A method for generating an image from within an optical scattering specimen by means of an optical coherence tomography imaging step (OCT) wherein the OCT step is preceded by pre-treating said specimen by subjecting it directly to a flash illumination within a wavelength range of 950 - 1250 nm.

**FIG. 1**

Comparative signal before/after flash

Size (pm thickness) number

Comparative signal before/after flash

Before

After flash

**Title**: IMPROVED METHOD FOR GENERATING AN IMAGE OF THE MORPHOLOGY AND CELLULAR ELEMENTS OF A SPECIMEN OR IN VIVO TISSUE BY MEANS OF OPTICAL HIGH RESOLUTION COHERENCE TOMOGRAPHY
Improved method for generating an image of the morphology and cellular elements of a specimen or in vivo tissue by means of optical high resolution coherence tomography.

[DESCRIPTION]

FIELD OF THE INVENTION

The present invention relates to non-invasive skin or biological tissue examination, more specifically to such examination by means of optical (high definition) coherence tomography (OCT).

BACKGROUND OF THE INVENTION

Optical coherence tomography (OCT) is a non-invasive imaging technique based on a measurement of optical reflections of internal microstructures inside a specimen or in vivo tissue.

Currently the most important areas of application of OCT in medicine are dermatology, ophthalmology, cardiology, surgery, diagnosis of cancer as well as follow-up treatment.

Due to its light scattering properties biological tissue (in vivo or ex vivo) is particularly suitable for diagnostic examination by means of OCT.

Since relatively low light intensities are sufficient for OCT examination and because the used wavelengths are situated in the near infrared range (750 nm to 1350 nm), unlike ionizing radiation the method does not contaminate biological tissue with radiation. It is therefore particularly significant for medical applications.

However the skin is due its protective nature against all kinds of radiation ranging from UV, visible light and NIR, very reflective and scattering towards spectral radiation and allows only penetration in certain "windows" of the infrared. Specially the horny layer (stratum corneum) of skin is highly in-penetrable for all kinds of light, preventing
outward transmission of information from deeper layers of the epidermis and the dermis.

The OCT technique is comparable to the ultrasound medical imaging technique. However, in the OCT technique broadband light with short coherence length is used instead of sound.

A sample is irradiated with light with short coherence length. Then, the time of flight of the light reflected on different reflective boundary layers and backscattering sides in a sample are recorded with the aid of an interferometer. This time of flight information in its turn yields spatial information about the specimen's microstructure.

With OCT typical resolutions higher than two orders of magnitude are achieved than with ultrasound, however, the achieved measuring depth is considerably smaller. Due to optical scattering the penetration depth is rather limited, image can be obtained that reach into the tissue up to a depth of 2 millimeters.


High resolution optical coherence tomography is obtained by applying dynamic focus tracking as described in EP 1 962 050. The described method provides high lateral resolution, i.e. parallel to the skin surface.

In this patent a system and a corresponding method for optical coherence tomography are described having an interferometer for emitting light with which a specimen is irradiated, the interferometer comprising a beam splitter and at least one reflector the optical distance of which from the beam splitter is changeable. Further a specimen objective is provided by means of which light
emitted by the interferometer is focused in a focus plane lying within the specimen, and a detector is provided for collecting light which is reflected by the specimen.

For simpler and quicker recording of the sharpest possible images of the specimen provision is made such that during a change of the optical distance between the reflector and the beam splitter the light respectively reflected at a number of different depths of the specimen is collected by the detector, and during the collection of the light respectively reflected at the different depths of the specimen the imaging properties of the specimen objective are changed such that the focus comes within the range of the respective depth of the specimen.

EP 2 498 048 describes the use of a light source with a Gaussian filter. The use of such a light source provides enhanced resolution in axial direction (i.e. perpendicular to the surface of the skin that is examined) .

In OCT lateral and axial resolution are decoupled. High lateral resolution is obtained by the resolution and magnification power of the optics that are used.

High lateral resolution may lead to low depths of focus (depth resolution being defined by the bandwidth of the light source) . This disadvantage can be solved by moving the focus while scanning the depth as performed in the focus tracking system described in EP 2 498 048.

By applying the adaptive focus concept a resolution up to 3µ can be obtained.

Using an incoherent light source with a Gaussian filter has the following effects:

- broad spectrum resulting in high axial resolution
- Gaussian spectrum resulting eliminating ghost images
Laterally coherent light resulting in the absence of image blur (by coherent cross-talk).

By applying the adaptive focus concept a resolution up to 3\(\mu\) can be obtained.

In most applications a small amount of optical gel is applied to the skin or to the examining probe in order to ensure good contact and to increase the penetration depth by adapting the optical reflection index of the skin by filling pores and skin asperities.

Whereas with the existing techniques skin or tissue examinations up to a depth of 1 mm can be performed, there is a constant aim to improve the examination depth.

**SUMMARY OF THE INVENTION**

The above-mentioned aspect is realised by a method having the specific steps set out in claim 1.

The invention provides improved depth penetration and improved image quality (contrast).

Specific features for preferred embodiments of the invention are set out in the dependent claims.

Further advantages and embodiments of the present invention will become apparent from the following description and drawings.

**DRAWINGS**

Figure 1 shows the results of an OCT examination of the skin without applying a pre-treatment as well as the results of the OCT examination of the same skin part that has been subjected to a pre-treatment according to the present invention.
The present invention describes an examination technique which can be applied for in vivo as well as non-invasive ex vivo diagnostic examination of tissue.

According to the present invention a specimen, more particularly a biological tissue (in vivo or ex vivo) is subjected to a pre-treatment prior to examination by means of a near-infrared optical imaging technique such as low-resolution optical coherence tomography (single or multibeam, in time domain or in Fourier domain), high-definition (also called high-resolution) optical coherence tomography and multi-photon tomography.

The pre-treatment is performed by subjecting the tissue (in vivo or ex vivo) to a flash irradiation with light in the wavelength range of 750 to 1250 nm.

The pre-treatment is applied directly to the tissue. In the context of the present invention 'Directly' means that no barrier of whatever kind is provided in the light path of the irradiation during the pre-treatment.

No optical clearing agents such as glycerol or oils are needed. Nor are means used to enhance the light absorption such as carbon, tattoo ink, small particles (micro or nano dots) etc.

The pre-treatment irradiation can be obtained by means of a calibrated pulse light (CPL) applicator.

An example of such a calibrated pulse light applicator is the CPL calibrated pulse light hand piece produced by Biotec, Italia and denominated by the trade name Xlase.

This product comprises a Xenon flashlamp and emits light flashes within adjustable wavelength ranges 550-1200 nm, 550-950 nm, 650 - 950 nm, 420 - 1200 nm.
In order to obtain a flash light illumination in the range of 750-1250 nm, which is the range in which the envisaged effects of the present invention are obtained, a spectral filter can be used to cut out light below 750 nm and over 1250 nm.

This spectral range is advantageous in that structures in the tissue which are situated below the epidermis of the skin are rendered visible when the tissue is subjected to a flash irradiation prior to being examined by means of OCT.

Irradiation with flash light in the wavelength range below 750 nm does not produce this effect.

Irradiation with flash light in the wavelength range higher than 1250 nm is disadvantageous because of strong absorption by water and the risk of skin burn.

In a preferred embodiment high resolution OCT is applied.

High lateral resolution may lead to low depths of focus (depth resolution being defined by the bandwidth of the light source). This disadvantage can be solved by moving the focus while scanning the depth as performed in the focus tracking system described in EP 2 498 048.

By applying the adaptive focus concept a resolution up to 3µ can be obtained.

Using an incoherent light source with a Gaussian filter further has the following effects:

- Broad spectrum resulting in high axial resolution
- Gaussian spectrum resulting eliminating ghost images
- Laterally coherent light resulting in the absence of image blur (by coherent cross-talk).

Applying high definition OCT in a method according to the present invention including the pre-treatment renders fibroblasts visible.
Hence this method can also be envisaged as a method for rendering visible fibroblasts in biological tissue (in vivo or ex vivo).

In order to obtain best results a pulse length of 10 - 50 msec is selected. A fluence of 10 - 14 J/cm² (depending on the photo type) is further preferred.

The flash light can also be applied as a pulse trains of up to 5 consecutive pulses.

Pulse duration (ms) or repetition (times) are exchangeable against pulse intensities (J/cm²) to reach the same effects of transparency in the skin.

The pre-treatment according to the present invention is applied directly onto the tissue. No contacting gel nor light absorbing products are applied to the tissue.

The OCT step is preferably performed immediately after the pre-treatment step. It is however possible to perform the OCT imaging step within a time limit of about 10 minutes after the pre-treatment step. After this period of time the effect of the pre-treatment is no longer obtained, the effect of the pre-treatment step thus being reversible.

By applying the method of the present invention the depth penetration and contrast (image quality) of near infrared (NIR) optical imaging techniques is enhanced. A further advantageous result is that cellular elements become visible and can be examined such as keratinocytes.

A comparison between the results of an OCT examination of a part of the skin of a patient's forearm (about 100x100 µm²) without and with pre-treatment according to the present invention is shown in figure 1.
In this figure the x-axis indicates the skin depth expressed as a number of pixels (which when multiplied by 3 expresses the corresponding depth in \( \mu m \)), the y-axis indicates histogram values representing the average value of the sum of signal values measured on the examined surface.

During the pre-treatment the skin was subjected to a flash illumination with light within the wavelength range of 950 nm to 125 nm (all other conditions un-changed).

Immediately following this pre-treatment the skin was subjected to an optical coherence tomography step by means of Skintell™ of Agfa Healthcare.

This optical coherence tomography device has a lateral resolution of 3 \( \mu m \), an axial resolution of 3 \( \mu m \) and an imaging depth of up to 570 \( \mu m \). The device has cellular resolution.

Comparison of the two curves in figure 1 shows that the pre-treatment diminishes considerably the image of the stratum corneum and renders the underlying layers of the skin better visible.

While the present invention has been described in connection with preferred embodiments thereof, it will be understood that it is not intended to limit the invention to those embodiments.
[CLAIMS]

1. A method for generating an image from within a tissue layer by means of an optical coherence tomography imaging step (OCT) characterized in that said OCT imaging step is preceded by pre-treating said tissue layer so as to reduce scattering and enhance the penetration depth by subjecting the tissue layer in the absence of an optical clearing agent directly to a flash illumination with light within a wavelength range of 950 - 1250 nm.

2. A method according to claim 1 wherein flash illumination is applied during at least one illumination step with a duration in between 0 and 50 ms.

3. A method according to claim 2 wherein said flash illumination is implemented as a pulse train comprising in between 1 and 5 pulses.

4. A method according to claim 1 wherein during said flash illumination an energy amount in the range of 10 to 14 J/cm² is applied.

5. A method according to claim 1 wherein said OCT step is high resolution OCT.

6. A method according to claim 1 wherein the time period between the end of said flash illumination step and said OCT step is less than or equal to 10 minutes.

7. A method according to claim 1 wherein said tissue is tissue in vivo.

8. A method according to claim 1 wherein said tissue is tissue ex vivo.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01B9/02 A61B5/00 G01N21/47

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)

G01B A61B G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

The minimum documentation was searched (classification system followed by classification symbols):

G01B A61B G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used):

EPO-Internal, WPI Data

Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance.

"E" earlier application or patent but published on or after the international filing date.

"L" document which may throw doubts on priority claim(s) or which establishes the publication date of the prior application or invention.

"O" document referring to an oral disclosure, use, exhibition or other means.

"P" document published prior to the international filing date but later than the priority date claimed.

Further documents are listed in the continuation of Box C.

Date of the actual completion of the international search: 21 June 2016

Date of mailing of the international search report: 28/06/2016

Authorized officer: Petel ski, Torsten
<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>V.V. TUCHIN ET AL: &quot;Optical clearing of skin using flashlamp-induced enhancement of epidermal permeability&quot;, LASERS IN SURGERY AND MEDICINE, vol. 38, no. 9, 23 October 2006 (2006-10-23), pages 824-836, XP055217145, ISSN: 0196-8092, DOI: 10.1002/lsm.20392 abstract page 825, left-hand column, last paragraph - page 827, left-hand column, paragraph 1</td>
<td>1-8</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>US 2014204391 Al</td>
<td>24-07-2014</td>
<td>CN 103443579 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2508843 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 2527004 T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2014204391 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wo 2012136338 Al</td>
</tr>
</tbody>
</table>