the present invention relates therefore to the diagnosis of all those pathologies that cause an alteration of the magnetic properties of one or more types of blood corpuscles and/or give rise to the formation of substances with different magnetic properties from plasma, said substances being absent or in a different concentration in physiological conditions. More particularly, pathologies are known that cause the alteration of the magnetic properties of the erythrocytes, or red blood cells, such as malaria, as well as pathologies which cause the alteration of the magnetic properties of the white blood cells. For example, in the case of malaria, it is known how the plasmodium, during the malaria pathogenesis, produces a particular substance mentioned above, which takes the name of haemozoin and is a paramagnetic substance. More particularly, the haemozoin is produced in the form of crystals which are accumulated in the infected erythrocytes, making them paramagnetic. Moreover, in the non-early phases of malaria, the membrane of the infected red blood cells breaks, giving rise to the release of the crystals of haemozoin in the plasma, which is, instead, diamagnetic. Pathologies are also known in which it is not the magnetic properties of corpuscles of the blood that vary but instead their density. An example of this type comes from sickle cell anaemia, where, while the diamagnetism of the red blood cells remains unchanged, their density changes. In this case, by adding a strongly paramagnetic substance to the plasma, such as for example gadolinium, it can be thought to exploit the magnetic difference between the red blood cells and the gadolinium solution added to the plasma together with the difference in density between the sick red blood cells and healthy red blood cells in order to obtain the separation and, therefore, perform the count of the pathological erythrocytes.

[0003] At the state of the art, techniques are known of separation of corpusculated components of the blood, based on the different magnetic behaviour assumed by these components in physiological and pathological conditions. More particularly, in *[*Chung Y K et al., An electrical biosensor for the detection of circulating tumor cells, Biosensors and Bioelectricity, 26, 2520-2526 (2011*)],* a method for the quantification of circulating tumor cells in a blood sample is disclosed. The first step of such a method consists in separating the circulating tumor cell in a blood sample from the rest of solution by immunomagnetic isolation, size filtration and magnetic trapping. The immunomagnetic isolation consisting in adding magnetic beads to the blood sample, the added magnetic beads being previously coated with antibodies able to interact with the circulating tumor cells. The circulating tumor cells bounded to antibodies are thus magnetically marked in order to be successively trapped via magnetic force. Afterwards, the separated components are concentrated in correspondence of microelectrode array, via magnetic sorting in microfluidic channels. Finally an output signal is produced, said signal being proportional to the measured produced by the microelectrode array, and the number of separated components is calculated, on the basis of said output signal. The method of *[*Chung Y K et al., An electrical biosensor for the detection of circulating tumor cells, Biosensors and Bioelectricity, 26, 2520-2526 (2011*)]* can be performed only on cell components in a blood sample because it requires that the cells interact with antibodies covering magnetic beads. Without such interaction, the cells cannot acquire magnetic properties and, thus, cannot be trapped via magnetic forces. The interaction with antibodies, is, obviously, possible only for the cells and not for the non-cell components of the blood sample. Therefore, the method of *[*Chung Y K et al., An electrical biosensor for the detection of circulating tumor cells, Biosensors and Bioelectricity, 26, 2520-2526 (2011*)]* is not suitable for the quantification of non-cell components of the blood. In the patent application US5985153A a device is described for the separation of cells or other magneto-sensitive biological entities comprising: a substrate, a generator of an external magnetic field and a microfluidic system for the loading and unloading of the blood. In document US0127222A a generic system is instead described for the immobilisation of cells previously marked with magnetic particles, in such a way that they can be attracted by ferromagnetic structures formed on a chip and placed in an external magnetic field. In the application

WO2010091874 a particular ferromagnetic structure is described, composed of magnetic conduits, capable of attracting magnetic particles in particular points in which magnetic domain walls are located. In all the prior art documents mentioned above, as well as in a part of the scientific literature listed *in the* bibliography *[*S. Bhakdi et al., Optimized high gradient magnetic separation for isolation of Plasmodium-infected red blood cells, Malaria Journal 2010, 9:38*]; [*J. Nam et al., Magnetic Separation of Malaria-Infected Red Blood Cells in Various Developmental Stages, Anal. Chem., 85, 7316-7323 (2013*)]; [*Ki-Ho Han and A. Bruno Frazier, Paramagnetic capture mode magnetophoretic microseparator for high efficiency blood cell separations, Lab Chip, 6, 265-273 (2006*)],* only the magnetophoretic separation of the components of interest from the rest of the blood sample is described, and no mention is made of the detection of the number of these components. In the patent application US20120003687A and in the scientific publications *[*E. Du, et al., Electric Impedance Microflow Cytometry for Characterization of Cell Disease States, Lab Chip. 2013 October 7; 13(19): 3903-3909*]* e *[*M. Ibrahim, J. Claudel, D. Kourtiche and M. Nadi, Geometric parameters optimization of planar interdigitated electrodes for bioimpedance spectroscopy, J Electr Bioimp, vol. 4, pp. 13-22, 2013*]* techniques of impedentiometric quantification of corpusculated components are described. These techniques have not however ever been used in association with magnetophoretic separation and concentration. Impedentiometric detection requires that the volumetric fraction of the corpuscles in proximity of the electrodes is sufficiently high, in order to obtain a signal-to-noise ratio in the output signal which is sufficient in order to guarantee a correct quantification of the separated components. This concentration is usually obtained with microfluidics techniques which considerably increase the degree of complexity of the system and make it poorly suited to a use by a non-specialised user, for example the actual patient. The device proposed intends to overcome these difficulties by replacing the microfluidics part with a system of magnetic separation and concentration of the components of interest on zones of the substrate in which the detection electrodes are located. In order to perform the measurement the non-specialised user has to dispense on the support a drop of newly sampled blood and then place it in contact with the substrate on which the concentrator elements and the electrodes are housed, in turn placed face downwards within an external magnetic field. For a volume of the drop of blood sampled of the order of around ten microlitres and supposing that the capture of the components of interest takes place at most at a distance from the concentrators comprised between 20 and 200 micrometres, the dimensions of the active area for the capture on the substrate must be of the order of a few cm^2 and, in particular, comprised between 0.5 and 5 cm^2. The support must also have approximately the same dimensions. On these values of active area a high concentration of components of interest is necessary in order to ensure an adequate signal-to-noise ratio. As will be explained in greater detail here below, this concentration can be quantified by means of a so-called concentration factor F_c which comes from the ratio between the active area of the substrate within which the drop containing the components which are to be quantified is confined and the area defined by the detection electrodes. In order to have an adequate signal-to-noise ratio in the output signal the concentration factor F_c must preferably be at least around 100.

[0004] The object of the present invention is therefore that of providing a device and relative method which are able to quantify the haematic components of interest starting from a quantity of blood such as that which can be extracted by means of pricking with a needle the finger of a patient (5 - 10 microL) and produce a signal in output with a signal-to-noise ratio such as to allow the detection of corpusculated and non-corpusculated components of the blood with lower limit of concentration up to 10 components per microlitre.

[0005] This object is achieved by the present invention with a measurement of the impedentiometric type performed by means of appropriate detection electrodes. The device of the present invention comprises, in fact:

- at least one pair of detection electrodes, said at least one pair of detection electrodes comprising at least one first electrode connected with a first input apt to receive a first signal in input (V^+) and a second electrode;
- at least one pair of reference electrodes; said at least one pair of reference electrodes comprising a first electrode connected with a second input configured to receive a second signal in input (V^+) of opposite polarity to the first input signal (V^+) and a second electrode connected to the second electrode of said at least one pair of detection electrodes, in a common point wherefrom an output signal (Out) is picked up;
- at least one concentrator of ferromagnetic material, configured to co-operate with a magnetic field external to the device, in such a way as to cause the concentration of said components on said at least one pair of detection electrodes;
- a substrate configured for the housing of: said at least one pair of detection electrodes; said at least one pair of reference electrodes and said at least one concentrator;
- a support configured to receive a sample of blood or of solution containing blood; and
- at least one spacer element, configured to confine in the plane of the substrate the blood sample and to distance said substrate from said support.

[0006] Said at least one concentrator can be a cylinder or a parallelepiped or an element of another shape placed on the substrate, placed at the detection electrodes and is constituted by ferromagnetic material. The concentrator, attracting towards itself the components to be quantified, ensures that the latter are not distributed everywhere in the area covered

by the substrate but concentrate, instead, in proximity of said concentrator, and therefore in proximity of the detection electrodes. In this way, dimensioning appropriately both the concentrator and the detection electrodes, the concentration factor can increase up to the value necessary for obtaining an adequate signal-to-noise ratio. The device described above co-operates with means for the generation of a static magnetic field, with which it forms an apparatus. These means for the generation of said field are permanent magnets configured so as to generate a magnetic field, optionally, characterised also by a macroscopic gradient. Said field is able to attract uniformly towards the substrate the components sought, when they are at a great distance from the concentrators, and of magnetising the aforementioned concentrators to create therefore an intense local magnetic field gradient, which completes the separation of the components to be quantified from the rest of the solution and produces their accumulation on the concentrators. This separation takes place thanks to the competition between the gravitational force which aims towards the ground and the magnetic attraction force in the direction opposite to that of the gravitational force. The detection electrodes are placed in proximity of said concentrator elements, while the reference ones are placed in areas without said concentrators. In this way the separated components accumulate selectively on the detection electrodes but not on the reference ones, causing a specific variation of the impedance between the detection electrodes with respect to the spurious one possibly recorded between the reference electrodes. The output signal of the impedentiometric quantification system is therefore proportional to the difference between the impedance variation recorded between the detection electrodes and the one between the reference electrodes. The number of components of interest of this output signal can then be estimated through comparison with an appropriate calibration curve, performed by means of a processor.

[0007] As mentioned above, the device and relative method of the present invention can be applied to the diagnosis of any pathology which is the cause of a variation of the magnetic properties of one more haematic components, as well as to the diagnosis of pathologies which cause a variation in the density of one or more components, in this case providing for the addition in the sample of blood to be analysed of a solution of different magnetic properties with respect to the plasma.

[0008] Among the various pathologies for the diagnosis of which it is possible to use the device of the present invention, malaria is however of particular interest, in that the diagnostic devices for this type of pathology, today present on the market, have some limitations which make them not always easy to use in particularly disadvantaged contexts, such as the typical ones of endemic zones, often located in developing countries. The most sensitive method currently available for the diagnosis of malaria is based in fact on gene recognition of the various strains of plasmodium by means of PCR (polymerase chain reaction). This type of method is particularly complex and delicate and, therefore, difficult to apply in contexts that are not technologically advanced. Moreover PCR is not a pan-plasmodium method but is targeted at specific strains and subject, therefore, to the problems arising from the continuous mutations of the plasmodium. The method, instead, of the "thin smear and/or thick drop" which consists in counting under the optical microscope the red blood cells infected by the plasmodium in a drop of blood, while not requiring complex instrumentation, needs highly expert staff, entails a certain variability in the interpretation of the results and long analysis times. The rapid tests (RDT) based on the antibody-antigen interaction are, instead, characterised by such low sensitivity as to prevent use thereof for early diagnosis. Moreover, due to the latent presence of the antigen in the body of patients in an endemic zone, the methods based on the antibody-antigen interaction give rise to a high number of false positives.

[0009] A second object of the present invention is therefore that of providing a device and relative method which also allow the early diagnosis of malaria, is pan-plasmodium, has adequate sensitivity and is of such simplicity and economical nature as to be able to be used also in those zones where the economic means available do not allow the use of complex instruments and specialist personnel.

[0010] This object is achieved by the device and method of the present invention, in that the latter is able to perform the magnetic separation and the quantification both of the infected erythrocytes and the magnetic separation and direct detection of the free haemozoin crystals in the plasma. The quantification of the infected erythrocytes allows a direct valuation of the parasitemia to be obtained, which is normally quantified by calculating the ratio between infected erythrocytes and healthy erythrocytes, optionally also in the early phase of the disease, before the completion of the first cycle of reproduction of the plasmodium (48-72 hours). The direct detection of the crystals of haemozoin is, instead, particularly useful, in the non-initial phases of the disease, such as for example concurrent with the first fever attack, since, in these phases, the erythrocytes have already undergone the breakage of the membrane, and the only thing which can effectively be quantified in circulation is the free haemozoin. These and further objects of the present invention will be made clearer by the reading of the following detailed description of some preferred embodiments of the present invention, to be understood by way of a non-limiting example of the more general concepts claimed, and from the examples relating to experimental tests performed on the present invention.

[0011] The following description refers to the accompanying drawings, in which:

- Figure 1 is an overall diagram of an apparatus comprising a device according to the present invention apt to be used for the diagnosis of malaria;
- Figure 2 is an example diagram of the positioning of the detection and reference electrodes with respect to the

concentrators, in a first embodiment of the present invention;
- Figure 3a shows a section of a first embodiment of the device of the present invention, said section being along a plane perpendicular to the greater dimension of said at least one concentrator;
- Figure 3b shows a detail of the section shown in Figure 3a, relating to said at least one concentrator;
- Figure 3c shows a detail of the section shown in Figure 3a, relating to said at least one pair of detection electrodes;
- Figure 4 is a view from above of a first embodiment of the device of the present invention;
- Figure 5 is a view from above of a detail of a second embodiment of the present invention;
- Figure 6 shows the trend of the percentage resistance variation between the detection electrodes and the reference electrodes as a function of the level of parasitemia generated by the capture of erythrocytes infected by the plasmodium of the malaria, in a second embodiment of the present invention.

[0012] Referring to Figures 1, 3a, 3b and 3c and 5, the device (1) of the present invention comprises:

- a plurality of detection electrodes (4,4', 5, 5', 6, 6', 34, 34'),
- a pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, 37') for each pair of detection electrodes (4,4', 5, 5' 6, 6', 34, 34');
- a concentrator (10, 10', 10", 14, 14', 14") for each pair of detection electrodes (4, 4', 5, 5', 6, 6'), said concentrator (10, 10', 10") being configured to attract magnetically the components (3, 3', 3") to be quantified and concentrate said components on the detection electrodes (4, 4', 5, 5', 6, 6', 34, 34');
- a substrate (11) configured for the housing of the detection electrodes (4, 4', 5, 5', 6, 6', 34, 34'), of the reference electrodes (7, 7', 8, 8', 9, 9', 37, 37') and of the concentrators (10, 10', 10", 14, 14', 14");
- a support (12) configured to receive a sample of blood or of solution containing blood; and
- at least one spacer element (13, 13') configured to confine the sample to be analysed and to distance said substrate (11) from said support (12).

[0013] Said at least one spacer element (13, 13') can be ring shaped.

[0014] The device (1) of the present invention can be inserted inside an apparatus (100) comprising also:

- an electronic unit for the generation of the input signals, the readings of the signals generated by the electrodes (7', 8', 9', 4', 5', 6', 34', 37') and their processing;
- a housing configured for the positioning of said device (1);
- a plurality of connectors for the connection between said device (1) and said electronic unit; and
- means for the generation of a static magnetic field (101, 102, 103), said means (101, 102, 103) being configured to generate a magnetic field able to cause the separation of the components (3, 3', 3") to be quantified from the rest of the solution.

[0015] In the particular case of malaria, said means (101, 102, 103) for the generation of a static magnetic field are able to generate a field which, preferably, has an intensity of at least 10^4 A/m and a macroscopic gradient of at least 10^8 A/m^2 aimed towards the substrate or exiting therefrom, respectively in the case of paramagnetic or diamagnetic components with respect to the liquid medium in which they are dispersed. Said means comprise a plurality of permanent magnets (101, 102, 103) positioned so that the field generated by said magnets (101, 102, 103) overcomes the resultant of the weight force and of that of Archimedes acting on the components of interest at a great distance from the substrate, preventing said components from precipitating on the surface of the support. Moreover the field generated by said magnets must be able to magnetise effectively the concentrator elements so that they produce an intense gradient of local magnetic field able to attract selectively and concentrate said components (3, 3', 3") only on the areas of the substrate (11), occupied by the detection electrodes (4, 4', 5, 5', 6, 6'), said components (3, 3', 3") being paramagnetic. It is obvious that in the cases wherein the components to be quantified are diamagnetic, said means for the generation of a static magnetic field comprise a plurality of permanent magnets positioned so that the gradient of the field generated by said magnets is exiting from the substrate, such as to overcome the weight force at a great distance. Similarly, the local field gradient produced by the magnetic concentrators must be exiting from the zones with the detection electrodes and ensure that said components accumulate at said detection electrodes, said components being diamagnetic.

[0016] Referring to Figure 2, in a first embodiment of the present invention each pair of detection electrodes (4,4', 5, 5', 6, 6'), comprises a first electrode (4, 5, 6) apt to receive a first signal in input (V^+) and a second electrode (4', 5', 6'). Each pair of reference electrodes (7, 7', 8, 8', 9, 9') comprises a first electrode (7, 8, 9) apt to receive a second signal in input (V-) of opposite polarity to the first input signal (V^+) and a second electrode (7', 8', 9') connected to the second electrode (4', 5', 6') of each pair of detection electrodes (4, 4', 5, 5', 6, 6'), in a common point from which the output signal (Out) is picked up.

[0017] Referring to Figures 3a, 3b, 3c, in a first embodiment of the present invention, apt for the diagnosis of malaria, the concentrators (10, 10', 10") are made of ferromagnetic material, such as Ni, Fe, Co, NiFe, CoFe, etc., and have the

shape of a parallelepiped with the greater dimension which extends perpendicularly to the plane shown in Figure 3a. In order to guarantee a sufficient concentration factor for obtaining an adequate signal-to-noise ratio, the dimensions of the concentrators (10, 10', 10") and of the detection electrodes (4, 4', 5, 5', 6, 6') must be, preferably, comprised within the ranges listed in Table 1.

Component	h_F (μm)	w_F (μm)	d_F (μm)	h_E (nm)	w_E (μm)	d_E (μm)
i-RBC	10-30	30-60	30-60	10-300	2-6	2-6
HC	5-10	15-30	15-30	10-300	1-3	1-5

[0018] Table 1: h_F is the smaller dimension of the base of a concentrator, w_F is the larger dimension of the base of a concentrator and d_F is the distance between one concentrator and the adjacent concentrator. h_E is the smaller dimension of the base of a detection electrode, w_F is the larger dimension of the base of a detection electrode and d_E the distance between two adjacent electrodes at the same concentrator.

[0019] In the first row of Table 1, the ranges are shown of the dimensions of the concentrators and of the detection electrodes necessary for a correct detection of the erythrocytes infected *(i-RBC)* by the plasmodium of the malaria. While in the second row of Table 1 the ranges are shown of the dimensions of the concentrators and of the detection electrodes necessary for a correct detection of the free crystals of haemozoin (HC). Referring to Figure 4, the substrate (11) and, therefore the actual device (1) of the present invention, the structure of the detection electrodes (4, 4', 5, 5', 6, 6') and of the reference electrodes (7, 7', 8, 8', 9, 9') shown in Figures 3a, 3b and 3c, can be replicated in nine square zones (300, 301, 302) into which the substrate (11) is divided. The division of the active area into several regions with independent readings allows an increase in the ratio between the variation in impedance produced by a single component attracted on the detection electrodes and the overall impedance between the electrodes, improving the signal-to-noise ratio in the case of low concentrations of components to be detected. Since for each zone an output contact is necessary towards the amplifier from which to emit the output signal (Out), while all the output signals (V^+) and (V-) for detection electrodes and reference electrodes need only two contacts, the total number of contacts to be formed on the chip is equal to 9+2=11. This number is compatible with the positioning of 11 terminals (401,402,403) of dimension equal to 400x400 μm on one side of the substrate (11).

[0020] Referring to Figure 5, a second embodiment of the device of the present invention provides for the use of a matrix of ferromagnetic concentrators of cylindrical shape (14, 14', 14") evenly distributed on the substrate (11). Figure 5 shows, in particular, six pairs of detection electrodes (34, 34') and six pairs of reference electrodes (37, 37'). The first electrode (34) of each pair of detection electrodes (34, 34') is connected to a first input configured for the reception of the first input signal (V^+) by means of a first connection path (44). The first electrode (37) of each pair of reference electrodes (37, 37') is connected to a second input configured for the reception of the second input signal (V-) by means of a second connection path (47). Similarly, the second electrode (34') of each pair of detection electrodes (34, 34') is connected to the node wherefrom the output signal (Out) is emitted by means of a third connection path (44') and the second electrode (37') of each pair of reference electrodes (37, 37') is connected to the node wherefrom said output signal (Out) is emitted by means of a fourth connection path (47').

[0021] Above the first connection path (44), the second connection path (47), the third connection path (44') and the fourth connection path (47') an insulating layer (40, 40', 50, 50') is placed for each path, said insulating layer (40, 40', 50, 50') having dielectric constant and thickness such as to make the impedance between said connection paths (44, 44', 47, 37') negligible.

[0022] The configuration of the concentrators provided by the second embodiment allows a concentration factor to be obtained which is even higher compared to that which can be obtained with respect to the first embodiment. To this end the dimensions of the concentrators (14, 14', 14") and of the detection electrodes (34, 34', 35, 35') must be, preferably, comprised within the ranges listed in Table 2.

Table 2: h_F is the height of a concentrator, w_F is the diameter of the base of a concentrator and d_F is the distance between one concentrator and the adjacent concentrator. h_E is the smaller dimension of the base of a detection electrode, w_F is the larger dimension of the base of a detection electrode and d_E the distance between the first detection electrode finger and the second finger of said detection electrode.

Component	h_F (μm)	w_F (μm)	d_F (μm)	h_E (nm)	w_E (μm)	d_E (μm)
i-RBC and HC	10-30	10-30	50-150	10-300	1-3	1-5

[0023] Table 2 shows the ranges of the dimensions of the concentrators and of the detection electrodes necessary for a correct detection both of the erythrocytes infected *(i-RBC)* by the plasmodium of the malaria and of the free crystals

of haemozoin (HC). With these dimensions, supposing a length L of the electrodes equal to 6 μm, a concentration factor is obtained $F_C = \frac{(d_F+w_F)^2}{L\cdot(2w_E+d_E)}$ equal to approximately 400.

EXAMPLE

[0024] The example described here below relates to the calculation of the percentage variation of impedance between the detection electrodes and the reference electrodes in a second embodiment of the present device and with reference to the detection of i-RBC. In the particular case a substrate (11) of area equal to 1 cm^2 was considered. The substrate (11) (of dimension 1 cm^2) was divided into nine square zones (as in Fig. 4), each one provided with a matrix of 550 concentrators. At the centre of each concentrator a pair of detection electrodes is placed with length L equal to 6 μm, width w_E equal to 2 μm and distance between the electrodes d_E of 2 μm. The containment ring with a plurality of spacer elements (13, 13') is such as to impose a distance between the substrates 11 and 12 of 50 μm and the concentrators (10,10', 10')' allow the capture of all the infected erythrocytes which are found in the volume defined by substrate, support and containment ring. By measuring the impedance at a frequency of the order of 1-20MHz it is possible to obtain the electrical resistance R of the material between the electrodes, given mainly by the solution and by the possible presence of infected erythrocytes i-*RBC* captured by the magnetic concentrators. Figure 6 shows the percentage resistance variation $\Delta R/R_0$ as a function of the infected erythrocytes *i-RBC* captured on the surface of the detection electrodes, obtained by means of finite element simulation (FEM) (full squares) and by means of the following formula (empty squares):

$$\frac{\Delta R}{R_0} = \frac{3}{2}\cdot\frac{V_p}{N\cdot(w_E+d_E)\cdot L\cdot H}$$

where *Vp* represents the total volume occupied by the *i-RBC* captured on the surface of the electrodes, while N, , H are, respectively, the number of pairs of detection electrodes which share a same output, and the height up to which a pair of detection electrodes is sensitive to the presence of the components of interest, equal to approximately 1 - 2 times the distance between the electrodes d_E. The volume V_p is equal to the volume of a single *i-RBC* multiplied by the number of erythrocytes captured. The latter is equal to the concentration of infected erythrocytes multiplied by the volume of capture of the concentrators, 1 $cm^2 \cdot d_{capture}$ = 5 μl.

[0025] $\Delta R/R_0$ is in fact proportional to the fraction of the effective volume, to which the impedentiometric measurement is sensitive, occupied by the components of interest. It should be noted in the case of parasitemia equal to 10 parasites/μL (on average 5.5 parasites for each of the nine zones of our geometry), the expected resistance variation, $\Delta R/R_0$, is found to be equal to about 0.4%, corresponding to a resolution required of the reading electronics, in the resistance measurement, equal to approximately 1000 ppm).

[0026] Should the system for magnetic concentration (i.e. the whole constituted by external magnets and concentrators) be able to capture the infected erythrocytes at a distance ten times greater, $d_{capture}$ = 500 μm, and the distance between the substrates 11 and 12 increase correspondingly by a factor 10, it would be possible to arrive at a $\Delta R/R_0$ ten times greater with respect to the previous case, at the same concentration of parasites and active area of the substrate but increasing by a factor 10 the volume of the drop of blood. Or, again with $d_{capture}$ = 500 μm and height of the container ring with a plurality of spacer elements of 500 μm, the volume of the drop could be kept unchanged at 5 microlitres and a $\Delta R/R_0$ equal to that in Figure 6 obtained, reducing the active area on the chip.

Claims

1. Device (1) for the quantification of cell and non-cell components (3, 3', 3") in a solution containing a blood sample comprising:

   - at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34'), said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34') comprising at least one first electrode (4, 5, 6, 34) connected with a first input apt to receive a first signal in input (V^+) and a second electrode (4', 5', 6', 34');
   - at least one pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, 37'), said at least one pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, 37') comprising a first electrode (7, 8, 9, 37) connected with a second input configured to receive a second signal in input (V-) of opposite polarity to the first input signal (V^+) and a second electrode (7', 8', 9', 37') connected to the second electrode (4', 5', 6', 34') of said at least one pair of detection electrodes

(4,4', 5, 5', 6, 6', 34, 34'), in a common point wherefrom an output signal (Out) is picked up;

said device (1) being **characterised in that** it comprises:

- at least one concentrator (10, 10', 10", 14, 14', 14") in ferromagnetic material, configured to co-operate with a magnetic field external to the device (1), in such a way as to cause the concentration of said components (3, 3', 3") on said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34'), said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, (10, 10', 10", 14, 14', 14") 34') being placed in proximity of said at least one concentrator (10, 10', 10", 14, 14', 14") and said one pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, (10, 10', 10", 14, 14', 14") 37'), being placed in areas without said at least one concentrator (10, 10', 10", 14, 14', 14")
- a substrate (11) configured for the housing of: said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34'), said at least one pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, 37') and said at least one concentrator (10, 10', 10", 14,14', 14");
- a support (12) configured to receive a sample of blood or of solution containing blood; and
- at least one spacer element (13, 13') configured to confine in the plane of the substrate the blood sample and to distance said substrate (11) from said support (12).

2. Device (1) according to the preceding claim, wherein:

- the first electrode (34) of each pair of detection electrodes (34, 34') is connected to the first input by means of a first connection path (44);
- the first electrode (37) of each pair of reference electrodes (37, 37') is connected to the second input by means of a second connection path (47);
- the second electrode (34') of each pair of detection electrodes (34, 34') is connected to the node wherefrom the output signal (Out) is emitted by means of a third connection path (44'); and
- the second electrode (37') of each pair of reference electrodes (37, 37') is connected to the node wherefrom said output signal (Out) is emitted by means of a fourth connection path (47');

above each of said connection paths (44, 44', 47, 37') an insulating layer (40, 40', 50, 50') being placed with such dielectric constant and thickness as to make the impedance between said connection paths (44, 44', 47, 37') negligible.

3. Device (1) according to claim 1 or 2, wherein said at least one concentrator (10, 10', 10", 14, 14', 14") is cylindrical in shape, the diameter of the base surface of said concentrators (14, 14', 14") being comprised between 10 and 30 μm, the height of said concentrators (14, 14', 14") being comprised between 10 and 30 μm and the distance between said concentrators (14, 14', 14") being comprised between 50 and 150 μm.

4. Device according to any one of the preceding claims, wherein the first electrode (5, 6, 34) of said at least one pair of detection electrodes (5, 5', 6, 6', 34, 34') and the second electrode (5', 6', 34') of said at least one pair of detection electrodes (5, 5', 6, 6', 34, 34') are with rectangular section, with base comprised between 10 and 300 nm and height comprised between 1 and 3 μm.

5. Device (1) according to the preceding claim, wherein the distance between the first electrode (5, 6, 34) of said at least one pair of detection electrodes (5, 5', 6, 6', 34, 34') and the second electrode (5', 6', 34') of said at least one pair of detection electrodes (5, 5', 6, 6', 34, 34') is comprised between 1 and 5 μm.

6. Apparatus (100) for the quantification of cell and non-cell components (3, 3', 3") in a solution containing a blood sample comprising:

- a device (1) according to any one of the preceding claims;
- an electronic unit for the generation of the first input signal (V^+) and the second input signal (V-) and for the readings and the processing of the output signal (Out);
- a housing configured for the positioning of said device (1);
- a plurality of connectors for the connection between said device (1) and said electronic unit; and
- means for the generation of a static magnetic field (101, 102, 103), said means (101, 102, 103) being configured to generate a magnetic field able to cause, in combination with the concentrators, the separation of the components (3, 3', 3") to be quantified from the rest of the solution and the concentration on the detection electrodes (5, 5', 6, 6', 34, 34').

7. Apparatus (100) according to the preceding claim, wherein said means (101, 102, 103) for the generation of a static magnetic field comprise a plurality of permanent magnets (101, 102, 103) positioned so that the field generated by said magnets (101, 102, 103) in combination with the gradient generated by said at least one concentrator (10, 10', 10", 14, 14', 14") is such as to ensure that said components (3, 3', 3") accumulate on the detection electrodes (5, 5', 6, 6', 34, 34') of the substrate (11), said components (3, 3', 3") being paramagnetic.

8. Apparatus (100) according to claim 6, wherein said means for the generation of a static magnetic field comprise a plurality of permanent magnets positioned so that the field generated by said magnets in combination with the gradient generated by said at least one concentrator is such as to ensure that said components (3, 3', 3") accumulate on the detection electrodes of the substrate (11), said components (3, 3', 3") being diamagnetic.

9. Apparatus (100) according to claim 6, wherein said magnetic field has an intensity of at least 10^4 A/m and a gradient of at least 10^8 A/m^2.

10. Method for the quantification of cell and non-cell components (3, 3', 3") in a solution containing a blood sample comprising:

- separating of at least one cell or non-cell component (3, 3', 3") from the rest of the solution; the separating being caused by a static magnetic field in combination with at least one concentrator (10, 10', 10", 14, 14', 14") in ferromagnetic material, configured to co-operate with said magnetic field;
- concentrating of said at least one separated component in correspondence of at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34'), the concentrating being caused by said static magnetic field in combination with at least one concentrator (10, 10', 10", 14, 14', 14"), said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34') being placed in proximity of said at least one concentrator (10, 10', 10", 14, 14', 14") and said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34') comprising at least one first electrode (4, 5, 6, 34) connected with a first input apt to receive a first signal in input (V^+) and a second electrode (4', 5', 6', 34') and said at least one concentrator;
- measuring of the difference in impedance between said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34') and at least one pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, 37'), producing an output signal proportional to the difference in impedance between said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34') and said at least one pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, 37'), said at least one pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, 37') being placed in areas without said at least one concentrator (10, 10', 10", 14, 14', 14") and said at least one pair of reference electrodes (7, 7', 8, 8', 9, 9', 37, 37') comprising a first electrode (7, 8, 9, 37) connected with a second input configured to receive a second signal in input (V-) of opposite polarity to the first input signal (V^+) and a second electrode (7', 8', 9', 37') connected to the second electrode (4', 5', 6', 34') of said at least one pair of detection electrodes (4, 4', 5, 5', 6, 6', 34, 34'), in a common point wherefrom an output signal (Out) is picked up; and
- calculating, by means of a processor and comparison with appropriate calibration curve, the number of components (3, 3', 3") separated on the basis of said output signal.

11. Method according to claim 10, wherein the cell components (3, 3', 3"), are blood corpuscles whose magnetic properties can be altered by pathologies.

12. Method according to claim 10 or 11 wherein the non-cell components (3, 3', 3") are substances with different magnetic properties from plasma, said substances being absent in physiological conditions, or said substances being present in a concentration which is different between physiological and pathological conditions.

Patentansprüche

1. Vorrichtung (1) zur Quantifizierung von Zell- und Nicht-Zellkomponenten (3, 3', 3") in einer Lösung, die eine Blutprobe enthält, umfassend:

- mindestens ein Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34'), wobei das besagte mindestens eine Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34') mindestens eine erste Elektrode (4, 5, 6, 34) umfasst, die mit einem ersten Eingang verbunden ist, geeignet, ein erstes Eingangssignal (V^+) und eine zweite Elektrode (4', 5', 6', 34') zu empfangen;
- mindestens ein Referenzelektrodenpaar (7, 7', 8, 8', 9, 9', 37, 37'), wobei das besagte mindestens eine

Referenzelektrodenpaar (7, 7', 8, 8', 9, 9', 37, 37') eine erste Elektrode (7, 8, 9, 37) umfasst, die mit einem zweiten Eingang verbunden ist, so konfiguriert, dass er ein zweites Eingangssignal (V-) mit einer zum ersten Eingangssignal (V+) entgegengesetzten Polarität empfängt, und eine zweite Elektrode (7', 8', 9', 37) umfasst, die mit der Elektrode (4', 5', 6', 34') des besagten mindestens einen Detektionselektrodenpaares (4, 4', 5', 6, 6', 34, 34') in einem gemeinsamen Punkt verbunden ist, von dem ein Ausgangssignal (Out) abgenommen wird;

wobei die besagte Vorrichtung (1) **dadurch gekennzeichnet ist, dass** sie Folgendes umfasst:

- mindestens einen Konzentrator (10, 10', 10", 14, 14', 14") aus ferromagnetischem Material, konfiguriert, um mit einem Magnetfeld außerhalb der Vorrichtung (1) so zusammenzuwirken, dass er die Konzentration der besagten Komponenten (3, 3', 3") auf dem besagten mindestens einen Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34') bewirkt, wobei das besagte mindestens eine Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34') in der Nähe des besagten mindestens einen Konzentrators (10, 10', 10", 14, 14', 14") angeordnet ist und das besagte eine Referenzelektrodenpaar (7, 7', 8, 8', 9, 9', 37, 37') in Bereichen ohne den besagten mindestens einen Konzentrator (10, 10', 10", 14, 14', 14") angeordnet ist;
- ein Substrat (11), konfiguriert für die Unterbringung: des besagten mindestens einen Detektionselektrodenpaares (4, 4', 5, 5', 6, 6', 34, 34'), des besagten mindestens einen Referenzelektrodenpaares (7, 7', 8, 8', 9, 9', 37, 37') und des besagten mindestens einen Konzentrators (10, 10', 10", 14, 14', 14");
- einen Träger (12), der zur Aufnahme einer Blutprobe oder einer bluthaltigen Lösung konfiguriert ist; und
- mindestens ein Abstandselement (13, 13'), das so konfiguriert ist, dass es die Blutprobe in der Ebene des Substrats begrenzt und das besagte Substrat (11) von dem besagten Träger (12) trennt.

2. Vorrichtung (1) gemäß dem vorhergehenden Anspruch, wobei:

- die erste Elektrode (34) jedes Detektionselektrodenpaares (34, 34') mit dem ersten Eingang mittels eines ersten Verbindungspfades (44) verbunden ist;
- die erste Elektrode (37) jedes Referenzelektrodenpaares (37, 37') mit dem zweiten Eingang mittels eines zweiten Verbindungspfades (47) verbunden ist;
- die zweite Elektrode (34') jedes Detektionselektrodenpaares (34, 34') mit dem Knoten verbunden ist, von dem das Ausgangssignal (Out) mittels eines dritten Verbindungspfades (44') ausgegeben wird; und
- die zweite Elektrode (37') jedes Referenzelektrodenpaares (37, 37') mit dem Knoten verbunden ist, von dem das besagte Ausgangssignal (Out) mittels eines vierten Verbindungspfades (47') ausgegeben wird;

wobei über jedem der besagten Verbindungspfade (44, 44', 47, 37') eine Isolierschicht (40, 40', 50, 50') mit einer solchen Dielektrizitätskonstante und Dicke angeordnet ist, dass die Impedanz zwischen den besagten Verbindungspfaden (44, 44', 47, 37') vernachlässigbar gemacht wird.

3. Vorrichtung (1) gemäß Anspruch 1 oder 2, wobei der besagte mindestens eine Konzentrator (10, 10', 10", 14, 14', 14") eine zylindrische Form aufweist, wobei der Durchmesser der Basisfläche der besagten Konzentratoren (14, 14', 14") zwischen 10 und 30 μm liegt, wobei die Höhe der besagten Konzentratoren (14, 14', 14") zwischen 10 und 30 μm und der Abstand zwischen den besagten Konzentratoren (14, 14', 14") zwischen 50 und 150 μm liegt.

4. Vorrichtung gemäß einem jeden der vorhergehenden Ansprüche, wobei die erste Elektrode (5, 6, 34) des besagten mindestens eines Detektionselektrodenpaars (5, 5', 6, 6', 34, 34') und die zweite Elektrode (5', 6', 34') des besagten mindestens eines Detektionselektrodenpaars (5, 5', 6, 6', 34, 34') einen rechteckigen Querschnitt mit einer Basis zwischen 10 und 300 nm und einer Höhe zwischen 1 und 3 μm aufweisen.

5. Vorrichtung (1) gemäß dem vorhergehenden Anspruch, wobei der Abstand zwischen der ersten Elektrode (5, 6, 34) des besagten mindestens einen Detektionselektrodenpaars (5, 5', 6, 6', 34, 34') und der zweiten Elektrode (5', 6', 34') des besagten mindestens einen Detektionselektrodenpaars (5, 5', 6, 6', 34, 34') zwischen 1 und 5 μm liegt.

6. Gerät (100) zur Quantifizierung von Zell- und Nicht-Zellkomponenten (3, 3', 3") in einer Lösung, die eine Blutprobe enthält, umfassend:

- eine Vorrichtung (1) gemäß einem der vorhergehenden Ansprüche;
- eine elektronische Einheit zur Erzeugung des ersten Eingangssignals (V^+) und des zweiten Eingangssignals (V^-) sowie zum Ablesen und zur Verarbeitung der Ausgangssignals (Out);
- ein Gehäuse, das für die Positionierung der besagten Vorrichtung (1) konfiguriert ist;

- eine Vielzahl von Verbindungsstücken für die Verbindung zwischen der besagten Vorrichtung (1) und der besagten elektronischen Einheit; und
- Mittel zur Erzeugung eines statischen Magnetfeldes (101, 102, 103), wobei die besagten Mittel (101, 102, 103) so konfiguriert sind, dass sie ein Magnetfeld erzeugen, das in Kombination mit den Konzentratoren die Trennung der zu quantifizierenden Komponenten (3, 3', 3") vom Rest der Lösung und die Konzentration auf den Detektionselektroden (5, 5', 6, 6', 34, 34') bewirken kann.

7. Gerät (100) gemäß dem vorhergehenden Anspruch, wobei die besagten Mittel (101, 102, 103) zur Erzeugung eines statischen Magnetfeldes eine Vielzahl von Permanentmagneten umfassen, die so angeordnet sind, dass das von den besagten Magneten (101, 102, 103) erzeugte Feld in Kombination mit dem Gradienten, der von dem besagten mindestens einen Konzentrator (10, 10', 10", 14, 14', 14 ") erzeugt wird, so beschaffen ist, dass sich die besagten Komponenten (3, 3', 3") auf den Detektionselektroden (5, 5', 6, 6', 34, 34') des Substrats (11) ansammeln, wobei die besagten Komponenten (3, 3', 3") paramagnetisch sind.

8. Gerät (100) gemäß Anspruch 6, wobei die besagten Mittel zur Erzeugung eines statischen Magnetfeldes eine Vielzahl von Permanentmagneten umfassen, die so angeordnet sind, dass das von den besagten Magneten erzeugte Feld in Kombination mit dem Gradienten, der von dem besagten mindestens einen Konzentrator erzeugt wird, so beschaffen ist, dass sie gewährleisten, dass sich die besagten Komponenten (3, 3', 3") auf den Detektionselektroden des Substrats (11) ansammeln, wobei die besagten Komponenten (3, 3', 3") diamagnetisch sind.

9. Gerät (100) gemäß Anspruch 6, wobei das besagte Magnetfeld eine Intensität von mindestens 10^4A/m und einen Gradienten von mindestens 10^8 A/m^2 aufweist.

10. Verfahren zur Quantifizierung von Zell- und Nicht-Zellkomponenten (3, 3', 3") in einer Lösung, die eine Blutprobe enthält, umfassend:

- Abtrennung von mindestens einer Zell- oder Nicht-Zellkomponente (3, 3', 3") vom Rest der Lösung; wobei die Abtrennung durch ein statisches Magnetfeld in Kombination mit mindestens einem Konzentrator (10, 10', 10", 14, 14', 14") aus ferromagnetischem Material verursacht wird, der so konfiguriert ist, dass er mit dem besagten Magnetfeld zusammenwirkt;
- Konzentrieren der besagten mindestens einen abgetrennten Komponente in Übereinstimmung mit mindestens einem Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34'), wobei das Konzentrieren durch das besagte statische Magnetfeld in Kombination mit mindestens einem Konzentrator (10, 10', 10", 14, 14', 14") verursacht wird, wobei das besagte mindestens eine Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34') in der Nähe des besagten mindestens einen Konzentrators (10, 10', 10", 14, 14', 14") angeordnet ist und das besagte mindestens eine Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34') mindestens eine erste Elektrode (4, 5, 6, 34), die mit einem ersten Eingang verbunden ist, geeignet, ein erstes Eingangssignal (V^+) zu empfangen, und eine zweite Elektrode (4', 5', 6', 34') und den besagten mindestens einen Konzentrator umfasst;
- Messen der Differenz in der Impedanz zwischen dem besagten mindestens einen Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34') und dem mindestens einen Referenzelektrodenpaar (7, 7', 8, 8', 9, 9', 37, 37'), wobei ein Ausgangssignal erzeugt wird, das proportional ist zur Differenz in der Impedanz zwischen dem besagten mindestens einen Detektionselektrodenpaar (4, 4', 5, 5', 6, 6', 34, 34') und dem besagten mindestens einen Referenzelektrodenpaar (7, 7', 8, 8', 9, 9', 37, 37'), wobei das besagte mindestens eine Referenzelektrodenpaar (7, 7', 8, 8', 9, 9', 37, 37') in Bereichen ohne den besagten mindestens einen Konzentrator (10, 10', 10", 14, 14', 14") angeordnet ist und das besagte mindestens eine Referenzelektrodenpaar (7, 7', 8, 8', 9, 9', 37, 37') eine erste Elektrode (7, 8, 9, 37) umfasst, die mit einem zweiten Eingang verbunden ist, der so konfiguriert ist, dass er ein zweites Eingangssignal (V^-) mit einer dem ersten Eingangssignal (V^+) entgegengesetzten Polarität empfängt, und eine zweite Elektrode (7', 8', 9', 37') umfasst, die mit der zweiten Elektrode (4', 5', 6', 34') des besagten mindestens einen Detektionselektrodenpaares (4, 4', 5, 5', 6, 6', 34, 34') in einem gemeinsamen Punkt verbunden ist, von dem ein Ausgangssignal (Out) abgenommen wird; und
- Berechnen, mithilfe eines Prozessors und Vergleich mit einer geeigneten Kalibrierkurve, der Anzahl von Komponenten (3, 3', 3"), die auf der Grundlage des besagten Ausgangssignals getrennt wurden.

11. Verfahren gemäß Anspruch 10, wobei die Zellkomponenten (3, 3', 3") Blutkörperchen sind, deren magnetische Eigenschaften durch Pathologien verändert werden können.

12. Verfahren gemäß Anspruch 10 oder 11 wobei die Nicht-Zellkomponenten (3, 3', 3") Substanzen mit anderen magnetischen Eigenschaften als Plasma sind, wobei die besagten Substanzen unter physiologischen Bedingungen

nicht vorhanden sind oder wobei die besagten Substanzen in einer Konzentration vorliegen, die zwischen physiologischen und pathologischen Bedingungen anders ist.

Revendications

1. Dispositif (1) pour la quantification des composants cellulaires et non cellulaires (3, 3', 3") dans une solution contenant un échantillon de sang, comprenant :

    - au moins une paire d'électrodes de détection (4, 4', 5, 5', 6, 6', 34, 34'), ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34') comprenant au moins une première électrode (4, 5, 6, 34) connectée à une première entrée apte à recevoir un premier signal en entrée (V+) et une seconde électrode (4', 5', 6', 34') ;
    - au moins une paire d'électrodes de référence (7, 7', 8, 8', 9, 9', 37, 37'), ladite paire d'électrodes de référence au minimum (7, 7', 8, 8', 9, 9', 37, 37') comprenant une première électrode (7, 8, 9, 37) connectée à une seconde entrée configurée pour recevoir un seconde signal en entrée (V-) de polarité opposée au premier signal d'entrée (V+) et une seconde électrode (7', 8', 9', 37') connectée à la seconde électrode (4', 5', 6', 34') de ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34'), en un point commun à partir duquel un signal de sortie (Out) est capté ;

    ledit dispositif (1) étant **caractérisé par le fait qu'**il comprend :

    - au moins un concentrateur (10, 10', 10", 14, 14', 14") en matériau ferromagnétique, configuré pour coopérer avec un champ magnétique externe au dispositif (1), de manière à provoquer la concentration desdits composants (3, 3', 3") sur ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34'), ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34') étant placée à proximité dudit concentrateur au minimum (10, 10', 10", 14, 14', 14") et ladite paire d'électrodes de référence au minimum (7, 7', 8, 8', 9, 9', 37, 37') étant placée dans des zones sans ledit concentrateur au minimum (10, 10', 10", 14, 14', 14") ;
    - un substrat (11) configuré pour le logement de : ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34'), ladite paire d'électrodes de référence au minimum (7, 7', 8, 8', 9, 9', 37, 37') et ledit concentrateur au minimum (10, 10', 10", 14, 14', 14") ;
    - un support (12) configuré pour recevoir un échantillon de sang ou de solution contenant du sang ; et
    - au moins un élément d'espacement (13, 13') configuré pour confiner dans le plan du substrat l'échantillon de sang et pour éloigner ledit substrat (11) dudit support (12).

2. Dispositif (1) selon la revendication précédente, où :

    - la première électrode (34) de chaque paire d'électrodes de détection (34, 34') est connectée à la première entrée au moyen d'un premier chemin de connexion (44) ;
    - la première électrode (37) de chaque paire d'électrodes de référence (37, 37') est connectée à la seconde entrée au moyen d'un deuxième chemin de connexion (47);
    - la seconde électrode (34') de chaque paire d'électrodes de détection (34, 34') est connectée au nœud à partir duquel le signal de sortie (Out) est émis au moyen d'un troisième chemin de connexion (44') ; et
    - la seconde électrode (37') de chaque paire d'électrodes de référence (37, 37') est connectée au nœud à partir duquel ledit signal de sortie (Out) est émis au moyen d'un quatrième chemin de connexion (47') ;

    au-dessus de chacun desdits chemins de connexion (44, 44', 47, 37'), une couche isolante (40, 40', 50, 50') étant placée avec une constante diélectrique et une épaisseur telles qu'elles rendent négligeable l'impédance entre lesdits chemins de connexion (44, 44', 47, 37').

3. Dispositif (1) selon la revendication 1 ou 2, où ledit concentrateur au minimum (10, 10', 10", 14, 14', 14") est de forme cylindrique, le diamètre de la surface de base desdits concentrateurs (14, 14', 14") étant compris entre 10 et 30 μm, la hauteur desdits concentrateurs (14, 14', 14") étant comprise entre 10 et 30 μm et la distance entre lesdits concentrateurs (14, 14', 14") étant comprise entre 50 et 150 μm.

4. Dispositif selon l'une des revendications précédentes, où la première électrode (5, 6, 34) de ladite paire d'électrodes de détection au minimum (5, 5', 6, 6', 34, 34') et la seconde électrode (5', 6', 34') de ladite paire d'électrodes de détection au minimum (5, 5', 6, 6', 34, 34') sont de section rectangulaire, avec une base comprise entre 10 et 300 nm et une hauteur comprise entre 1 et 3 μm.

5. Dispositif (1) selon la revendication précédente, où la distance entre la première électrode (5, 6, 34) de ladite paire d'électrodes de détection au minimum (5, 5', 6, 6', 34, 34') et la seconde électrode (5', 6', 34') de ladite paire d'électrodes de détection au minimum (5, 5', 6, 6', 34, 34') est comprise entre 1 et 5 μm.

6. Appareil (100) pour la quantification de composants cellulaires et non cellulaires (3, 3', 3") dans une solution contenant un échantillon de sang comprenant :

- un dispositif (1) selon l'une des revendications précédentes ;
- une unité électronique pour la génération du premier signal d'entrée (V^+) et du second signal d'entrée (V-) et pour les lectures et le traitement du signal de sortie (Out) ;
- un boîtier configuré pour le positionnement dudit dispositif (1) ;
- une multitude de connecteurs pour la connexion entre ledit dispositif (1) et ladite unité électronique ; et
- des moyens pour la génération d'un champ magnétique statique (101, 102, 103), lesdits moyens (101, 102, 103) étant configurés pour générer un champ magnétique capable de provoquer, en combinaison avec les concentrateurs, la séparation des composants (3, 3', 3") à quantifier du reste de la solution et la concentration sur les électrodes de détection (5, 5', 6, 6', 34, 34').

7. Appareil (100) selon la revendication précédente, où lesdits moyens (101, 102, 103) pour la génération d'un champ magnétique statique comprennent une multitude d'aimants permanents (101, 102, 103) positionnés de sorte que le champ généré par lesdits aimants (101, 102, 103) en combinaison avec le gradient généré par ledit concentrateur au minimum (10, 10', 10", 14, 14', 14") soit tel que lesdits composants (3, 3', 3") s'accumulent sur les électrodes de détection (5, 5', 6, 6', 34, 34') du substrat (11), lesdits composants (3, 3', 3") étant paramagnétiques.

8. Appareil (100) selon la revendication 6, où lesdits moyens pour la génération d'un champ magnétique statique comprennent une multitude d'aimants permanents positionnés de sorte que le champ généré par lesdits aimants en combinaison avec le gradient généré par ledit concentrateur au minimum soit tel que lesdits composants (3, 3', 3") s'accumulent sur les électrodes de détection du substrat (11), lesdits composants (3, 3', 3") étant diamagnétiques.

9. Appareil (100) selon la revendication 6, où ledit champ magnétique a une intensité d'au moins 10^4 A/m et un gradient d'au moins 10^8 A/m^2.

10. Méthode pour la quantification des composants cellulaires et non cellulaires (3, 3', 3") dans une solution contenant un échantillon de sang comprenant :

- la séparation d'au moins un composant cellulaire ou non cellulaire (3, 3', 3") du reste de la solution ; la séparation étant provoquée par un champ magnétique statique en combinaison avec au moins un concentrateur (10, 10', 10", 14, 14', 14") en matériau ferromagnétique, configuré pour coopérer avec ledit champ magnétique. ;
- la concentration dudit au moins un composant séparé en correspondance d'au moins une paire d'électrodes de détection (4, 4', 5, 5', 6, 6', 34, 34'), la concentration étant provoquée par ledit champ magnétique statique en combinaison avec au moins un concentrateur (10, 10', 10", 14, 14', 14"), ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34') étant placée à proximité dudit concentrateur au minimum (10, 10', 10", 14, 14', 14") et ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34') comprenant au moins la première électrode (4, 5, 6, 34) connectée à une première entrée apte à recevoir un premier signal en entrée (V^+) et une seconde électrode (4', 5', 6', 34') et ledit concentrateur au minimum ;
- la mesure de la différence d'impédance entre ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34') et paire d'électrodes de référence au minimum (7, 7', 8, 8', 9, 9', 37, 37') produisant un signal de sortie proportionnel à la différence d'impédance entre ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34') et ladite paire d'électrodes de référence au minimum (7, 7', 8, 8', 9, 9', 37, 37'), ladite paire d'électrodes de référence au minimum (7, 7', 8, 8', 9, 9', 37, 37') étant placée dans des zones sans ledit concentrateur au minimum (10, 10', 10", 14, 14', 14") et ladite paire d'électrodes de référence au minimum (7, 7', 8, 8', 9, 9', 37, 37') comprenant une première électrode (7, 8, 9, 37) connectée à une seconde entrée configurée pour recevoir un second signal en entrée (V-) de polarité opposée au premier signal en entrée (V^+) et une seconde électrode (7', 8', 9', 37') connectée à la seconde électrode (4', 5', 6', 34') de ladite paire d'électrodes de détection au minimum (4, 4', 5, 5', 6, 6', 34, 34'), en un point commun à partir duquel un signal de sortie (Out) est capté ; et
- le calcul, au moyen d'un processeur et par comparaison avec une courbe d'étalonnage appropriée, du nombre de composants (3, 3', 3") séparés sur la base dudit signal de sortie.

11. Méthode selon la revendication 10, où les composants cellulaires (3, 3', 3"), sont des corpuscules sanguins dont les propriétés magnétiques peuvent être modifiées par des pathologies.

12. Méthode selon la revendication 10 ou 11 où les composants non cellulaires (3, 3', 3") sont des substances ayant des propriétés magnétiques différentes de celles du plasma, lesdites substances étant absentes dans les conditions physiologiques, ou lesdites substances étant présentes dans une concentration qui est différente entre les conditions physiologiques et pathologiques.

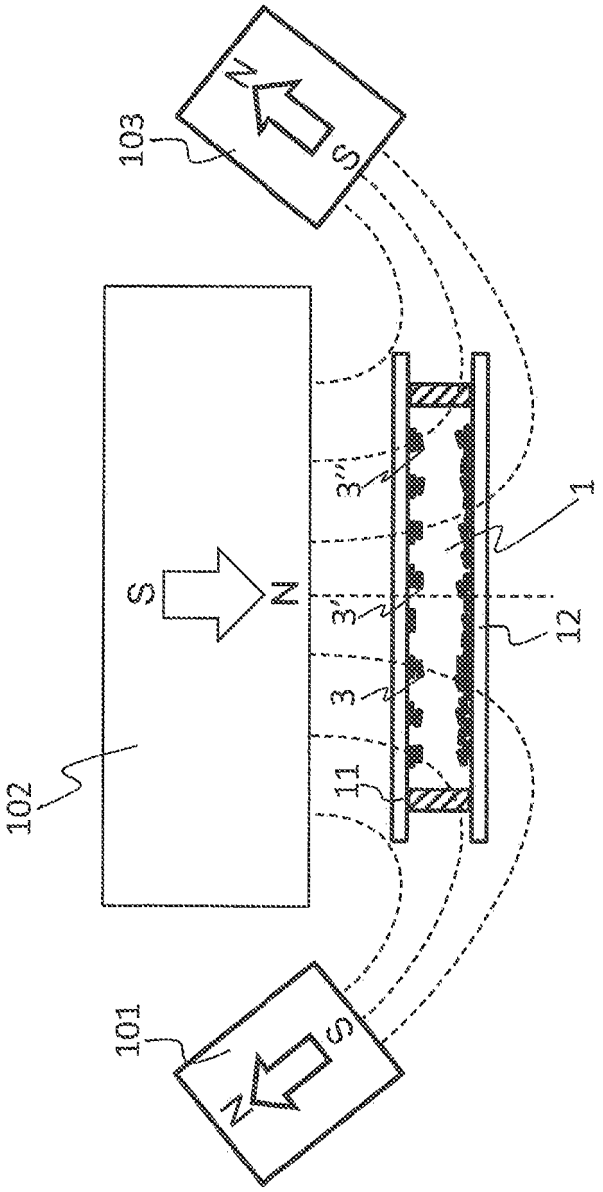

Fig.1

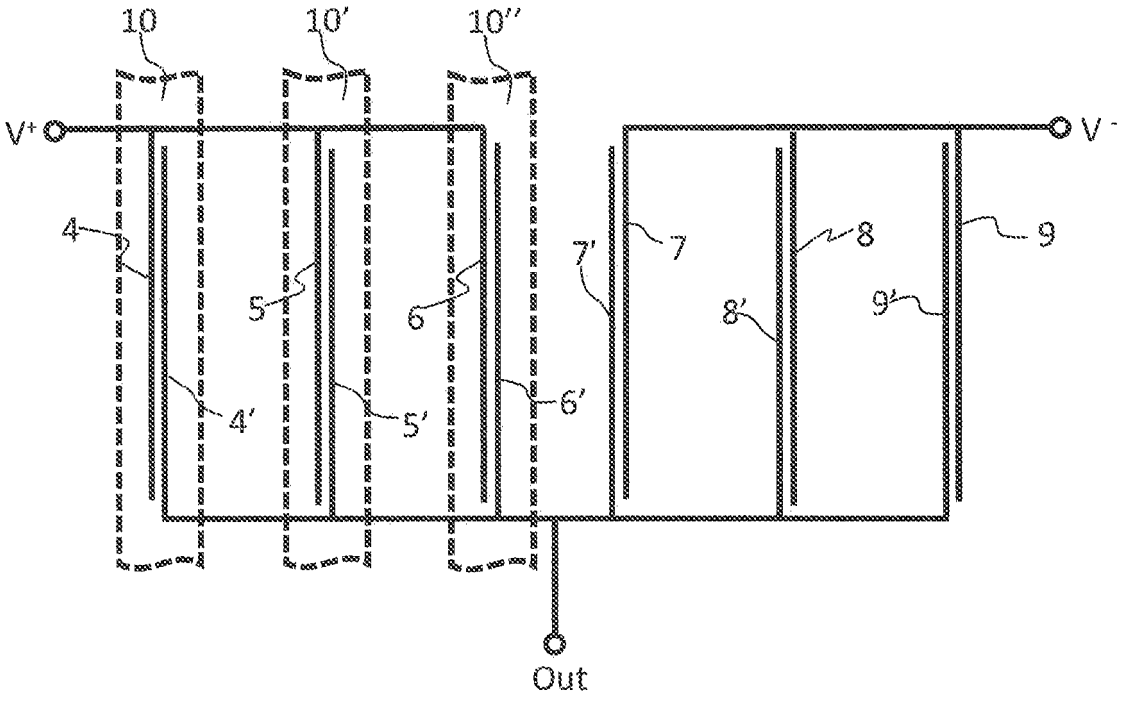

Fig.2

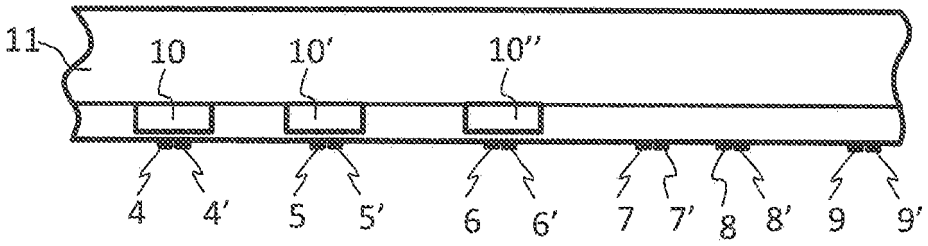

Fig.3a

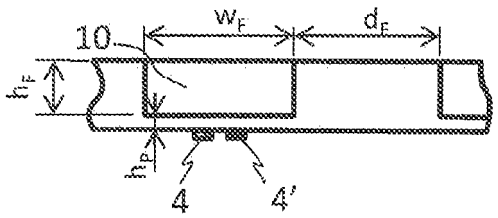

Fig.3b

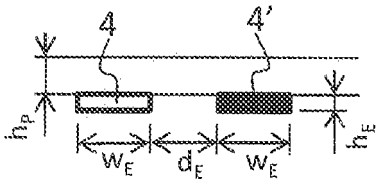

Fig.3c

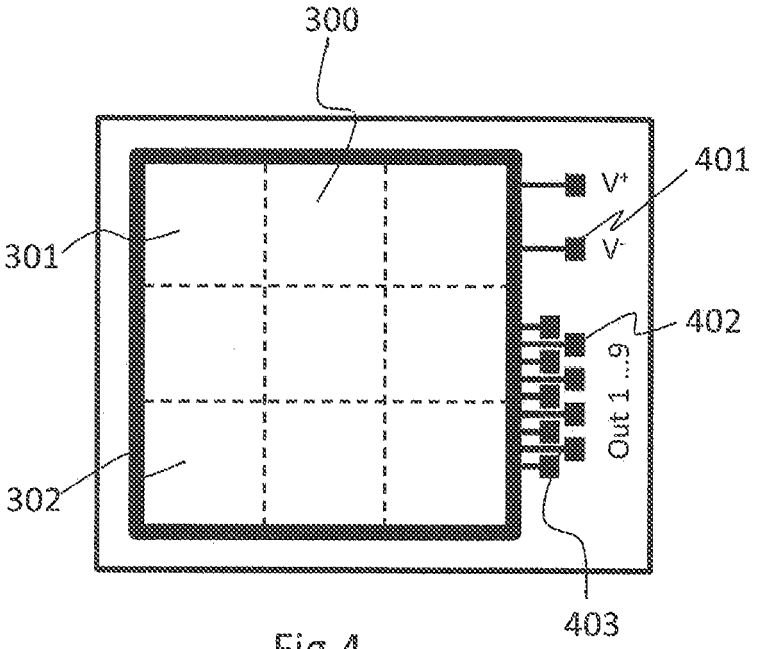

Fig.4

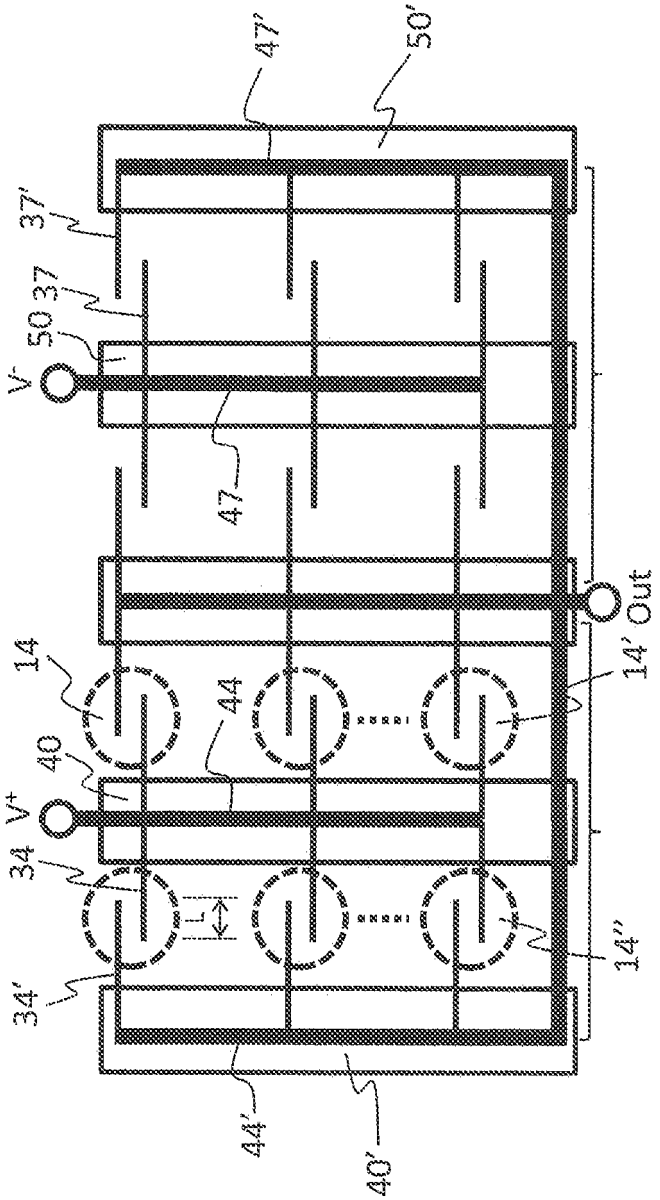

Fig.5

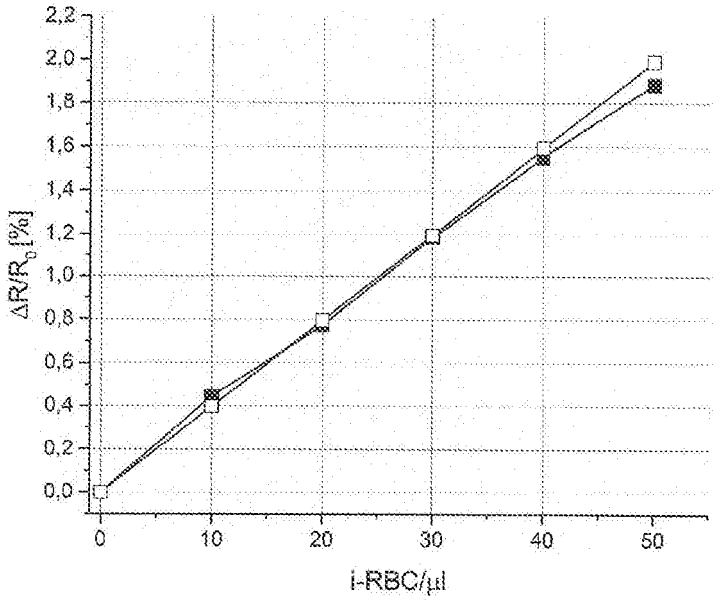

Fig.6

REFERENCES CITED IN THE DESCRIPTION

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