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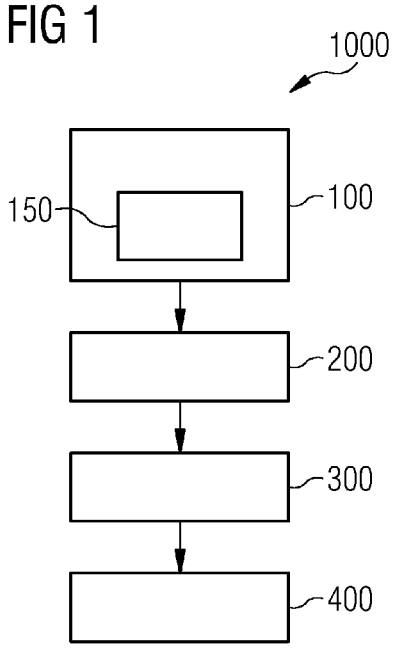
[Continued on next page]

(54) Title: A METHOD FOR DETERMINING A CHARACTERISTIC OF A BIOLOGICAL CORPUSCULAR ENTITY

(57) Abstract: The present technique presents a method for determining a characteristic of a biological corpuscular entity in a biological sample obtained from a subject. In the method, the biological sample including the biological corpuscular entity in its native morphological form is provided. To the biological sample, a reagent mixture is mixed including at least a fixative in the reagent mixture. The fixative maintains the native morphological form of the biological corpuscular entity. Subsequently, the biological sample is inspected with an interferometric microscopy device to obtain an interference pattern representing the biological corpuscular entity in the native morphological form. Finally in the method, the interference pattern is analyzed to determine the characteristic of the biological corpuscular entity in the native morphological form. Thus a volumetric and/or morphological study of the biological corpuscular entity is performed in the native morphological form.

FIG 1



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Description

A method for determining a characteristic of a biological corpuscular entity

The present invention relates to interferometric techniques, and more particularly to a method for determining a characteristic of a biological corpuscular entity in a biological sample by an interferometric microscopy device.

In present day hematology analyzer for determining volume of a specimen, such as biological corpuscular entities for example Red blood cells (RBCs) i.e. erythrocyte, White blood cells (WBCs), bacterial cells, etc, in a biological sample such as in a plasma or other medium, the biological sample, hereinafter also referred to as the sample, is diluted by addition of a buffer. The biological corpuscular entity for example a RBC is rounded up from its native morphological state or form, in case of the RBC, the RBC is rounded up i.e. changed into a spherical form, which is a distorted morphological form, from its native biconcave form. The rounding up of the biological corpuscular entity is achieved by adding a surfactant and a regulator to adjust the osmotic pressure to prepare the biological corpuscular entity for conducting volumetric measurements using Mie scattering. However volumetric measurements done by the above described method are not exact as the rounding up of the biological corpuscular entity creates the distorted morphological form and thereby affects the volume of the biological corpuscular entity. Moreover, simultaneous study of morphology of the biological corpuscular entity is not possible as the morphology has been changed in the rounding up, i.e. from native morphological form to a distorted morphological form, during preparation of the corpuscular entity to suit requirements of Mie scattering technique.

Furthermore, when no distortion of the native morphological form, as in rounding up, is willingly performed, still the

morphological form of the biological corpuscular entities are continuously changing when the biological corpuscular entities are brought out from the subject and most importantly when the biological corpuscular entities are made to contact a foreign surface such as surface of a glass slide or a polymer film as part of preparing the sample that is to be studied by the microscopic device, for example by an interferometric microscopy device such as a digital holographic microscope. The biological corpuscular entity with actively distorted morphological form or which is continuously changing when in contact with the glass slide does not yield accurate volumetric measurements or morphological studies. The output of the interferometric microscopy device using the sample having the biological corpuscular entity with actively distorted morphological form or with continuously changing morphological form do not yield accurate and/or reproducible results.

Thus it is desirable that a determination of a characteristic, such as volumetric or morphological measurements, of a biological corpuscular entity in a biological sample is performed in such as way that the morphological state of the biological corpuscular entity represented in the output, i.e. image or interference pattern, of the interferometric microscopy device is same or substantially similar to a native morphological form of the biological corpuscular entity, and thus more accurately representing the characteristic of the biological corpuscular entity than when the biological corpuscular entity is distorted before inspection or undergoes distortion during inspection. Furthermore, the output of the interferometric microscopy device is desired to be reproducible.

Thus the object of the present disclosure is to provide a method for determining a characteristic of a biological corpuscular entity in a biological sample such that the characteristic determined by the method of the present technique

represents characteristic of the biological corpuscular entity in its native morphological form.

The above objects are achieved by a method according to claim 1 of the present technique. Advantageous embodiments of the present technique are provided in dependent claims. Features of claim 1 may be combined with features of dependent claims, and features of dependent claims can be combined together.

The present technique presents, a method for determining a characteristic of a biological corpuscular entity in a biological sample obtained from a subject. In the method the biological sample is provided. The biological sample includes the biological corpuscular entity. The biological corpuscular entity has a native morphological form in the biological sample. Thereby, a reagent mixture is mixed with the biological sample. The reagent mixture includes at least a fixative that maintains the native morphological form of the biological corpuscular entity. Subsequently in the method, the biological sample is inspected with an interferometric microscopy device to obtain an interference pattern representing the biological corpuscular entity in the native morphological form. Finally in the method, the interference pattern is analyzed to determine the characteristic of the biological corpuscular entity in the native morphological form.

Thus a volumetric and/or morphological study of the biological corpuscular entity is performed in the native morphological form, hereinafter also referred to as the native form, i.e. without pre-inspection rounding up of the biological corpuscular entity, and thereby the biological corpuscular entity has not undergone a change in morphology and thus the characteristic of the and/or morphological that is determined is precise and exact for example if the characteristic determined is volumetric data and/or morphological data of the biological corpuscular entity then the data is precise. Furthermore since the biological corpuscular entity is not rounded up and thereby is maintained in its native form dur-

ing the inspection by the interferometric microscopy device, the morphological study and the volumetric study can be performed simultaneously from the same interference pattern.

In an embodiment of the method, the native morphological form is same as a form in which the biological corpuscular entity existed in the subject. Thus characteristics of the biological corpuscular entity determined by the method of the present technique represent characteristics of the biological corpuscular entity inside the subject, for example, when the biological corpuscular entity is an RBC and if the characteristic to be determined is a volume of the RBC, then by the method of the present technique, the volume of the RBC that is determined represents the volume of the RBC when the RBC was present in the subject from whom the RBC has been obtained. Thus the biological sample which is blood in this case is drawn from the subject and fixatives are added before the RBCs undergo any desiccation, degeneration or morphological alteration.

In another embodiment of the method, the native morphological form is a non-spherical shape. Thus the biological corpuscular entity that have native form as non-spherical are included in the method of the present technique. Examples of the biological corpuscular entity that are non-spherical exclude rounded up cellular entities prepared by adding surfactant to the biological corpuscular entity, but include blood cells that are non-spherical for example non-spherical WBC, sickle-cell shaped RBC, platelets, microorganisms that are non-spherical, and so and so forth.

In another embodiment of the method, the biological corpuscular entity is an erythrocyte and the native morphological form is an oval biconcave disk shape. Thus characteristic such as volume and/or morphology of the RBC is determined.

In another embodiment of the method, the fixative is a cross-linking fixative. Cross-linking fixatives provide a simple

and quick way of cross-linking of proteins or phospholipids with free amino groups or unsaturated lipids or like chemical entities in the cell wall to preserve or maintain the native morphological form of the biological corpuscular entity.

In another embodiment of the method, the cross-linking fixative comprises an aldehyde group. Aldehyde group containing fixatives, for example formaldehyde, are efficient for fixing the biological corpuscular entity in its native morphological form.

In another embodiment of the method, the cross-linking fixative is Glutaraldehyde. Glutaraldehyde provides a more rigid or tightly linked fixed corpuscular entity and thus the native form of the biological corpuscular entity is well preserved until the inspection of the biological corpuscular entity and while the inspection of the biological corpuscular entity is being performed.

In another embodiment of the method, the method includes mixing an anticoagulant to the biological sample before inspecting the biological sample. Thus the biological corpuscular entity in the biological sample is not coagulated with another corpuscular entity or with another component of the biological sample.

In another embodiment of the method, the anticoagulant is one of EDTA, Heparin, Acid citrate dextrose, and a combination thereof. These provide effective anticoagulants.

In another embodiment of the method, in inspecting the biological sample, the interferometric microscopy device is a common path interferometric microscopy device. The common path interferometric microscopy device performs common path interferometry and provides advantageous outputs, for example volume information, compared to other forms of imaging or microscopy.

In another embodiment of the method, the common path interferometric microscopy device is a Digital Holographic Microscopy device. This provides an easy way of implementing the method of the present technique.

In another embodiment of the method, in inspecting the biological sample, the interferometric microscopy device is a different path interferometric microscopy device. This provides an easy way of implementing the method by using a simple setup because different path interferometry works on a simpler setup as compared to the common path interferometry.

In another embodiment of the method, in analyzing the interference pattern to determine the characteristic of the biological corpuscular entity in the native morphological form, a morphological feature of the biological corpuscular entity is determined. Thus morphological feature of the biological corpuscular entity in its native form is determinable.

In another embodiment of the method, in analyzing the interference pattern to determine the characteristic of the biological corpuscular entity in the native morphological form, a volumetric measurement of the biological corpuscular entity is determined. Thus, volumetric measurement of the biological corpuscular entity in its native form is determinable. Furthermore, the morphological feature determination and the volumetric measurement of the biological corpuscular entity may be performed at the same time with the one and the same interference pattern output of the interferometric microscopy device.

The present technique is further described hereinafter with reference to illustrated embodiments shown in the accompanying drawing, in which:

FIG 1 depicts a flow chart illustrating an exemplary embodiment of a method of the present technique;

FIG 2 schematically illustrates a biological sample with biological corpuscular entities and further depicts a native morphological form and a distorted morphological form of the biological corpuscular entity;

FIG 3 schematically illustrates exemplary interferometric microscopy outputs of the biological corpuscular entities without fixative at different sequential instances of time; and

FIG 4 schematically illustrates exemplary interferometric microscopy outputs of the biological corpuscular entities with fixative at different sequential instances of time; in accordance with aspects of the present technique.

Hereinafter, above-mentioned and other features of the present technique are described in details. Various embodiments are described with reference to the drawing, wherein like reference numerals are used to refer to like elements throughout. In the following description, for purpose of explanation, numerous specific details are set forth in order to provide a thorough understanding of one or more embodiments. It may be noted that the illustrated embodiments are intended to explain, and not to limit the invention. It may be evident that such embodiments may be practiced without these specific details.

The basic idea of the present technique is to acquire image or interference pattern using an interferometric microscopy device while the biological corpuscular entity is maintained in its native morphological form i.e. neither the biological corpuscular entity is distorted willfully, for example by rounding up of the biological corpuscular entity, nor the biological corpuscular entity is allowed to undergo desiccation or change in morphological form when the biological corpuscular entity is positioned on a surface, such as a glass slide or polymer film, for purposes of inspection by the imaging

device. The important aspect is to not use any chemical substance, such as a surfactant or a chemical entity that promote hyper-osmotic or hypo-osmotic conditions and/or that promotes distortion of the native morphological form of the biological corpuscular entity, but to fix the biological corpuscular entity by using a fixative while the biological corpuscular entity is in its native morphological form, for example RBC in blood plasma. This way the biological corpuscular entity is fixed or made rigid or mechanically strong in its native morphological form i.e. no distortion of the biological corpuscular entity is performed and at the same time the biological corpuscular entity is not allowed to undergo desiccation or change in morphological form, deviating from the native morphological form, when the biological corpuscular entity is positioned on the surface for purposes of inspection by the imaging device.

FIG 1 depicts a flow chart illustrating an exemplary embodiment of a method 1000 of the present technique. The method 1000 has been explained hereinafter using FIG 1 in combination with FIGs 2, 3 and 4. As shown in FIG 2, the method 1000 of FIG 1 is for determining a characteristic of a biological corpuscular entity 5, hereinafter also referred to as the entity 5, in a biological sample 7, hereinafter also referred to as the sample 7. The sample 7 is obtained from a subject (not shown). The entity 5 may be, but not limited to, erythrocyte, white blood cell, a microorganism, a cell, and so on and so forth. The sample 7 may be, but not limited to, whole blood, diluted blood, plasma, and so on and so forth.

In FIG 2, the entity 5, say for example an erythrocyte or a red blood cell 5 hereinafter referred to as the RBC 5, has been portrayed to be present in the sample 7. The RBC 5 may have two morphological forms, i.e. structures, - a native morphological form 4 or a distorted morphological form 6. The native morphological form 4 is a form in which the entity 5, in this case the RBC 5, exists naturally. The native morphological form 4 may be as the entity 5 exists generally in the

subject for example when the entity 5 is the RBC 5 the native morphological form generally is biconcave form or shape or structure for the RBC 5. The native morphological form 4 may also be as the entity 5 exists under some specific physiological condition in the subject. In general the native morphological form 4 of the entity 5 may be understood as that morphological form in which the entity 5 was when collected from the subject. The distorted morphological form 6 is a form in which the entity 5, in this case the RBC 5, does not exist naturally. The distorted morphological form 6 may be as the entity 5 exists when the native morphological form 4 of the entity 5 has been changed or distorted or disturbed for example by using a surfactant to round up or make the RBC 5 spherical. In general the distorted morphological form 4 of the entity 5 may be understood as that morphological form in which the entity 5 is after the native morphological form 4 of the entity 5 has been distorted by an external cause such as by use of a chemical substance like a surfactant. Thus as shown in FIG 2, the RBC 5 may have either the native morphological form 4 or the distorted morphological form 6.

In the method 1000, the sample 7 is provided in a step 100. The sample 7 provided in the step 100 includes the entity 5. The entity 5 is in the native morphological form 4, i.e. no distortion of the native morphological form 4 has been performed on the entity 5. The native biological form 4 may be a form in which the entity 5 existed in the subject, for example non-spherical shape, or for example biconcave disk shape when the entity 5 is the RBC 5.

Additionally, in the method 1000, when the sample 7 is such that the sample 7 may undergo coagulation, the sample 7 is mixing with an anticoagulant in a step 150. The anticoagulant may be, but not limited to, EDTA (Ethylenediaminetetraacetic acid) and/or Heparin and/or Acid citrate dextrose. There are several types of anticoagulants, which differ in their mechanism of action. Heparin binds to antithrombin III and accelerates the inactivation of thrombin and other clotting fac-

tors. EDTA chelates metals, such as calcium and magnesium. Acid citrate dextrose also chelates calcium.

In the method 1000, subsequent to step 100, in a step 200, a reagent mixture is mixed with the sample 7. The reagent mixture includes at least a fixative. The fixative maintains the native morphological form 4 of the entity 5. The fixative either inhibits onset of or slows downs a rate of autolysis or putrefaction or desiccation of the entity 5 and thereby maintains the native morphological form 4 of the entity 5. The fixative basically functions to mechanically strengthen an outer covering for example a cell wall of the entity 5 by an action on the cell wall of the entity 5.

The fixative, as used in the present disclosure, includes a chemical substance used to preserve or stabilize the entity 5, i.e. to make the entity 5 physically and/or chemically robust, in a state in which the fixative is provided to the entity 5. In the method 1000, the fixative is provided to the entity 5 in the step 200 following the step 100 wherein the entity 5 is in the native morphological form 4. It may be noted that it is essential for the method 1000 that no surfactant is used in the reagent mixture or is provided to the sample 7 or is contacted with the entity 5 before or during the step 200. The fixative used herein are primarily, but not limited to, a cross-linking fixative for example Aldehydes such as Glutaraldehyde, Formaldehyde, etc, that act by creating covalent chemical bonds between proteins in biological membranes or cell walls of the entity 5 such as the cell wall of the RBC 5. The cross-linking of proteins in the cell wall anchors soluble proteins in the cell wall and provides overall rigidity to at least the external biological membrane or cell wall of the entity 5 and thus preserving the structural or morphological integrity of the entity 5 i.e. maintaining the entity 5, i.e. the RBC 5, in the its native morphological form 4.

Glutaraldehyde, like other similar fixatives, operates by causing deformation of the alpha-helix structures in proteins similar to formaldehyde action. However, glutaraldehyde fixation provides a more rigid or tightly linked fixed product – the greater length of a Glutaraldehyde molecule and two aldehyde groups in the Glutaraldehyde molecule allow the Glutaraldehyde molecule to 'bridge' and link more distant pairs of protein molecules on the cell wall of the RBC 5. Glutaraldehyde causes rapid and irreversible changes in the entity 5 in form of the cross-linking of the proteins as mentioned hereinabove, fixes the entity 5 quickly and fixes the entity 5 well even at lower temperature such as at 4°C. Thus outcome of the step 200 is that the entity 5, i.e. the RBC 5, is fixed in the native morphological form 4 and is not in the distorted morphological form 6.

In a next step 300 of the method 1000, the sample 7 is inspected with an interferometric microscopy device to obtain an interference pattern or an image. The interference pattern or an image provided as an output of the interferometric microscopy device represents the entity 5 in the native morphological form 4.

In one embodiment of the method 1000, in the step 300, the interferometric microscopy device is a common path interferometric microscopy device, for example a digital holographic microscopy device. In the common path interferometric microscopy device (not shown), for example the digital holographic microscopy device, a beam of light, for example laser light, is shone or impinged on the sample 7 and then the beam emerges after interacting with the sample 7 and continues into a microscope objective and then the beam is subsequently split into an object beam and a reference beam with help of a beam splitter and associated optics. The object beam travels along an object beam path that substantially overlaps with a reference beam path along which the reference beam travels. With help of a spatial filter, for example a pinhole, present in the common path interferometric

microscopy device, object information is deleted or erased or removed from the reference beam. The object beam and the reference beam are then overlapped with each other at an optical detector on which the image or the interference pattern is formed by superimposition of the object beam and the spatially filtered reference beam. The principle of working and optical setup of the common path interferometric microscopy device, for example the digital holographic microscopy device, are well known in field of microscopy and so not explained herein in details for sake of brevity.

In another embodiment of the method 1000, in the step 300, the interferometric microscopy device is a different path interferometric microscopy device (not shown). In the different path interferometric microscopy device, with a beam splitter a light beam, for example laser light, is split to generate an object beam and a reference beam before the light beam is shone upon the sample 7. The object beam interacts with the sample 7, collects object information from the sample 2, more particularly from the entity 5, and continues through an optical setup to finally meet the object beam that is devoid of object information as the object beam did not interact with the sample 7. The object beam and the reference beam are then overlapped with each other at an optical detector on which the image or the interference pattern is formed by superimposition of the object beam and the reference beam. The principle of working and optical setup of the different path interferometric microscopy device are well known in the field of microscopy and so not explained herein in details for sake of brevity.

FIG 3 schematically illustrates exemplary interferometric microscopy outputs, i.e. an interference pattern or an image representing the entities 5, at different sequential instances of time without the fixative effect. The entities 5 represented in FIG 3 have not been contacted or provided with the fixative in step 200. In contrast to FIG 3, FIG 4 schematically illustrates exemplary interferometric microscopy out-

puts, i.e. an interference pattern or an image representing the entities 5, at different sequential instances of time with the fixative effect. The entities 5 represented in FIG 4 have been contacted or provided with the fixative in step 200.

As is clearly depicted in FIG 3, when the entities 5 are not provided with the fixative in step 200, the entities 5 undergo a morphological change, for example initiated by a contact between the entities 5 and a surface of glass or polymer, between two sequential time instances i.e. at a begin time, represented in the FIG 3 as 't=0 min' in section 10 of the FIG 3, when the inspection 300 of the sample 7 was performed in a state represented by the sample 7 immediately after the sample 7 was obtained from the subject and at a later instance of time, represented in the FIG 3 as 't=5 min' in section 15 of the FIG 3, when the inspection 300 of the sample 7 was performed in a state represented by the sample 7 at end of 5 minutes after the sample 7 was obtained from the subject. The entities 5 change from the native morphological form 4 in the section 10 of the image represented in FIG 3 to the distorted morphological form 6, also referred to as echinocytes for RBCs in the section 15 of the image represented in FIG 3.

In contrast, as is clearly depicted in FIG 4, when the entities 5 are provided with the fixative in step 200, the entities 5 do not undergo a morphological change or remain substantially same morphologically between two sequential time instances i.e. at a begin time, represented in the FIG 4 as 't=0 min' in section 20 of the FIG 4, when the inspection 300 of the sample 7 was performed in a state represented by the sample 7 immediately after the sample 7 was obtained from the subject and at a later instance of time, represented in the FIG 4 as 't=5 min' in section 25 of the FIG 4, when the inspection 300 of the sample 7 was performed in a state represented by the sample 7 at end of 5 minutes after the sample 7 was obtained from the subject. Furthermore, when the entities

5 are provided with the fixative in step 200, the entities 5 do not undergo a morphological change or remain substantially same morphologically even at a further later time instance i.e. at a further later time, represented in the FIG 4 as‘t=15 min’ in section 35 of the FIG 4, when the inspection 300 of the sample 7 was performed in a state represented by the sample 7 at end of 15 minutes after the sample 7 was obtained from the subject. The entities 5 do not change morphologically and remain substantially same morphologically, i.e. in the native morphological form 4, in the sections 20, 25 and 35 of the image represented in FIG 4.

In the method 1000, after the step 300, a step 400 is performed in which the interference pattern is analyzed to determine the characteristic of the entity 5 in the native morphological form 4. It may be noted that in the present disclosure, the term image and the term interference pattern have been used interchangeably and mean the output of the interference microscopy device. From the interference pattern obtained as an output of the step 300 representing the entity 5 in the native morphological form 4, a morphological feature of the entity 5 is determined. For example a specific structural feature such as dimensions of the entity 5. From the interference pattern obtained as the output of the step 300 representing the entity 5 in the native morphological form 4, a volumetric measurement of the entity 5 is determined, i.e. an amount of volume of the entity 5.

While the present technique has been described in detail with reference to certain embodiments, it should be appreciated that the present technique is not limited to those precise embodiments. Rather, in view of the present disclosure which describes exemplary modes for practicing the invention, many modifications and variations would present themselves, to those skilled in the art without departing from the scope and spirit of this invention. The scope of the invention is, therefore, indicated by the following claims rather than by

the foregoing description. All changes, modifications, and variations coming within the meaning and range of equivalency of the claims are to be considered within their scope.

Patent claims

1. A method (1000) for determining a characteristic of a biological corpuscular entity (5) in a biological sample (7) obtained from a subject, the method (1000) comprising:
- providing (100) the biological sample (7), wherein the biological sample (7) includes the biological corpuscular entity (5) and wherein the biological corpuscular entity (5) has a native morphological form;
- mixing (200) a reagent mixture with the biological sample (7), wherein the reagent mixture comprises at least a fixative, and wherein the fixative is configured to maintain the native morphological form of the biological corpuscular entity (5);
- inspecting (300) the biological sample (7) with an interferometric microscopy device to obtain an interference pattern representing the biological corpuscular entity (5) in the native morphological form; and
- analyzing (400) the interference pattern to determine the characteristic of the biological corpuscular entity (5) in the native morphological form.

2. The method (1000) according to claim 1, wherein the native morphological form is same as a form in which the biological corpuscular entity (5) existed in the subject.

3. The method (1000) according to claim 1 or 2, wherein the native morphological form is a non-spherical shape.

4. The method (1000) according to any of claims 1 to 3, wherein the biological corpuscular entity (5) is an erythrocyte and the native morphological form is a biconcave disk shape.

5. The method (1000) according to any of claims 1 to 4, wherein the fixative is a cross-linking fixative.

6. The method (1000) according to claim 5, wherein the cross-linking fixative comprises an aldehyde group.

7. The method (1000) according to claim 6, wherein the cross-linking fixative is Glutaraldehyde.

8. The method (1000) according to any of claims 1 to 7, comprising mixing (150) an anticoagulant to the biological sample (7) before inspecting (300) the biological sample (5).

9. The method (1000) according to claim 8, wherein the anticoagulant is one of EDTA, Heparin, Acid citrate dextrose, and a combination thereof.

10. The method (1000) according to any of claims 1 to 9, wherein in inspecting (300) the biological sample (7), the interferometric microscopy device is a common path interferometric microscopy device.

11. The method (1000) according to claim 10, wherein the common path interferometric microscopy device is a digital holographic microscopy device.

12. The method (1000) according to any of claims 1 to 9, wherein in inspecting (300) the biological sample (7), the interferometric microscopy device is a different path interferometric microscopy device.

13. The method (1000) according to any of claims 1 to 12, wherein in analyzing (400) the interference pattern to determine the characteristic of the biological corpuscular entity (5) in the native morphological form, a morphological feature of the biological corpuscular entity (5) is determined.

14. The method (1000) according to any of claims 1 to 13, wherein in analyzing (400) the interference pattern to determine the characteristic of the biological corpuscular entity

(5) in the native morphological form, a volumetric measurement of the biological corpuscular entity (5) is determined.

FIG 1

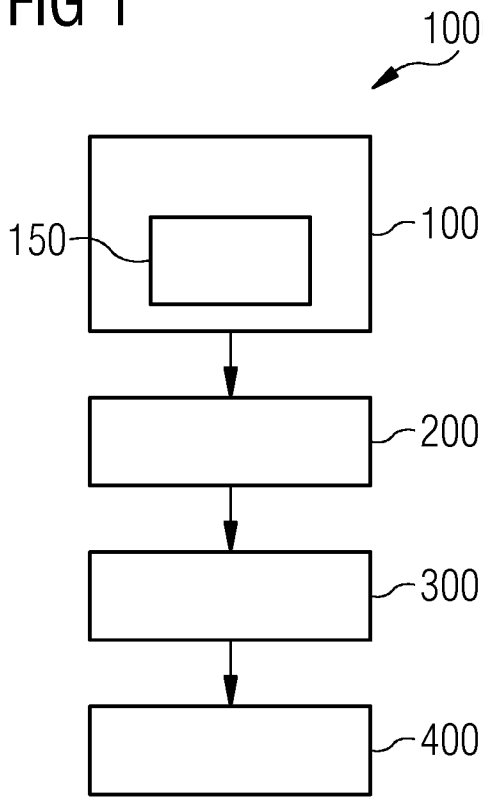


FIG 2

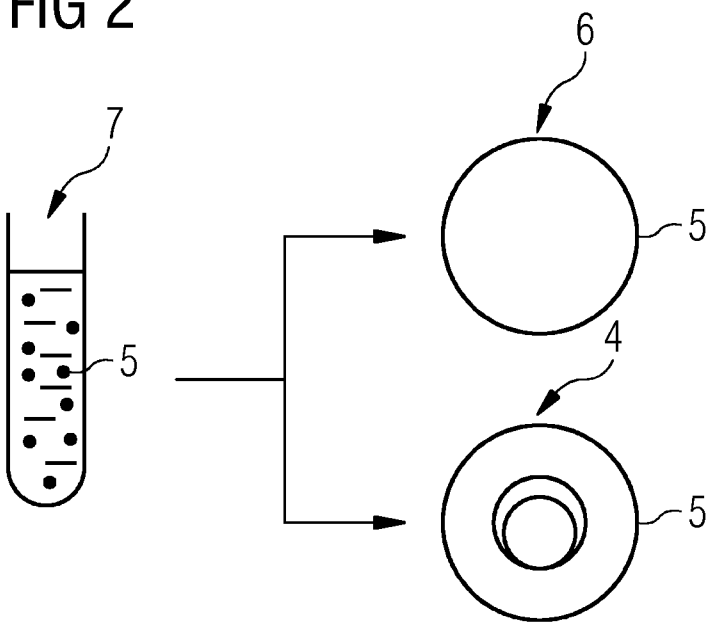
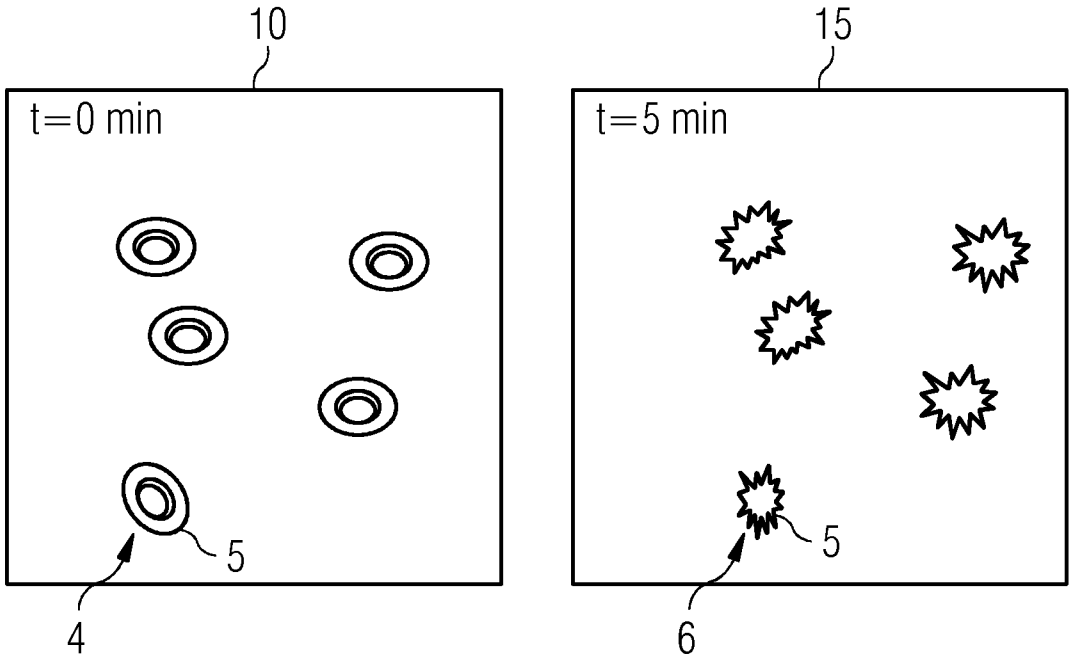


FIG 3



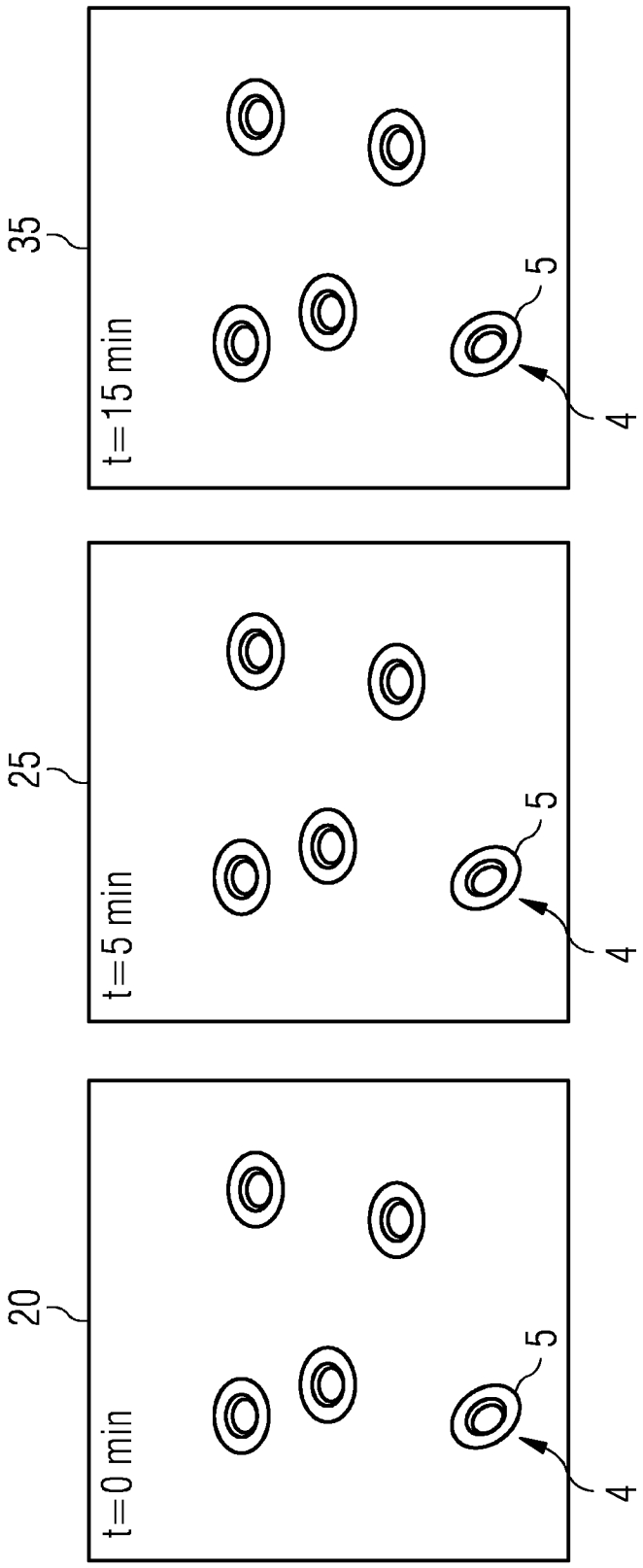


FIG 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/072261

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/80 G01N1/30 G01N21/45 G02B21/00 G03H1/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N G02B G03H

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OLGA KOMAROVA ET AL: "Effective chemical preservation of morphology of urinary erythrocytes", PEDIATRIC NEPHROLOGY., vol. 18, no. 7, 1 July 2003 (2003-07-01), pages 665-666, XP055262450, DE ISSN: 0931-041X, DOI: 10.1007/s00467-003-1149-6	1-14
Y	the whole document abstract tables 1, 2	1-14

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

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Date of the actual completion of the international search	Date of mailing of the international search report
1 June 2016	10/06/2016

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HERBERT J KAYDEN ET AL: "Morphology of Normal Erythrocyte and Acanthocyte Using Nomarski Optics and the Scanning Electron Microscope", BLOOD, vol. 35, 1 January 1970 (1970-01-01), pages 427-436, XP055262455,	1-14
Y	the whole document abstract page 427 - page 430 figure 2	1-14

X	GENNADY I KOZINETS ET AL: "Blood cell research using methods of microinterferometry", PROCEEDINGS SPIE, vol. 2982, 2 May 1997 (1997-05-02), XP055262457, US ISSN: 0277-786X, DOI: 10.1117/12.273652	1-14
Y	the whole document abstract figures 2, 5 page 494 - page 495	1-14

Y	PAUL N MCMILLAN ET AL: "Preservation of Erythrocyte Ghost Ultrastructure Achieved by Various Fixatives (plasma membranes/electron microscopy/fixation procedures/membrane proteins)", PNAS, vol. 70, 1 November 1973 (1973-11-01), pages 3060-3064, XP055262461, the whole document abstract	1-14

Y	CHRISTIAN J. SCHWARZ ET AL: "Imaging interferometric microscopy", OPTICS LETTERS, vol. 28, no. 16, 15 August 2003 (2003-08-15), page 1424, XP055076319, ISSN: 0146-9592, DOI: 10.1364/OL.28.001424 the whole document	1-14

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