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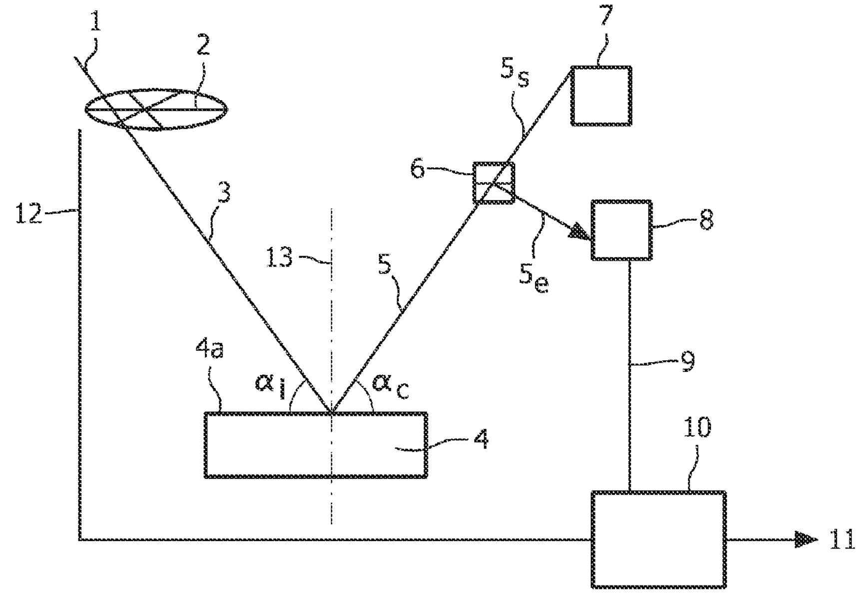
(54) Title: REFLECTION OR SINGLE SCATTERING SPECTROSCOPY AND IMAGING



(57) Abstract: The invention relates to a method for the analysis of a probe medium (4) by interaction between an incident light beam (3), having an intensity, and the probe medium (4), the incident light beam (3) also passing through a scattering medium, the method comprising: - modulating the amplitude of at least two polarization components of the incident light beam (3), while keeping the intensity of the incident light beam constant, - after interaction between the modulated incident light beam (3) and the probe medium (4) and scattering by the scattering medium, generating an outgoing interacted and backscattered light beam (5, 5'), collecting a collected interacted and backscattered light beam (5p, 5s) from the outgoing light beam (5, 5'), - demodulating the collected light beam (5), in order to measure a value related to the interaction, free from scattering which does not preserve modulation.

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Reflection or single scattering spectroscopy and imaging

FIELD OF THE INVENTION

The invention relates to the analysis of a probe medium by interaction between an incident light beam and the probe medium, and in particular to reflection spectroscopy on biological samples, such as skin, and imaging of biological samples, such as skin, with an optical scanning microscope.

BACKGROUND OF THE INVENTION

Reflection spectroscopy may be used to analyze the structure or composition of a biological sample. An incident light beam is irradiated on the sample; part of the light is reflected; the amount of reflection is typical of the material that the sample is composed of. The amount of reflected light is therefore measured in order to obtain information on the sample.

An example of a diffuse reflection spectroscopy method, for the diagnosis of pre-cancerous changes on skin, is given in the article “Polarized light scattering spectroscopy for quantitative measurements of epithelial cellular structures in situ”, by V. Backman, R. Gurjar, K. Badizadegan, I. Itzkan, R.R. Dasari, L.T. Perelman and M.S. Feld, in IEEE, Journal of Selected Topics in Quantum Electronics, vol.5, no.4, July/August 1999, pages 1019-1026.

A problem may arise when the probe sample is in the presence of a scattering environment, for instance, if the light signal has to pass through a fluid, with high scattering properties, submerging the sample, or if the signal is distorted by a scattering layer underlying the sample to be analyzed, such as underlying tissues when analyzing biological cells. The sample itself may also form a scattering environment.

In biological applications, the contribution of multiple scattering is much higher than the contribution of direct (i.e. specular) reflection or single scattering; this makes it very difficult to do an accurate measurement of the reflection spectrum.

In the prior art, for instance in the above-quoted article by V. Backman et al., this problem is solved by using polarized excitation light. The light polarization is preserved by direct reflection or single scattering while, when the light has scattered multiple times, the incoming polarization state is lost. This property is used in order to determine the respective contributions of reflection and scattering in the collected light, by analyzing the two polarization components of the collected light.

Before presenting further this prior art method, a few definitions will now be given.

A light beam may be decomposed into a P-polarization component and a S-polarization component. The P and S polarization directions constitute a vector basis for the polarization. The P-polarization component is the component comprised in the plane of incidence, this latter being defined by the propagation direction and a vector normal to the plane of the reflecting surface; the S-polarization component is the component perpendicular to the plane of incidence.

In the foregoing, the following notations will be taken:

- α is the reflection efficiency coefficient of a sample;

- β is the scattering efficiency coefficient of a sample;

- $I_{x,y}$ is the intensity (i.e. the energy) of a light beam polarization component, x representing its polarization (e.g. x = P or S for the P or S polarization components) and y representing the nature of the beam (i for incident, r for reflected, s for scattered and o for outgoing); therefore, as an example, $I_{P,r}$ represents the intensity of the P-polarization component of a reflected light beam; as another example, $I_{S,s}$ represents the intensity of the S-polarization component of a scattered light beam;

- the intensity of an incident light beam will be denoted I_i, the intensity of a reflected light beam will be denoted I_r, the intensity of a scattered light beam will be denoted I_s and the intensity of an outgoing light beam will be denoted I_o, the outgoing light being the light coming back from a medium; these values I_i, I_r, I_s and I_o represent the total energy of the corresponding beam, taking into account all its polarization components;

- let us notice that the intensity of the P-polarization component of the outgoing light beam is denoted $I_{P,o}$ and the intensity of the S-polarization component of the outgoing light beam is denoted $I_{S,o}$; these values represent the energy of the corresponding polarization components, taking into account all the types (i.e. nature) of light coming back from the medium (in the present description, they are therefore the sum, for each polarization component, of the reflected and scattered light intensities).

With the definitions above, the following simplified equations describe the behaviour of light:

[1] $I_i = I_{P,i} + I_{S,i}$

[2] $I_r = I_{P,r} + I_{S,r}$

[3] $I_s = I_{P,s} + I_{S,s}$

[4] $I_r = \alpha.I_i$

[5] $I_s = \beta.I_i$

[6] $I_o = I_{P,o} + I_{S,o} = I_r + I_s$

[7] $I_{P,o} = I_{P,r} + I_{P,s}$

[8] $I_{S,o} = I_{S,r} + I_{S,s}$

Reverting to the prior art, the sample is illuminated with a linearly polarized light beam, for instance a P-polarized light beam, the intensity of which is $I_i = I_{P,i}$ (no S-polarization component). Since reflection does not change the polarization, the reflected light only has a P-polarization component, such as $I_r = I_{P,r} = \alpha.I_{P,i}$, with $I_{S,r} = 0$. Scattering provokes a randomization of the polarization, that is to say, the scattered light is un-polarized: the P-polarization and S-polarization components of the scattered light beam are therefore equal to a constant B. As a consequence, the energy of the scattered light is $I_s = I_{P,s} + I_{S,s}$, with $I_{P,s} = I_{S,s} = B$.

The outgoing light beam, which comprises both the scattering and reflection contributions, is split into two light beams, each corresponding to one polarization component, each light beam being collected and its intensity measured, that is to say, $I_{P,o}$ and $I_{S,o}$. Those measured values are subtracted, which gives:

$$I_{P,o} - I_{S,o} = (I_{P,r} + I_{P,s}) - (I_{S,r} + I_{S,s}) = (\alpha.I_{P,i} + B) - (0 + B) = \alpha.I_{P,i}$$

Since $I_{P,i} = I_i$, this latter value being the known energy of the incident light beam, from the subtraction of the measured intensity of the P and S components of the outgoing light, the value of α can be calculated, thus giving the information required on the sample.

For a measurement under strong scattering conditions, this method is not accurate, because the intensities of the individual P and S polarization components due to scattering (i.e. $I_{P,s}$ and $I_{S,s}$) are very high, while the contribution of direct reflection (i.e. $I_{P,r}$) is very small. Therefore, the subtraction between the P and S polarization components (i.e. $I_{P,o}$ - $I_{S,o}$) cannot lead to precise results, since the noise on the P and S polarization components due to scattering will be in the order of magnitude of the value of the direct reflection contribution, or even much larger.

Thus, a problem exists on how to accurately measure the intensity of light that has interacted with a medium in the presence of a scattering environment.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a method for the analysis of a probe medium by interaction between an incident light beam and the probe medium, which is not influenced by scattering in the medium or in an environing scattering medium.

In accordance with the present invention there is provided a method for the analysis of a probe medium by interaction between an incident light beam, having an intensity, and the probe medium, the incident light beam also passing through a scattering medium, the method comprising:

- decomposing the incident light beam into at least two polarization components,
- modulating the amplitude of the polarization components of the incident light beam, while keeping the intensity of the incident light beam constant,
- after interaction between the modulated incident light beam and the probe medium and scattering by the scattering medium, generating an outgoing interacted and backscattered light beam, collecting a collected interacted and backscattered light beam from the outgoing light beam,
- demodulating the collected light beam, in order to measure a value related to the interaction, free from scattering which does not preserve modulation.

In fact, the "decomposing step" of the method of the invention is not necessarily a material step; it just means that the person skilled in the art shall choose any decomposition of the incident light into at least two polarization components in order to modulate those components. It is the fact of performing the modulation which defines those components, the light not necessarily being materially and actively decomposed into those components before the modulation, by splitting the light beam or any other means. Indeed, as well as any vector may always be "decomposed" into at least two components of a vector basis, that is to say, described as the sum of those components, any light beam may always be "decomposed" into at least two polarization components of a polarization basis which, combined, form the complete light beam.

As a consequence, the method of the invention may also be presented as follows: a method for the analysis of a probe medium by interaction between an incident light beam, having an intensity, and the probe medium, the incident light beam also passing through a scattering medium, the method comprising:

- modulating the amplitude of at least two polarization components of the incident light beam, while keeping the intensity of the incident light beam constant,
- after interaction between the modulated incident light beam and the probe medium and scattering by the scattering medium, generating an outgoing interacted and backscattered light beam, collecting a collected interacted and backscattered light beam from the outgoing light beam,
- demodulating the collected light beam, in order to measure a value related to the interaction, free from scattering which does not preserve modulation.

A method for the analysis of a probe medium shall be understood with a wide meaning and notably comprises reflection spectroscopy and imaging with a scanning microscope.

Thanks to the invention, in spite of the presence of a scattering environment, a value linked to the interaction between the light and the medium can be measured, without influence of scattering which does not preserve polarization. This permits to make more accurate measurements.

According to an embodiment, the interaction is reflection.

The problem solved by the invention has been presented in relation with reflection, but the invention shall not be restricted to this application. The invention applies to any interaction, between an incident light beam and a medium, which preserves polarization.

According to another embodiment, the interaction is single backscattering. Single backscattered light is light which has been scattered only once or very few times, as explained in the article, cited above, by V. Backman et al. Such single backscattered light presents the same property as reflected light: the polarization of the light beam is unchanged, while the polarization of a multiple backscattered light beam is randomized.

It should be understood that, in the method presented above, the scattering medium can be the probe medium and/or another medium, situated above, below or around the probe medium.

It should also be understood that the outgoing light beam refers to the interacted and scattered light beam, which may propagate in various directions and is not restricted to a ray. According to an embodiment, the outgoing light beam is analyzed in a particular analysis direction. In the present description, and as will be seen below, analyzing the outgoing light beam may comprise splitting the light beam in order to only collect part of it, or directly collecting the whole outgoing light beam.

According to one embodiment, light is analyzed in the reflection direction, in order to measure the intensity of the reflected light beam.

According to another embodiment, light is analyzed in a direction different from the reflection direction, in order to measure the intensity of a single backscattered light beam.

In the method presented above, the "polarization components" should be understood as being any polarization components into which a light beam may be decomposed.

According to an embodiment, the incident light beam is decomposed into two perpendicular linear polarization components (P,S).

According to an embodiment, the incident light beam is decomposed into right and left handed circular polarizations.

The invention especially applies to a medium in the presence of a strong scattering environment, but it also applies to weak scattering environments.

According to an embodiment, the method comprises, before collecting the collected interacted and backscattered light beam, the step of:

- decomposing the outgoing interacted and backscattered light beam into at least two polarization components.

This "decomposing step" may be a materially performed step, that is to say, the outgoing interacted and backscattered light beam may indeed be separated into at least two light beams, each light beam carrying a polarization component.

According to an embodiment in that case, the collected light beam is one of the polarization components of the outgoing interacted and backscattered light beam.

According to an embodiment, the outgoing interacted and backscattered light beam is decomposed into components having same polarizations as those of the incident light beam, that is to say, same polarizations as the ones of the incident light beam that has been modulated.

According to an embodiment, the collected light beam is the outgoing light beam.

According to a particular embodiment in that case:

- the interaction is reflection;
- the incident light beam is decomposed into P and S polarization direction components;
- the probe medium has different reflection coefficients (α_P, α_S) in the P and S polarization directions of the incident light beam;
- the measured value related to reflection is (α_P - α_S).

According to an embodiment, the demodulation is made at the same frequency as the modulation.

In accordance with the present invention there is also provided a device for the analysis of a probe medium by interaction between an incident light beam, having an intensity, and the probe medium, the incident light beam also passing through a scattering medium, the device comprising:

- means for emitting an incident light beam,
- means for modulating the amplitude of at least two polarization components of the incident light beam, while keeping the intensity of the incident light beam constant,
- means for collecting a collected interacted and backscattered light beam,
- means for demodulating the collected light beam, in order to measure a value related to the interaction, free from scattering which does not preserve modulation.

According to an embodiment, the device further comprises means for decomposing a light beam outgoing from the probe medium into at least two polarization components.

According to an embodiment, the modulating means comprise a mechanical polarizing chopper.

According to an embodiment, the modulating means comprise a photoelastic modulator.

According to an embodiment, the modulating means comprise a rotating half wave plate and a stationary quarter wave plate.

According to an embodiment, the modulating means comprise a rotating polarizer arranged to be put on the light path of a circularly polarized (or un-polarized) incident light beam.

According to an embodiment, the modulating means comprise a rotating half wave plate arranged to be put on the light path of a polarized light beam.

According to an embodiment, the modulating means comprise a rotating quarter wave plate arranged to be put on the light path of a polarized light beam.

According to an embodiment, the modulating means are provided on the light path of the incident light beam as well as on the light path of the outgoing light beam. Therefore, in such a case, the incident light beam passes through the modulating means, which modulates the amplitude of at least two polarization components of the incident light beam, and the outgoing interacted and backscattered light beam also passes through this modulating means. The modulating means may change the polarization of the outgoing light beam, thus leading to a collected light beam with a polarization decomposition differing from the polarization decomposition of the outgoing light beam; however, the modulation information contained in the outgoing light beam polarization components is kept and is retrieved when the collected light beam is demodulated, even if it is not retrieved in the same polarization components as the ones of the outgoing light beam carrying the modulation information. The person skilled in the art only needs to know the influence of the modulating means on the outgoing light beam in order to adapt the collection of the collected light beam and its demodulation.

According to an embodiment, the device is an optical scanning microscope. Such a microscope presents all the advantages described above for the device of the invention.

Such a scanning microscope, when it comprises modulating means provided on the light path of the incident light beam as well as on the outgoing light beam, presents the advantages of permitting to image an object surrounded by a scattering medium, while being very compact.

According to an embodiment, a beam splitter is provided on the light path of the incident and outgoing light beams, in order to split the paths of the incident light beam and of the collected light beam.

These and other aspects of the invention will be more apparent from the following description with reference to the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

- Fig.1 is a block diagram of an exemplary device for implementing the method of the invention;

- Fig.2 is a block diagram of a device according to a particular embodiment of the invention;

- Fig.3a-3e are schematic views of an exemplary polarization modulation scheme for the device of Fig.2;

- Fig.4 is a block diagram of a scanning microscope according to the invention;

- Fig.5a-5d are schematic views of an exemplary polarization modulation scheme for the scanning microscope of Fig.4;

- Fig.6a-6d are schematic views of the polarization of the outgoing interacted and backscattered light beam in the scanning microscope of Fig.4 and

- Fig.7 is a diagram showing a signal measured by the detector of the scanning microscope of Fig.4.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The method of the invention will be described, with reference to a particular embodiment, where reflection spectroscopy is performed on human skin. The light is decomposed, in this embodiment, into a P-polarization component and a S-polarization component. The notations which have been defined above for the intensities of the different light beams will be kept.

According to the method of the invention, the amplitude of the P and S polarization components $I_{P,i}$ and $I_{S,i}$ of an incident light beam are modulated in time, that is to say, with a certain time-dependence, while the intensity I_i of the incident light beam is kept constant in time. This constant will be denoted A.

The P and S polarization components are separately intensity modulated, each with a certain time-dependence periodic function, respectively f(t) and f*(t), where f*(t) is chosen to be out of phase with f(t).

Therefore, the incident light beam is modulated as follows:

$I_i = I_{P,i}.f(t) + I_{S,i}.f^*(t) = A$

According to an embodiment, the sum f(t) + f*(t) is chosen constant in time, for instance, f(t) + f*(t) = 1.

For example, two square waves complementary to each other may be used, in which:

- in the first half period, f(t) = 1 and f*(t) = 0,
- in the second half period, f(t) = 0 and f*(t) = 1.

According to another embodiment, f(t) and f*(t) are cosine and/or sine functions with a constant offset.

According to a particular embodiment, the intensities $I_{P,i}$ and $I_{S,i}$ of the P and S components of the non-modulated incident light beam are equal. In such a case, the functions f and f* should be chosen such that $f(t) + f^*(t)$ = constant, this constant being equal to $A / I_{P,i}$. In the case above where f(t) + f*(t) = 1, the P polarization component and the S polarization component both have the same intensity, equal to the total intensity of the incident beam $I_i = A$.

It should be noticed that the intensities $I_{P,i}$ and $I_{S,i}$ of the P and S components of the non-modulated incident light beam are not necessarily equal. When it is not the case, the function f and f* are adapted so that the total intensity of the modulated incident light beam is constant in time.

The modulated incident light beam is irradiated on the medium to be analyzed, here on human skin, at an incidence angle α_i from the skin surface.

The light beam coming back from the tissue, that is to say, the outgoing light beam, is analyzed, in this embodiment, in the reflection direction, that is to say at an analysis angle α_c from the skin surface symmetrical to the incidence angle α_i.

One part of the outgoing light originates from the direct (specular) reflection on skin. This reflected light keeps the polarization state of the incident light, while its intensity I_r depends on the reflection efficiency coefficient α.

Therefore, $I_r = I_{P,r} + I_{S,r} = \alpha.I_{P,i}.f(t) + \alpha.I_{S,i}.f^*(t)$.

Thus, the directly reflected light still has the same time-modulated polarization dependence as the incident light, that is to say f(t) for the P-polarization component and f*(t) for the S-polarization component. In other words, since the polarization is preserved, the modulation is preserved.

The other part of the outgoing light originates from scattering. This scattered light has lost its polarization state, the contribution of all polarization components having been randomized, in such a way that the P and S polarization components of the scattered outgoing light are equal in intensity ($I_{P,s} = I_{S,s}$). The value of these intensities is constant in time; it depends on the scattering efficiency coefficient β and is directly proportional to the value (A) of the intensity of the incident light beam; it will be considered equal to c.β.A, with c a constant. Since the polarization has not been preserved, the modulation has not been preserved neither.

The outgoing light comprises both the reflection and scattering contributions, which cannot be directly distinguished.

According to a first embodiment, before being collected, the outgoing light beam is decomposed into components having same polarization as the incident light beam; here, the outgoing light beam is split into a P-polarization component light beam and a S-polarization component light beam and one of those two light beams is collected, in order to measure its intensity. In the described embodiment, the P-polarization component light beam is collected and measured. From what has been seen above, its signal is in the form:

$$I_{P,o} = I_{P,r} + I_{P,s} = \alpha.I_{P,i}.f(t) + c.\beta.A$$

This signal is de-modulated at the frequency of the modulation frequency. Therefore, the part of the collected signal which is at the modulation frequency is extracted from that signal.

Indeed, since the scattered part of the signal ($I_{P,s} = c.\beta.A$) is constant in time, its demodulated value is zero. The result of the demodulation is therefore $\alpha.I_{P,I}$, which is the de-modulated amplitude of the intensity of the light reflected by skin.

From this value, and since $I_{P,i}$ is known, the coefficient α can be calculated directly, or several measurements may be performed at different wavelengths in order to make spectroscopy measurements. A value (here the coefficient α) related to the reflection has therefore been measured thanks to the invention.

Optionally, the de-modulated component is filtered in order to cancel the measurement noise (which is mainly electronic noise, such as flicker noise).

For filtering, it is possible to integrate the de-modulated collected signal along a certain period of time. The amount of noise diminishes with high modulation frequencies.

Let us notice that, should the S-polarization component be collected and measured, its signal would be in the form: $I_{S,o} = I_{S,r} + I_{S,s} = \alpha.I_{S,i}.f^*(t) + c.\beta.A$. The demodulation of such a signal is similar to the demodulation for the P-polarization component.

According to a particular embodiment of the first embodiment which has just been described, the outgoing light beam is decomposed into two polarization components, those components not being exactly the same polarization components as those in which the incident light beam has been decomposed and modulated. Indeed, due to the so-called Kerr effect, the polarization state may be rotated by reflection, and thus, the decomposing step is done at a slightly different (rotated) polarization, a light beam of at least one polarization component being collected and its intensity measured, in order to get the right information.

According to a second embodiment, the outgoing light beam is not split into two polarization components before being collected and measured, that is to say, the collected light beam is the outgoing light beam, not only part of it.

Such an embodiment may be implemented for a medium of which the reflection coefficients in the P and S polarization directions are different. In such a case, after interaction between the incident light beam and the medium, the amount of reflected light in the P and S polarization directions is different, due to which a small modulation appears in the outgoing light beam, without any need to decompose it. The outgoing light beam may therefore be directly demodulated.

Indeed, let us denote α_P and α_S the reflection coefficients for the P and S polarization directions. The intensity I_o of the outgoing light beam reads as follow:

$$I_o = I_{P,o} + I_{S,o} = I_{P,r} + I_{P,s} + I_{S,r} + I_{S,s} = \alpha_P.I_{P,i}.f(t) + \alpha_S.\ I_{S,i}.f^*(t) + I_s$$

This signal I_o is demodulated, which gives, as a result, the difference between the amplitudes of oscillation of the P and S components at the modulation frequency, that is to say, a value proportional to $(\alpha_P - \alpha_S)$, the scattering part of the signal being constant and therefore giving zero as a result of demodulation. A value (α_P - α_S) related to the reflection is therefore obtained.

An example of a device for implementing the method of the present invention is shown in Fig.1 and will now be described.

The device comprises a light source, not shown, adapted to emit a non-modulated incident light beam 1 toward a polarization modulator 2. The non-modulated incident light beam 1 is either polarized or not polarized. For instance, it may be polarized with a 45° polarization angle with respect to the polarizers of the polarization modulator 2, because such a polarization angle is half from the P and S directions. Alternatively, the polarization angle can be changed in order to modify the ratio between the P and S polarization components. In any case, the function of the polarization modulator 2 is to modulate the P and S polarization components of the non-modulated incident light beam 1, in such a way that the intensity I_i of the modulated incident light beam 3 is constant, that is to say:

$$I_i = I_{P,i}.f(t) + I_{S,i}.f^*(t) = A.$$

The polarization modulator 2 can for instance be a mechanical polarizing chopper with P and S polarizers on its blades; such a mechanical chopper is well known in the art and does not need to be detailed further. The difference with prior art mechanical choppers is that a chopper, used for the method of the invention, comprises polarizers on its blades. Other embodiments for the polarization modulator 2 will be described hereinafter.

Therefore, the polarization modulator 2 changes the non-modulated incident light beam 1 into a polarization modulated incident light beam 3. The light intensity I_i of this beam is constant (equal to A), but the intensity of the individual P and S polarization components are intensity modulated at a frequency ν and 180° out of phase with each other.

The polarization modulated incident light beam 3 is directed onto a medium 4 to be analyzed, here a human skin 4, at an incidence angle α_i. The medium 4 is in the presence of a scattering environment. Part of the light is reflected (I_r) and part of the light is scattered (I_s).

The modulation at the modulation frequency ν is only preserved in the reflected light I_r, while the amount of scattered light stays substantially constant.

The outgoing light 5, reflected and scattered from the sample 4, is analyzed in a particular analysis direction. Since the method presently described is a reflection spectroscopy method, the analysis direction is symmetrical to the incidence direction, with respect to the plane 13 perpendicular to the reflection surface 4a of skin 4, at an analysis angle α_c equal to the incidence angle α_i.

The P and S components of the outgoing light 5 are separated using a polarizing beam splitter 6, placed on the path of the outgoing light beam 5. The polarizing beam splitter 6 separates the outgoing light beam into a light beam 5_P only comprising the P-polarization component of the outgoing light beam 5 and a light beam 5_S only comprising the S-polarization component of the outgoing light beam 5. With the polarizing beam splitter 6 used herein, the light beams 5_P, 5_S are directed in perpendicular directions. Those split light beams 5_P, 5_S are collected and their light intensities are measured within light detectors 8, 7, where the respective collected light beams 5_P, 5_S are directed. In fact, both light beams 5_P, 5_S can be collected or only one of them, as has been seen above.

The signal 9 from one of these detectors 7, 8, here the detector 8 measuring the intensity of the P-polarization component, is sent to an electronic module 10 for demodulation of the collected signal 9, in order to extract therefrom the contribution of reflection in the outgoing light beam 5, as explained above. The electronic module 10 here may comprise a lock-in amplifier, well known in the art. The entries of the lock-in amplifier are the signal 9 of the collected P-polarization component of the outgoing light 5 and a reference signal 12, at the frequency of the polarization modulator 2 (i.e. at the modulation frequency ν). For instance, those two signals may be multiplied and entered into a narrow band filter centered on the frequency ν, for canceling the noise and the continuous part of the signal 9 (i.e. the constant scattering contribution), or into a Fourier analyzer giving the PSD (Power Spectral Density) of the signal 9. The signal 11 out of the electronic module 10 is linearly depending on the amount of light $\alpha.I_{P,i}$ that has reflected off the sample 4.

Subsequently, the wavelength of the light source 1 may be tuned, in order to perform a plurality of similar measurements at different wavelengths and get spectroscopic information on the medium 4.

In alternative embodiments, the polarization modulator 2 is not a polarizing chopper but is replaced by one of the five other examples of modulation devices described hereinafter.

According to a first alternative embodiment, the polarization modulator may comprise a rotating polarizer, put on the light path of a circularly polarized (or un-polarized) incident light beam. This results in a modulated polarized light beam, which rotates when the polarizer is rotated (at the same modulation frequency).

According to a second alternative embodiment, the polarization modulator may comprise a polarization rotator, such as a half wave plate, put on the light path of a polarized incident light beam. This results in a modulated polarized light beam, which rotates at a frequency of twice the rotation frequency of the half wave plate.

According to a third alternative embodiment, instead of a modulation in two perpendicular linear polarization directions, the incident light beam may be modulated in left-handed and right-handed circular polarizations. The advantage of such a polarization modulation is that the device for implementing the method of the invention is not sensitive to differences in reflectivity for the P and S polarizations. Such a modulation may be achieved sending a polarized incident light beam on a polarization modulator comprising a rotating quarter wave plate. This results in a modulated incident light beam with a modulation at twice the frequency of the rotation of the quarter wave plate, with light that is periodically linearly polarized, followed by left-handed circular polarization, followed by the same linear polarization, followed by right-handed circular polarization.

According to a fourth alternative embodiment, the polarization modulator comprises a Photo Elastic Modulator (PEM). The basic principle of operation of a PEM is the photo-elastic effect: a mechanically stressed photo-elastic material exhibits birefringence proportional to the strain caused by the induced stress. A light beam which passes through a photo-elastic material has its polarization changed, based on the stress applied to the material, since light moves faster along one axis than along another one. Therefore, a modulator can be provided, comprising a photo-elastic material, on which different stresses are induced at a given frequency, which corresponds to the modulation frequency.

According to a fifth embodiment, and with reference to Fig.3a-3e, the polarization modulator 2' may comprise a rotating half wave plate 2a and a stationary quarter wave plate 2b, put on the light path of a polarized incident light beam 14. This results in a modulation of twice the frequency of the rotating half wave plate 2a, with both a modulation in the linear polarization components and in the left and right-handed circular polarization components.

The Fig.3a-3e are five figures of such a modulator 2', showing the orientation of the rotating half wave plate 2a and the change in polarization at successive times t = 0, t = T/16, t = T/8, t = 3T/16, t = T/4, where T=1/ν is the period of rotation of the half wave plate 2a.

At t = 0 (Fig.3a), the incident beam 14 has a linear polarization 15 incident on the half wave plate 3, which has a slow axis 16 parallel to the incident polarization 15. The polarization 17a immediately after the half wave plate 3 is unchanged, and thus parallel to the incident polarization 15. The beam with polarization 17a is subsequently incident on the quarter wave plate 4, which has a slow axis 18 at an angle π/4 (i.e. 45°) with the incident linear polarization 15. The exit polarization 19a is therefore left-handed circular polarization.

At t = T/16 (Fig.3b), the half wave plate 3 is rotated over an angle π/16 (i.e. 11.25°). The intermediate polarization 17b is rotated over an angle π/4 and is therefore parallel to the slow axis of the quarter wave plate 4. The exit polarization 19b is thus linear and parallel to the intermediate polarization 17b.

At t = T/8 (Fig.3c), the half wave plate 3 is rotated over an angle π/8 (i.e. 22.5°). The intermediate polarization 17c is thus rotated over an angle π/2 (90 deg). The exit polarization 19c is thus circular with a handedness opposite to the circular polarization 19a of Fig.3a (i.e. with a right-handed circular polarization).

At t = 3T/16 (Fig.3d), the half wave plate 3 is rotated over an angle 3π/16 (i.e. 33.75°). The intermediate polarization 17d is thus rotated over an angle 3π/4 (i.e. 135°) and is therefore perpendicular to the slow axis of the quarter wave plate 4. The exit polarization 19d is therefore linear and parallel to the intermediate polarization 17d.

At t = T/4 (Fig.3e), the half wave plate 3 is rotated over an angle π/2. The intermediate polarization 17e is thus parallel to the incident polarization 15, just as at time t = 0. The exit polarization 19e is then the same (left-handed) circular polarization as polarization 19a of Fig.3a.

A device comprising the modulator 2' which has just been described is shown on Fig.2.

The device comprises a light source 1a, which emits linearly polarized light. The incident polarized light beam 14 is modulated by the modulator 2', i.e. by the rotating half wave plate 2a and the quarter wave plate 2b, and is incident on a medium 4'.

The outgoing (reflected and scattered) light beam 5' is incident on a normal beamsplitter 6', which separates the outgoing light beam 5' into two light beams 5'a, 5'b, in a manner known in the art.

The first light beam 5'a is incident on a quarter wave plate 7' and a polarizing beamsplitter 8'. Detectors 9' and 10' collect and measure the left and right-handed circularly polarized components of the outgoing light beam 5'.

The other light beam 5'b is incident on a polarizing beamsplitter 11'. Detectors 12' and 13' collect and measure the two linearly polarized components of the outgoing light beam 5', in a manner known in the art.

After demodulation of the signals from one of the detectors 9', 10', 12' or 13', the signal corresponding to the reflection on the sample 4' is retrieved, as well as presented above concerning the device of Fig.1.

In Fig.2, four detectors 9', 10', 12', 13' are provided in the device. However, according to the method of the invention only one of the four detectors 9', 10', 12', 13' is actually necessary and used as an input for the demodulation. The use of more detectors gives essentially the same information but has the advantage of further efficiency for the suppression of noise.

Other devices for the modulator could of course be contemplated, if they fulfill the required function of polarization modulation of the incident light beam.

The method of the invention applies to the measurement of interacted light on a wide range of media, in a scattering environment. The scattering environment may be, for instance, one or a combination of the following:

- a liquid submerging the surface of a probe medium,
- underlying layers of a tissue such as skin,
- the probe medium itself.

An interesting application of the invention will now be described. The article cited above, by V. Backman et al., cites reports of an *in situ* method of probing the structure of living epithelial cells, based on light scattering spectroscopy with polarized light. It is shown that it is possible to distinguish between single backscattering from uppermost epithelial cells and multiple scattered light. The spectrum of the backscattering signal can be further analyzed to provide histological information about the epithelial cells such as the size distribution of the cell nuclei and their refractive index. These are valuable quantities to detect and diagnose precancerous changes in human tissues.

As indicated above, the method of the invention applies to the analysis of any light beam which has interacted with a sample, if the interacted light has its polarization preserved; notably, it applies to the detection of single backscattered light. The method of the invention can therefore be used to improve the method reported in the article by V. Backman et al.

A device for the implementation of such a method can be similar to the one described on Fig.1. In such a case, the light is typically incident on the sample surface at a certain incidence angle α_i (for example 15°) and the analysis of light is done at a different angle. Indeed, if the angles were the same, specular reflection would be detected, while at a different angle, only backscattered light is detected. Both single and multiple scattered light reach the detector, but for single scattered light the polarization state is preserved. Therefore, at the output of the demodulation, the signal only contains the contribution of single backscattered light. The accuracy of the measurements can thus be improved thanks to the method of the invention.

It should also be noticed that the invention may be combined with other optical techniques such as OCT, Raman, diffuse reflection spectroscopy, etc.

With reference to Fig.4-7, another device for implementing the method of the invention will now be described. This device is an optical scanning microscope.

A scanning microscope is an apparatus which is used for forming an image of an object. In a scanning microscrope, a focused light beam is scanned across an object and the reflected light beam coming back from the object is collected so as to obtain an image of the object. A problem to be solved with scanning microscopes is that, when an object in the proximity of a scattering medium is imaged, the contribution of scattering is much higher that the contribution of reflection (or single scattering), which makes it very difficult to measure an accurate image.

In the prior art, a way to improve the microscopes performance is to use confocal microscopy. In this case a pinhole is placed in front of the detector, which is conjugated to the position of the focused light beam; as a result, only light that is back reflected from the focal point and travels back along the same optical path as the incident light beam is transmitted through the pinhole. Although this gives results to a certain extent, photons arising from out of focus positions can reach the detector, through multiple scattering coming back to the focal point when scattering becomes extensive. Therefore, the depth that can be imaged by confocal microscopy is limited in this way.

The scanning microscope of the invention comprises:

- means for emitting an incident light beam,
- means for modulating the amplitude of at least two polarization components of the incident light beam, while keeping the intensity of the incident light beam constant,
- means for collecting a collected interacted and backscattered light beam and
- means for demodulating the collected light beam, in order to measure a value related to the interaction, free from scattering (which does not preserve modulation).

Such an optical scanning microscope is therefore adapted to perform the method of the invention.

According to an embodiment, the modulating means are provided on the light path of the incident light beam as well as on the light path of the outgoing light beam.

In other words, the light paths of the incident and outgoing light beams are close or even collinear, which permits a much more compact scanning microscope.

The interaction may be reflection or single backscattering.

With reference to Fig.4, the scanning microscope 20 comprises a light source 21, such as a laser diode, for instance. The light source 21 is herein adapted to generate an incident polarized light beam 22, which is in the embodiment described a linearly polarized light beam 22, herein vertically polarized.

The scanning microscope 20 comprises a polarizing beam splitter 23, adapted to reflect light of linear polarization perpendicular to the plane of the drawing - that is to say vertical linear polarization - and transmits light of linear polarization in the plane of the drawing - that is to say horizontal linear polarization.

The incident vertically linearly polarized light beam 22 is incident on the beam splitter 23 according to a light path which is perpendicular to the optical axis 25 of the microscope 20. The incident light beam 22 is reflected on the beam splitter 23 in the direction of the optical axis 25. The incident light beam 22 is then collimated into a parallel beam of light by a first lens 24 (collimator lens), centered on the optical axis 25.

The incident light beam 22 passes through a modulator 26, which in this embodiment comprises a rotating quarter wave plate 26, which rotates around an axis 26' parallel to the optical axis 25, at a frequency f. The function of the modulator 26 is to modulate the polarization state of the non-modulated incident light beam 22 in order to obtain, after the modulator 26, a polarization modulated incident light beam 27.

This polarization modulated incident light beam 27 is focused by a second lens 28 (objective lens) into a tiny focal point (or region) at position 29 in an object 30 to be imaged, which is in the presence of a scattering environment. Part of the light is reflected and part of the light is scattered, as explained before.

The outgoing light beam 31, reflected and scattered, is analyzed in the same direction as the direction of the light path of the incident light beam 27, that is to say, on the optical axis 25. The optical axis 25 of the microscope 20 is placed substantially perpendicular to the surface of the object 30 to be imaged, so that a big amount of reflected light is collected in the outgoing reflected and scattered light beam 31. This also makes it easier to scan over the surface of the object 30.

Therefore, since the light paths of the incident modulated light beam 27 and the analyzed outgoing light beam 31 are collinear, the modulator 26, which is on the light path of the incident light beam 27, is also on the light path of the outgoing light beam 31. The outgoing light beam 31 is captured by the second lens 28 and passes through the modulator 26, in the inverse way compared to the incident modulated light beam 27. The polarization of the outgoing light beam 31 is changed in the modulator 26 and the obtained light beam, which contains the collected light beam 32, passes through the first lens 24 and enters the polarizing beam splitter 23. As will be explained later, the polarization of this light beam has changed compared to the one of the incident non-modulated light beam 22 and this light beam is thus at least partly transmitted by the beam splitter 23, in the direction of a detector 33, preferably a silicon photo-diode, where its intensity is measured. The part of the light beam that is transmitted by the beam splitter 23 is the collected light beam 32.

As well as before, the signal from the detector 33 is sent to an electronic module for demodulation of the collected signal, in order to extract therefrom the contribution of reflection in the outgoing light beam 31.

The fact that the polarization of the outgoing light beam 31 is modified by the modulator 26 is taken into account, the information contained in the amplitude of the polarization components of the outgoing light beam 31 still being accessible in the amplitude of the polarization components of the collected light beam 32, even if the polarization basis may have changed.

Indeed, here is briefly what happens to the polarization components of the different light beams. Firstly, depending on the orientation of the quarter wave plate 26 compared to the polarization axis of the incident non-modulated light beam 22, this will periodically generate a light beam with an alternation of left-handed circular polarization, linear polarization, right-handed circular polarization and linear polarization. Secondly, the handedness of the circular polarization states reverses upon reflection at the surface of the object 30 to be imaged. Thirdly, the polarization of the outgoing light beam 31 changes as the beam passes through the quarter wave plate 26 and, after this second pass, the polarization state of the light is periodically linearly polarized, the linear polarizations alternating between the states parallel and perpendicular to the original linear polarization state of the incident non-modulated light beam 22. The beam splitter 23 only transmits the linear polarization state perpendicular to the original polarization state (the parallel polarization state being reflected and lost) and therefore the detector 33 measures a harmonic signal with four times the frequency of the rotating quarter wave plate 26. The signal 37 measured on the detector 33 is reproduced on Fig.7, as a function of time.

A significant part of the incident light is scattered in the turbid (scattering) medium. The polarization state of the scattered light is random and this depolarized light results in a strong background signal that is constant in time. As well as before, this background signal is filtered out by demodulating the signal on the detector 33 at four times the reference rotation frequency of the quarter wave plate 26.

The influence of the quarter wave plate 26 on the incident non-modulated light beam 22 and on the reflected light beam will now be described in more details, with reference to Fig.5a-5d for the incident light beam 22 and with reference to Fig.6a-6d for the reflected light beam.

Fig.5a-5d are four drawings of the modulator 26 of Fig.4, showing the orientation of the rotating quarter wave plate 26 and the change in polarization of the incident light beam at successive times t = 0, t = T/8, t = T/4, t = 3T/8, where T = 1/f is the period of rotation of the quarter wave plate 26. The incident non-modulated light beam 22 is incident on the rotating quarter wave plate 26.

At t = 0 (Fig.5a), the incident light beam 22, which has a linear vertical polarization 34, is incident on the quarter wave plate 26, which has a slow axis 35 parallel to the incident polarization 34. The exit polarization 36a, after the quarter wave plate 26, is unchanged, and thus parallel to the incident polarization 34.

At t = T/8 (Fig.5b), the quarter wave plate 26 is rotated over an angle π/4 (i.e. 45°). The exit polarization 36b is a left-handed circular polarization.

At t = T/4 (Fig.5c), the quarter wave plate 26 is rotated over an angle π/2 (i.e. 90°). The exit polarization 36c is then linear and parallel to the entrance polarization 34.

At t = 3T/8 (Fig.5d), the quarter wave plate 26 is rotated over an angle 3π/4 (i.e. 135°). The exit polarization 36d is a right-handed circular polarization.

At t = T/2 the situation of Fig.5a at t = 0 is recovered. Within one period of rotation this sequence of exit polarization states therefore occurs twice.

Upon reflection on the surface of the object, the handedness of the circular polarizations changes. This implies that at t = 0 and t = T/4, where the polarization of the modulated light beam 31 incident on the object 30 is linear, nothing happens to the polarization and at t = T/8 and t = 3T/8, the handedness of the circular polarizations is changed by reflection.

The reflected light beam therefore presents an alternation of (vertical) linear, right-handed circular, (vertical) linear and left-handed circular polarizations.

Fig.6a-6d are four drawings of the modulator 26 of Fig.4, showing the orientation of the rotating quarter wave plate 26 and the change in polarization of the reflected light beam at successive times t = 0, t = T/8, t = T/4, t = 3T/8, where T = 1/f still is the period of rotation of the quarter wave plate 26.

At t = 0 (Fig.6a), the reflected light beam, which has a linear vertical polarization 38a, is incident on the quarter wave plate 26, which has a slow axis 35 parallel to the incident polarization 38a. The exit polarization 39a, after the quarter wave plate 26, is unchanged, and thus parallel to the incident polarization 38a, that is to say, a linear polarization parallel to the polarization 34 of the incident non-modulated light beam 22.

At t = T/8 (Fig.6b), the quarter wave plate 26 is rotated over an angle π/4 (i.e. 45°). The reflected light beam is incident on the quarter wave plate 26 with a right-handed circular polarization 38b and the exit polarization 39b is a horizontal linear polarization, that is to say, a linear polarization perpendicular to the polarization 34 of the incident non-modulated light beam 22.

At t = T/4 (Fig.6c), the quarter wave plate 26 is rotated over an angle π/2 (i.e. 90°). The reflected light beam is incident on the quarter wave plate 26 with a vertical linear polarization 38c and the exit polarization 39c is a vertical linear polarization, that is to say, a linear polarization parallel to the polarization 34 of the incident non-modulated light beam 22.

At t = 3T/8 (Fig.6d), the quarter wave plate 26 is rotated over an angle 3π/4 (i.e. 135°). The reflected light beam is incident on the quarter wave plate 26 with a left-handed circular polarization 38d and the exit polarization 39d is a horizontal linear polarization, that is to say, a linear polarization perpendicular to the polarization 34 of the incident non-modulated light beam 22.

At t = T/2 the situation of Fig.6a at t = 0 is recovered. Within one period of rotation this sequence of exit polarization states therefore occurs twice.

The polarizing beam splitter 23 therefore transmits the signal at t = T/8 and t = 3T/8, whereas it reflects the signal at t = 0 and t = T/4 towards the laser 1, this signal being lost.

As briefly explained above, and with reference to Fig.7, the signal 37 measured at the detector 33 consists of two parts, one constant part related to scattering in the scattering medium, and a part related to the reflected light coming from the scanning spot 29. This last part of the signal varies periodically with time because of the polarization modulation scheme of the scanning microscope 20. The modulation frequency of the signal 37 is four times the rotation frequency of the quarter wave plate.

The scanning microscope 20 comprises means (not shown) for displacing the focused spot in the two directions perpendicular to the optical axis 25, as indicated for one direction by the arrow 40, and in the direction along the optical axis 25. In such a way, the object 30 may be scanned and imaged.

According to an embodiment not shown on the drawings, the scanning microscope 20 comprises, in front of the detector 33, a pinhole which is placed such that only backscaterred photons coming from the focal point of the objective lens 28 can reach the detector 33, in order to perform confocal microscopy.

In any case, with the light path of the incident light beam 27 and the outgoing light beam 31 collinear and the outgoing light beam 31 passing through the modulator 26, the scanning microscope 20 of the invention permits an accurate imaging of an object in the presence of a scattering medium, while this particular structure provides a very compact scanning microscope 20.

In alternative embodiments of the scanning microscope of the invention, the polarization modulator 26 is not a quarter wave plate but is replaced by one of the four other examples of modulation devices described hereinafter.

According to a first alternative embodiment, the polarization modulator may comprise a rotating polarizer, put on the light path of a circularly polarized (or un-polarized) incident light beam. This results in a modulated polarized light beam, which rotates when the polarizer is rotated (at the same modulation frequency). In this case, the beam splitter 23 is not a polarizing beam splitter, but instead a 50% beam splitter, well know by the person skilled in the art, can be used.

According to a second alternative embodiment, the polarization modulator may comprise a polarization rotator, such as a half wave plate, put on the light path of a polarized incident light beam. This results in a modulated polarized light beam, which rotates at a frequency of eight times the rotation frequency of the half wave plate (since, with the particular structure of the scanning microscope 20, the light passes through the half wave plate twice).

According to a third alternative embodiment, the polarization modulator comprises a rotating half wave plate and a stationary quarter wave plate, on which is incident a linearly polarized light.

According to a fourth alternative embodiment, the polarization modulator comprises a Photo Elastic Modulator (PEM), already presented above. A PEM may replace quarter and half wave plates.

The optical scanning microscope may use fluorescence or contrast agents to help localizing interesting areas.

The optical scanning microscope of the invention may be used in a bio-sensing application based on imaging. In particular, since the method of the invention permits imaging through turbid media, it is relevant for biological and/or medical applications. As an example, the optical scanning microscope of the invention may be used in cytometry or virtual pathology.

While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive; the invention is not limited to the disclosed embodiments.

Other variations to the disclosed embodiments can be understood and effected by those skilled in the art in practicing the claimed invention, from a study of the drawings, the disclosure, and the appended claims. In the claims, the word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. A single processor or other unit may fulfill the functions of several items recited in the claims. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage. A computer program may be stored/distributed on a suitable medium, such as an optical storage medium or a solid-state medium supplied together with or as part of other hardware, but may also be distributed in other forms, such as via the Internet or other wired or wireless telecommunication systems. Any reference signs in the claims should not be construed as limiting the scope.

CLAIMS

1- Method for the analysis of a probe medium (4, 4') by interaction between an incident light beam (3, 14), having an intensity, and the probe medium (4, 4'), the incident light beam (3, 14) also passing through a scattering medium, the method comprising:

- modulating the amplitude of at least two polarization components of the incident light beam (3, 14), while keeping the intensity of the incident light beam constant,
- after interaction between the modulated incident light beam (3) and the probe medium (4, 4') and scattering by the scattering medium, generating an outgoing interacted and backscattered light beam (5, 5'), collecting a collected interacted and backscattered light beam (5_P, 5_S) from the outgoing light beam (5, 5'),
- demodulating the collected light beam (5_P, 5_S), in order to measure a value related to the interaction, free from scattering which does not preserve modulation.

2- Method according to claim 1 comprising, before collecting the collected interacted and backscattered light beam (5_P, 5_S), the step of:

- decomposing the outgoing interacted and backscattered light beam (5, 5') into at least two polarization components (5_P, 5_S).

3- Method according to claim 2, wherein the collected light beam (5_P, 5_S) is one of the polarization components of the outgoing interacted and backscattered light beam (5, 5').

4- Method according to claim 2, wherein the outgoing interacted and backscattered light beam (5, 5') is decomposed into components having same polarizations as those of the incident light beam (3).

5- Method according to claim 1, wherein the collected light beam (5_P, 5_S) is the outgoing light beam (5, 5').

6- Method according to claim 5, wherein:

- the interaction is reflection;
- the incident light beam is decomposed into P and S polarization direction components;
- the probe medium has different reflection coefficients (α_P, α_S) in the P and S polarization directions of the incident light beam;
- the measured value related to reflection is (α_P - α_S).

7- Method according to claim 1, wherein the outgoing light beam (5, 5') is analyzed in a particular analysis direction.

8- Method according to claim 1, wherein the demodulation is made at the same frequency as the modulation.

9- Method according to claim 1, wherein the incident light beam (3, 14) is decomposed into two perpendicular linear polarization components (P,S).

10- Method according to claim 1, wherein the incident light beam (3, 14) is decomposed into right and left handed circular polarizations.

11- Method according to claim 1, wherein the interaction is reflection, or single backscattering, and the probe medium is human skin.

12- Device for the analysis of a probe medium (4, 4') by interaction between an incident light beam (3, 14), having an intensity, and the probe medium (4, 4'), the incident light beam (3, 14) also passing through a scattering medium, the device comprising:

- means (1a) for emitting an incident light beam (3, 14),

- means (2, 2') for modulating the amplitude of at least two polarization components of the incident light beam (3, 14), while keeping the intensity of the incident light beam constant,

- means (7, 8, 9', 10', 12', 13') for collecting a collected interacted and backscattered light beam (5, 5'),

- means (10) for demodulating the collected light beam (5, 5'), in order to measure a value related to the interaction, free from scattering which does not preserve modulation.

13- Device according to claim 12, further comprising means (6, 6') for decomposing a light beam (5, 5') outgoing from the probe medium (4, 4') into at least two polarization components.

14- Device according to claim 12, wherein the modulating means comprise a mechanical polarizing chopper (2).

15- Device according to claim 12, wherein the modulating means comprise a photoelastic modulator.

16- Device according to claim 12, wherein the modulating means comprise a rotating half wave plate (2a) and a stationary quarter wave plate (2b).

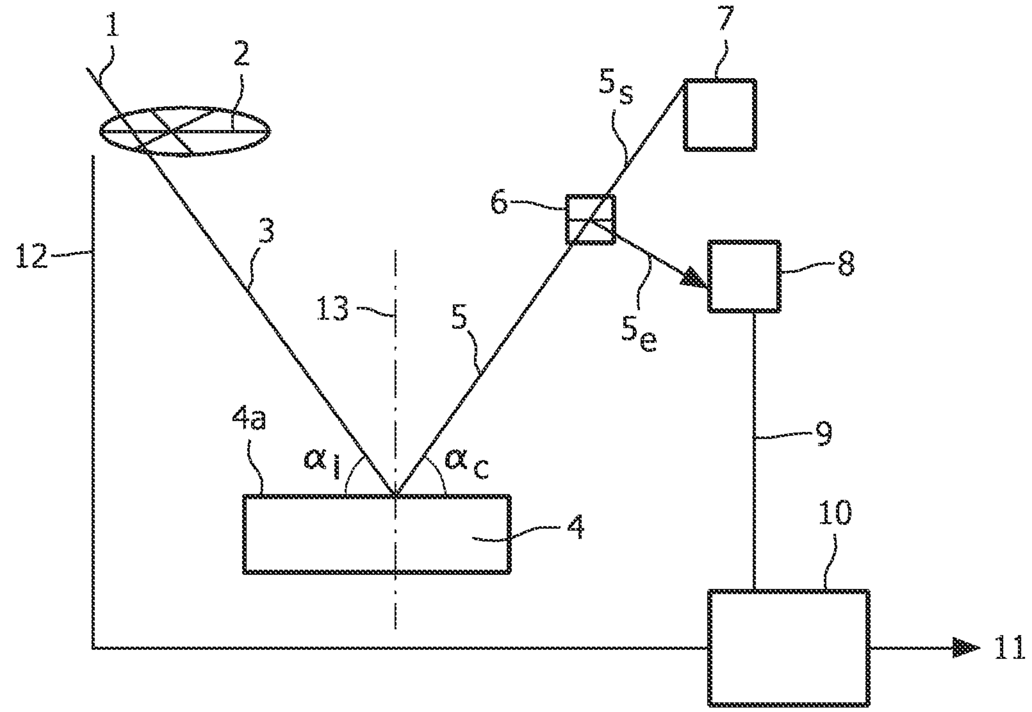
17- Device according to claim 12, wherein the modulating means comprise a rotating polarizer arranged to be put on the light path of a circularly polarized (or un-polarized) incident light beam.

18- Device according to claim 12, wherein the modulating means comprise a rotating half wave plate, or a rotating quarter wave plate, arranged to be put on the light path of a polarized light beam.

19- Device according to claim 12, where the modulating means (26) are provided on the light path of the incident light beam (27) as well as on the light path of the outgoing light beam (31).

20- Device according to claim 19, comprising a beam splitter (23) provided on the light path of the incident and outgoing light beams (27, 31) in order to split the paths of the incident light beam (27) and of the collected light beam (32).

21- Optical scanning microscope according to any of claims 12 to 20.


1
2
7
5_s
6
3
12
13
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8
5_e
4a
α_i
α_c
9
4
10
11

FIG. 1

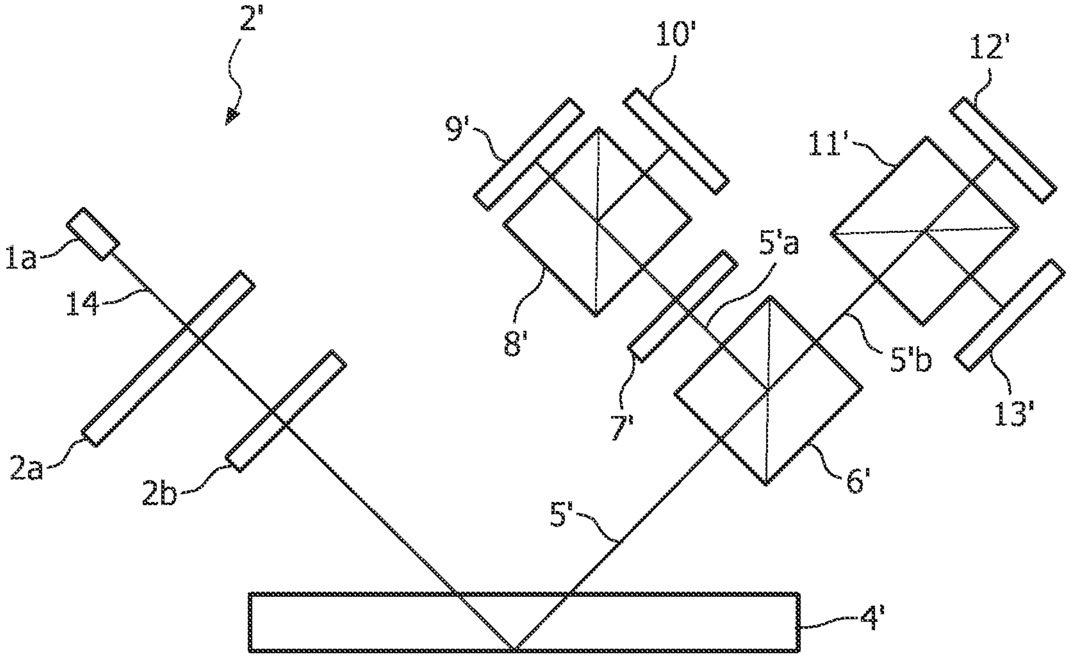
2'
10'
12'
9'
11'
5'a
1a
14
8'
5'b
7'
13'
2a
2b
6'
5'
4'

FIG. 2

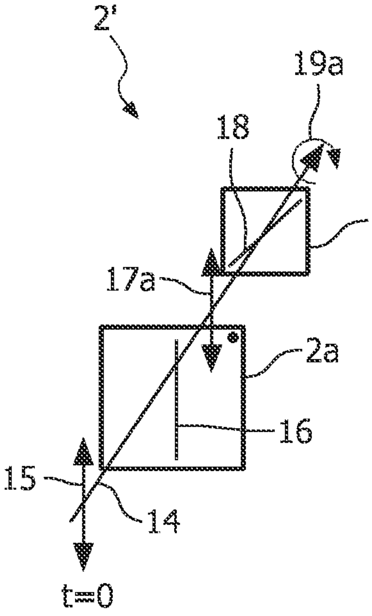

FIG. 3a

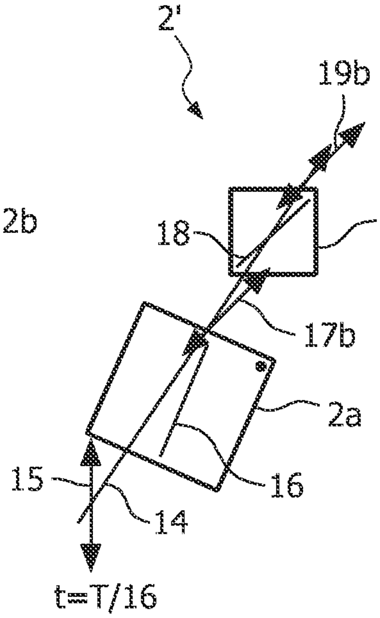

FIG. 3b

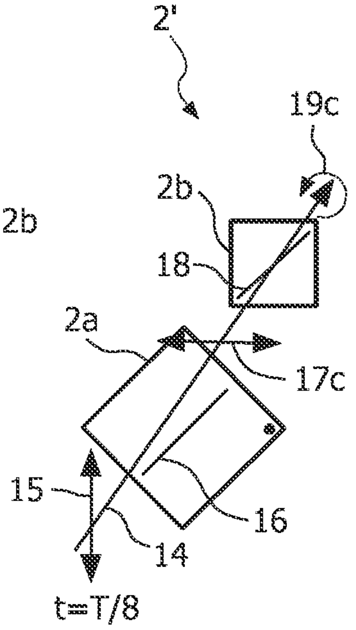

FIG. 3c

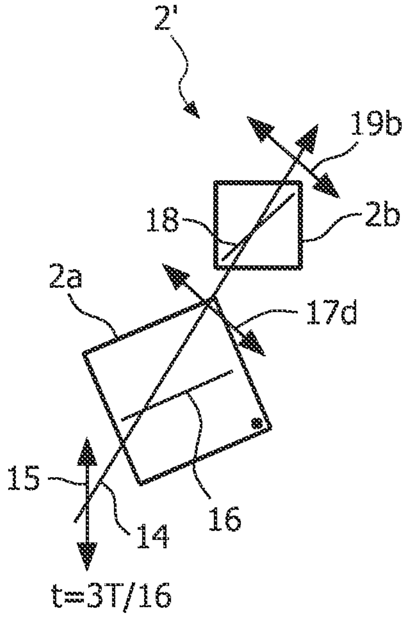

FIG. 3d

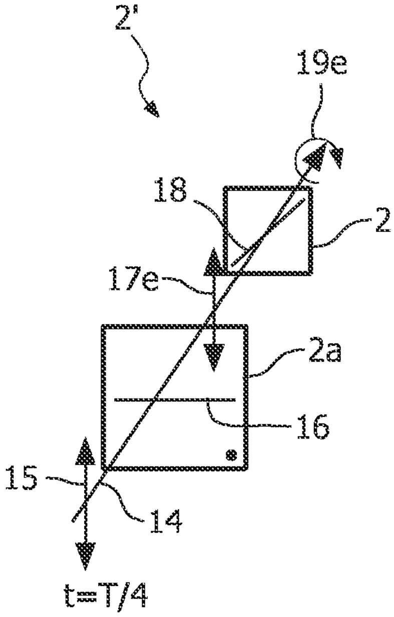

FIG. 3e

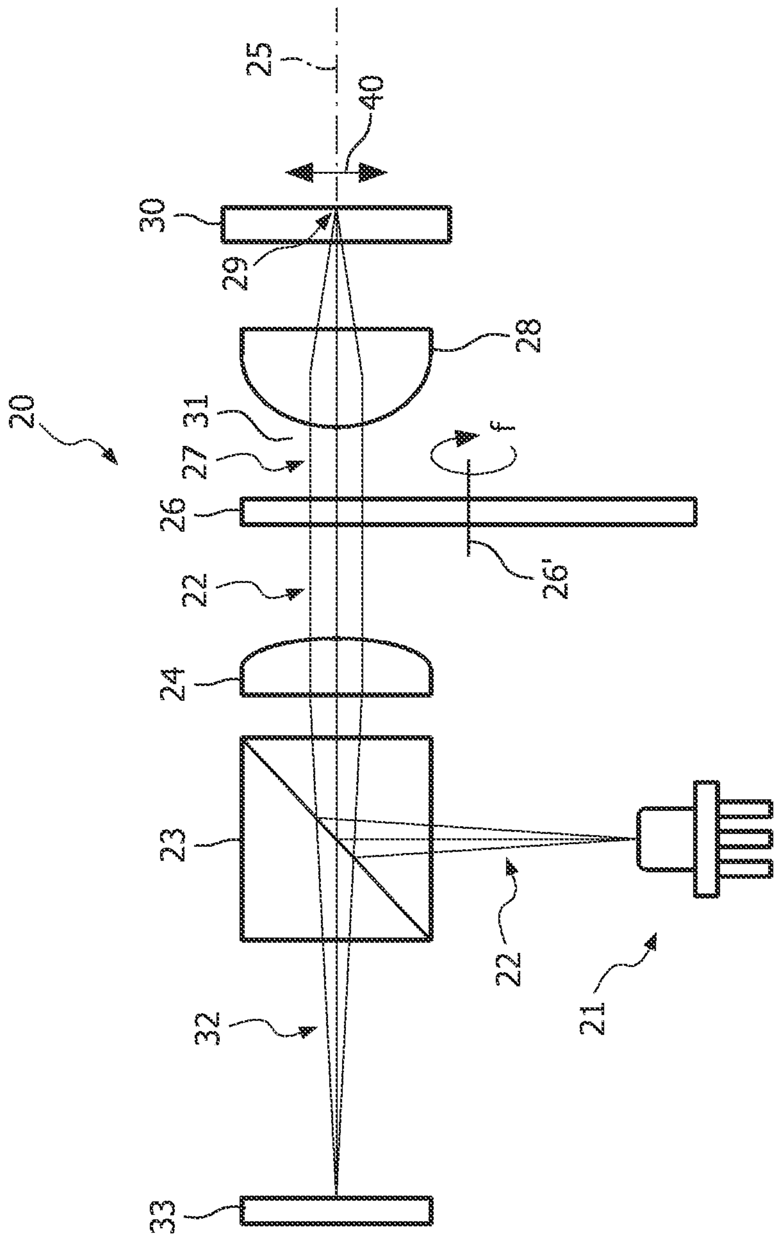

FIG. 4

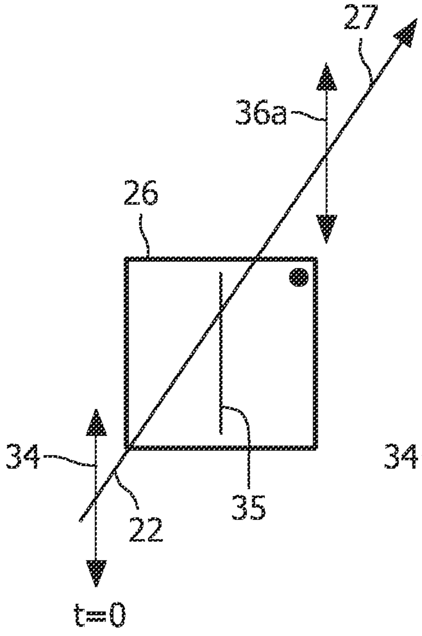

FIG. 5a

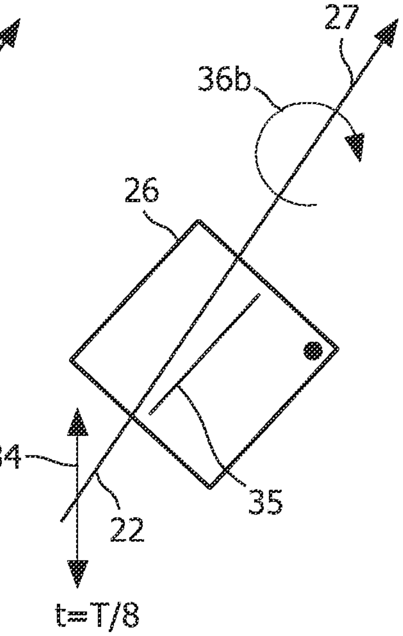

FIG. 5b

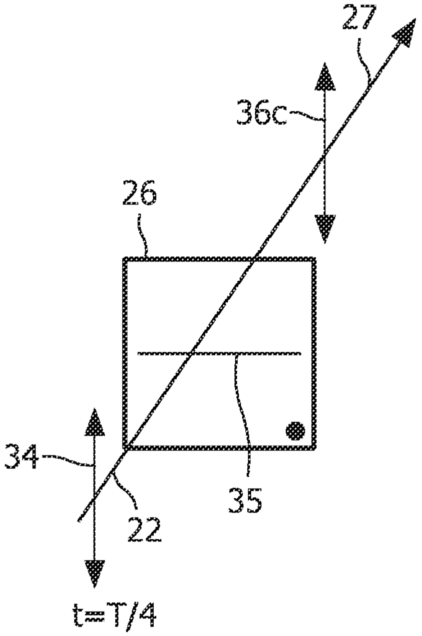

FIG. 5c

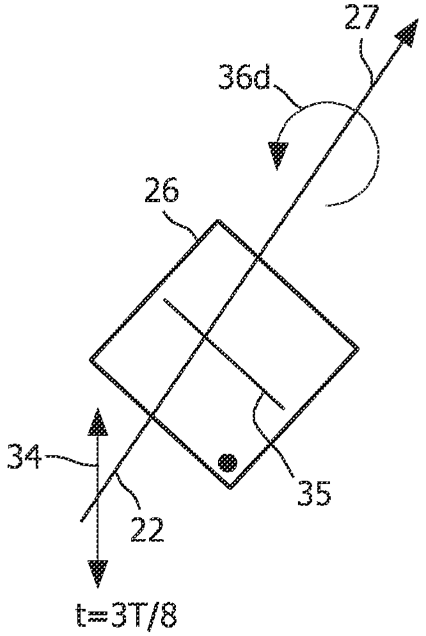

FIG. 5b

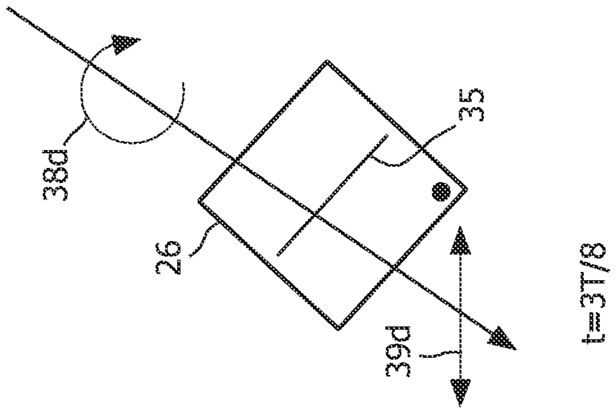

FIG. 6d

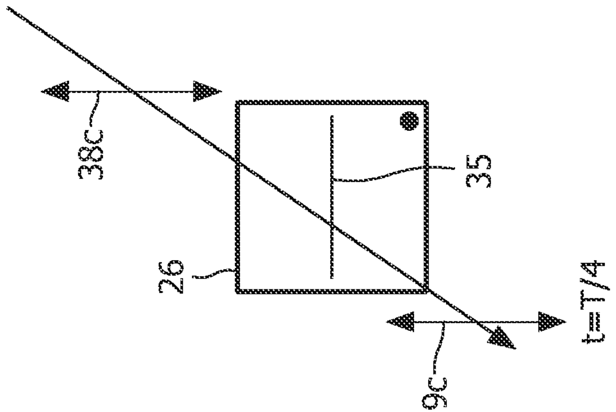

FIG. 6c

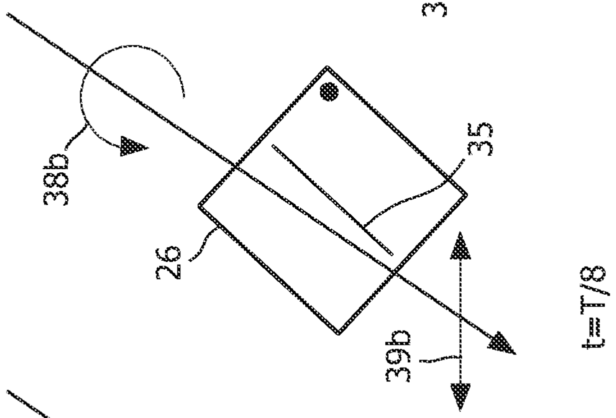

FIG. 6b

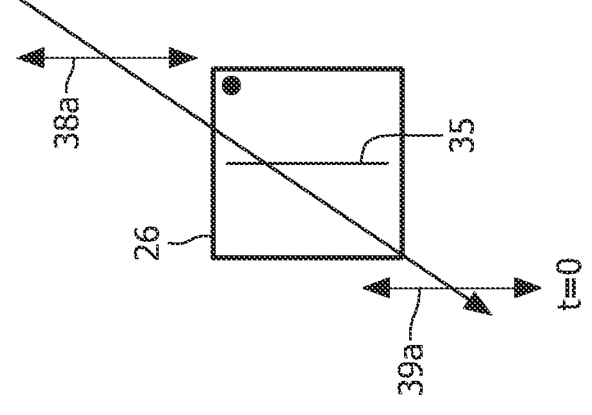

FIG. 6a

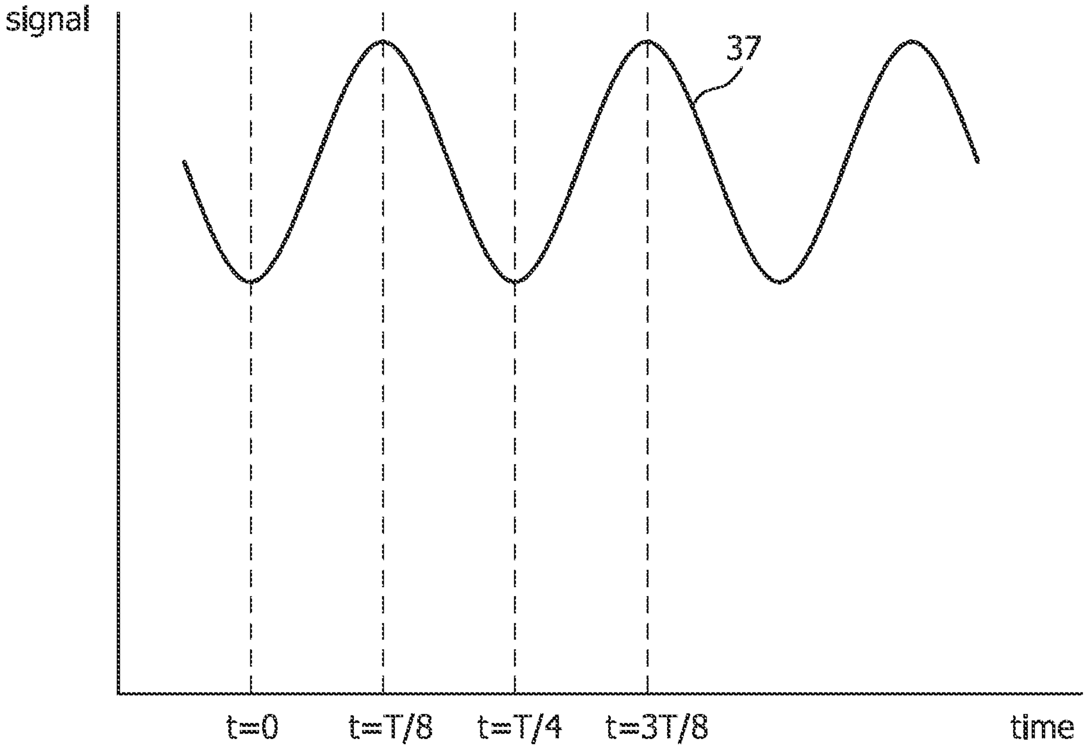

FIG. 7