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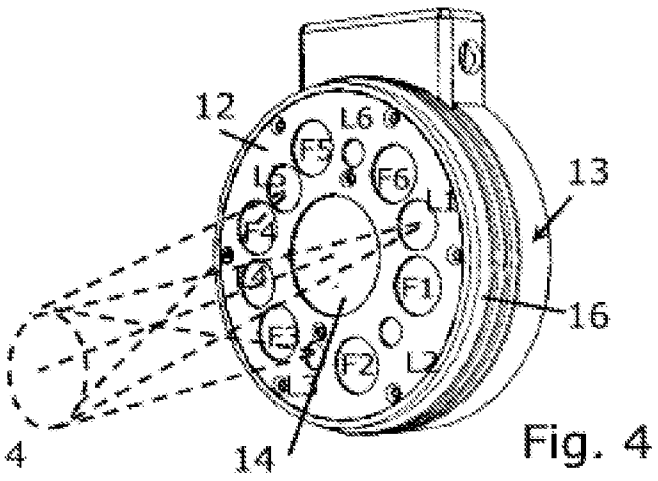



Fig. 4

(57) Abstract: In vivo fluorescence emitted from a skin region, into which a fluorescence generating component has been introduced, is determined by illuminating an illumination area within the skin region with non-focussed pulsed light, measuring light input to detector means arranged to receive light emitted by fluorescence from the illumination area, the detector means having a light-sensitivity range including at least one fluorescence emission wavelength of the fluorescence generating component, and processing light input measured by the detector means to obtain an averaged quantity of fluorescence from the illumination area and provide an objective quantification thereof with correction for influences from light sources other than the illumination area. The fluorescence detection apparatus may advantageously be designed as a handheld applicator having a housing (13) with support and spacer means for positioning the apparatus for substantially uniform illumination illumination of a relatively large illumination area (4) within the skin region. The applicator housing (13) may further be adapted for attachment of a digital still or video camera.

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Method and apparatus for in vivo determination of skin fluorescence.

**Technological field of the invention**

The present invention relates to a method and an apparatus for determination of in vivo fluorescence emitted from a fluorescence generating component in a mammal skin region, where the fluorescence generating component by illumination with light of a wavelength range including a fluorescence excitation wavelength for the component causes emission of fluorescence light of an emission wavelength specific to the fluorescence generating component.

Moreover, the invention specifically relates to the use of the method and apparatus during photodynamic procedures for treatment, investigation or diagnosing of skin conditions.

**Background of the invention**

In the examination of skin conditions the determination of fluorescence emitted in vivo from a skin region has gained wide-spread acceptance as a useful tool for detection of skin conditions ranging from assessment of aging phenomena as wrinkles or fine lines, photo-aged skin, pigmented lesions and telangiectasia to treatment and diagnosing of medical dermatologic conditions like acne, skin cancer and pre-cancer stages.

In WO 2006/099653, which in general discloses a photodynamic therapy light source for the treatment of skin conditions, a fluorescence measuring system is used to capture the amount of fluorescence from a skin region by means of a camera and thereby guide the treatment.

In photodynamic skin treatment procedures and dermatological practice it has equally become customary practice to pre-evaluate abnormal or adverse skin conditions and make assessment of treatment results with the aid of fluorescence determination.

By this prior art use of skin fluorescence measurement in connection with photodynamic procedures the assessment of a particular skin condition has primarily been of a subjective character in relying on visual observation of skin fluorescence pictures taken by a digital camera.

In EP 1 738 684 A1 a pen-like handheld device is disclosed, which is intended for determining fluorescence as indicator of an inherent parameter of a skin region such as skin age, proliferation status and photodamage level by non-professionals and is positioned during use to illuminate the skin region with focussed light in a small illumination point. This prior art device is neither adapted nor suitable for determination of fluorescence from a fluorescence generating component introduced into the skin region, e.g. during a photodynamic treatment or a diagnosing procedure.

**Summary of the invention**

On the background outlined above it is the object of the present invention to provide a method and an apparatus for determination of in vivo fluorescence from a fluorescence generating component introduced into a skin region in order to provide a useful tool for objective determination of skin fluorescence during a photodynamic procedure.

In line therewith additional objects of the invention comprise the provision of a fluorescence determination method and apparatus for particular use in monitoring of photodynamic treatment procedures for cosmetic as well as medical applications including investigation and monitoring of pre-exposure stages of such procedures, during which a photosensitizer is applied to a treatment target region and is allowed to incubate therein, and also as a useful tool in diagnosing of benign or malign dermatologic conditions.

To accomplish these and other objects, which will become clear from the following description, the invention provides a method for determination of in vivo fluorescence emitted by a fluorescence generating component introduced into a skin region of a patient, comprising the steps of illuminating an illumination area within the skin region with non-focussed pulsed light including a fluorescence excitation wavelength for the fluorescence generating component, measuring light input to detector means arranged to receive light emitted by fluorescence from the illumination area, the detector means having a light-sensitivity range including at least one fluorescence emission wavelength of the fluores-

cence generating component, and processing light input measured by the detector means to obtain an averaged quantity of fluorescence from the illumination area and provide an objective quantification thereof with correction for influences from light sources other than the illumination area.

The fluorescence generating component, which is introduced into the skin region from the outside, may comprise in particular a photosensitizing agent supplied to the skin region by a photosensitizer carrier fluid comprising either the photosensitizing agent as such or a precursor for production of the photosensitizing agent following entry of the precursor in epidermal cells in the skin region.

In one preferred implementation of the method, the photosensitizer fluid may comprise as photosensitizer agent a xanthene such as rose bengal.

In an alternative to this preferred implementation the photosensitizer fluid may comprise as photosensitizer agent a prophyrin.

In another preferred implementation, the photosensitizer fluid may comprise ALA or an ALA derivative as precursor for the production of protoporphyrin IX as photosensitizing agent. The precursor may favourably be encapsulated in a liposome-based carrier fluid in a relatively low concentration, e.g. between 0.1 % and 2.0 %.

In this implementation, the method is suitable, in particular, for use in a photodynamic treatment process for monitoring accumulation of a photosensitizing agent in a skin region or for diagnosing a dermatologic condition implying accumulation of a photosensitizing agent in a skin region. The invention is equally directed to both of these applications.

In yet another preferred implementation, the fluorescence generating component introduced into the skin region may comprise a skin tanning composition, preferably dihydroxyacetone, which is supplied to the skin region. Thereby, the method is particularly suited for use in an investigation procedure for determination of skin desquamation and stratum corneum renewal, to which the invention is equally directed.

In a further preferred implementation of the method the illumination area is uniformly illuminated. Thereby, the non-focussed pulsed

light may be controlled to provide an illumination area having a size between about 2 and about 20 $cm^2$, preferably between 10 and 18 $cm^2$.

It is further preferred, that the pulsed light is emitted with a pulse frequency equal to or constituting a whole fraction or multiple of the frequency of an operating AC voltage for ambient light sources with respect to a light source used for the non-focussed pulsed light.

By this measure the correction for influences from light sources other than the irradiation area may be implemented in a simple way by measuring the input to the detector means in light-on as well as light-off periods of the pulsed light.

By implementing the method in a way, such that the light input measured by the detector means is sampled and supplied to processor means for calculation of an averaged light input over a plurality of samples, the correction for influences from light sources other than the illumination area may be accomplished in said calculation by subtracting the light input measured by the detector means in said light-off periods the light input measured in said light-on periods,

For the performance of the method an apparatus for determination of in vivo fluorescence from a fluorescence generating component introduced into a skin region comprises a housing including a light source for illumination of an illumination area within the skin region by emission of non-focussed pulsed light including a fluorescence excitation wavelength for the fluorescence generating component, controlled power supply means for supplying and controlling operating power to the light source, first detector means arranged to receive light emitted by fluorescence from the illumination area and having a light-sensitivity range including at least one fluorescence emission wavelength of the fluorescence generating component, and processor means for processing light input measured by the first detector means to obtain an averaged quantity of fluorescence from the illumination area and provide an objective quantification thereof with correction for influences from light sources other than the illumination area.

For controlling the emission of non-focussed, pulsed light from the light source feed-back control means is provided in a preferred embodi-

ment of the apparatus, the feed-back control means including second detector means arranged to receive light input to the illumination field by emission from the light source.

In a preferred design of the apparatus, the illumination light source may comprise an arrangement of individual light sources positioned within the housing with substantially equal spacing along the perimeter of a circle for light emission through exit openings in a front surface part of the housing in the form of beams converging towards a common plane to produce substantially uniform illumination of a substantially circular illumination area, which could have a diameter between about 16 and about 50 mm, preferably between 36 and 48 mm.

Entry openings for the fluorescence emission from the illumination area may equally be provided by the front surface part of the housing to be positioned with regular spacing between the light exit openings.

To facilitate use of the apparatus during a photodynamic procedure the housing may in a still further preferred embodiment be adapted for attachment of a digital still or video camera such that a focal plane of the camera is positioned in the illumination area for imaging the fluorescence emitted therefrom. By attachment of a camera to the housing the camera could be connected, possibly via processing means such as a PC, with a display for reproduction of still or live images during use of the apparatus.

Advantageously, the housing may be adapted to be handheld.

To facilitate correct positioning of the housing in use of the apparatus support and spacer means may be connected with the front surface part of the housing for positioning of the illumination light source in a prescribed distance from the illumination area with the housing supported by the support and spacer means resting against the skin region outside the illumination field.

**Brief description of the drawings**

In the following the method and apparatus according to the invention will be further explained with reference to an illustrative, but not limiting embodiment as shown in the drawings, in which

figure 1 and 2 are graphic representations of typical excitation and emission wavelengths for examples of preferred fluorescence generating components introduced into a skin region in connection with implementation of the method and apparatus of the invention;

figure 3 is a schematic block diagram of an embodiment of the apparatus according to the invention;

figure 4 and 5 are front and rear perspective views, respectively, of a design of the apparatus as a handheld applicator; and

figure 6 is a graphic representation illustrating the feasibility of the invention for diagnosing an example of dermatologic disease.

**Detailed description**

One of the most common photosensitizing agents used in photodynamic treatment procedures is protoporphyin IX (PpIX), which is produced in epidermal cells by metabolic transformation of a so-called precursor such as 5-aminolevulinic acid (5-ALA), which is supplied to a treatment target region of the skin by means of a carrier fluid, which could be either a cream-based carrier usually incorporating a relatively high concentration about 20 % of 5-ALA or, more preferably, a liposome-based carrier fluid, in which 5-ALA may be encapsulated in a much lower concentration, e.g. between 0.1 % and 2.0 %, whereby a prolonged post-treatment phototoxicity, which is a known adverse side effect of a large accumulation of PpIX, may be significantly reduced. A further distinct advantage of using a liposome-based carrier fluid with a relatively low concentration of 5-ALA is that it can easily be applied to the skin by spraying, preferably in the form of a number of repeated spray doses delivered to the skin region as disclosed in copending international patent application "Method for non-theraoeutic and therapeutic photodynamic skin treatment" in the name of Kaare Christiansen et al.

As the rate of transformation of 5-ALA into PpIX is very dependent on cell activity or metabolism, cells or skin tissue affected by a condition like acne inflammation, skin cancer or pre-cancer stages will typically show large accumulations of PpIX.

The transformation rate for production of PpIX is further dependent

on the rate, at which the precursor such as 5-ALA penetrates through outer skin layers into the epidermal cells. Dependent on the type of carrier fluid used for the supply of the precursor there may be an incubation time of varying length before an accumulation of PpIX sufficient for activation by light exposure has been produced. The incubation time may typically be from 0.2 to 8 hours.

For this reason there is a need for monitoring the pre-exposure stages of a photodynamic treatment with respect to the production of PpIX.

For this purpose, determination of fluorescence from the target area is commonly used as a practical non-invasive method. It is based on the inherent and characteristic ability of PpIX for emission of red fluorescence light having a specific wavelength of 633 nm when excited by exposure to blue light of a wavelength of 407 nm.

In the graphic representation in fig 1 the excitation wavelength and the emission wavelength for fluorescence from PpIX are shown by the dashed indications $W_e$ and $W_f$, respectively.

For other applications of the method and apparatus according to the invention an alternatively preferred photosensitizing agent for introduction in a skin region as a fluorescence generating component would be a xanthene such as rose bengal, which by illumination with light including an excitation wavelength of 525 nm will generate a distinctive although relative broad fluorescence around an emission wavelength of 594 nm as shown in the graphic representation in figure 2. Rose bengal may be incorporated in any suitable photosensitizer carrier fluid such as a cream or gel, a liquid solution as e.g. ethanol or water or encapsulated in a liposome-based carrier fluid.

Figure 3 shows a schematic block diagram of main components of an embodiment of the apparatus according to the invention. A light source 1, which may be of any structure and design suitable for emission of pulsed light in a wavelength range including excitation wavelengths for one or more fluorescence generating components, with which the method an apparatus of the invention is to be used, is connected with a controlled power supply 2 controlling and supplying operating power to

the light source 1. As further explained in the following the light source may preferably include an arrangement of LED's configured to provide a substantial uniform illumination of a relatively large illumination area within a skin region subject to fluorescence determination

The controlled power supply 2, which acts as a programmable current generator, is controlled to supply a pulsed operating current to the light source 2. In a particularly preferred embodiment of the invention the pulse frequency of the operating current for the light source 1 is controlled to be equal to or constitute a whole fraction or multiple of an AC current used for ambient light sources in the vicinity of the apparatus in use. By this measure the potential influence of light from such ambient light sources may be compensated for as described in the following with the practical advantage that use of the apparatus of the invention will not be restricted to a dark environment. For a conventional 50 or 60 Hz AC operating power for such ambient light sources a pulse frequency between 25 Hz and 5000 Hz may be usable. In a typical design the pulse frequency could be 300 Hz.

In use of the apparatus in connection with a specific fluorescence generating composition, the effective emission spectrum for the illumination light from the light source 1 may be limited by a filter 3 arranged between the light source 1 and the illumination area 4 within a skin region under investigation. The specific function of the filter 3 would be, in particular, to exclude the expected wavelength of the fluorescence emission from the illumination area 4 from the effective emission spectrum from the light source 1, if this expected wavelength is included in the spectral emission range from the light source itself. Limitation of the emission bandwidth from the light source 1 to a desired range could alternatively be provided, however, as an integrated feature of the light source.

As a specific example for use of the apparatus for observation of the transformation of 5-ALA delivered to the skin region by means of a liposome-based carrier into protoporphyrin IX the light source 1 has thus been configured as an arrangement of six LED's of the type M3L1-HU supplied by Rothner Lasertechnik and having an emission range from

390 to 415 nm. The LED's are positioned along a circle with a diameter of 60 mm and with converging directions of light emission for uniform illumination of a substantially circular illumination area 4 at a distance of 70 mm from the light source 1 and having a diameter of 45 mm and thus an area of about 15.9 $cm^2$. In combination therewith the filter 3 was an absorption filter type BG3 with OD>3 in the wavelength range from 520 to 675 nm.

By a semi-permeable beam splitting mirror 5 arranged in the optical path between the light source 1 and the illumination area 4 the light beam emitted from the light source 1 is split into two unequal fractions, the larger of which is directed to the illumination area 4, whereas the smaller fraction reflected from the mirror 5 is supplied to a light detector 6 forming a feed-back control means for the emission of non-focussed, pulsed light from the light source 1.

The spectral bandwidth of the light input to the detector 6 is limited by means of a filter 7 arranged in the optical path between the beam-splitting mirror 5 and the detector 6, to exclude light of wavelengths outside the upper limit of the spectral range of the light impinging on the beam-splitting mirror 5, in particular IR light originating from the light source 1 or ambient light sources. In the specific example described above the filter 7 was a reflection filter for IR light beyond a cut-off wavelength of 720 nm.

By measuring the intensity of the light input the detector 6 generates a control signal providing a feed-back control for the light emission from the light source 1 to insure a constant light output.

By phase comparison of the phase of the control signal from the detector 6 with the measuring signal obtained from detection of the light emitted by fluorescence from the illumination area 4 in a manner known per se a phase control of the light emission from the light source 1 may further be obtained.

The light emitted by fluorescence from the illumination area 4 is received by a light detector 8, the light input to which is limited by a filter 9 to a relatively narrow wavelength range including the expected fluorescence emission wavelength from the fluorescence generating compo-

nent, which has been introduced in the skin region including the illumination area 4. In the specific example described above, the filter 9 was formed by a combination of a RG610 filter from Schott and an IR reflection filter of the same type as used for the filter 7, thereby limiting the light input to the fluorescence emission detector 8 to a spectral range from 610 to 720 nm.

It should be recalled, however, that this example was specifically related to the use of protoporphyrin IX produced in the skin region by metabolic transformation of 5-ALA. For other types of fluorescence generating components introduced into the skin region other spectral limitation ranges and filter characteristics may apply dependent on the specific excitation and emission wavelengths of such fluorescence generating components. As an example, the spectral sensitivity range of the fluorescence emission detector 9 could be from about 580 nm to about 800 nm to cover the emission wavelengths of various fluorescence generating components including e.g. rose Bengal, or the filter 9 may be exchangeable for adaptation to specific fluorescence generating components.

The electrical measuring signals obtained from the detector 8 in response to the intensity of the detected light input emanating from the fluorescence emission from the illumination area 4 is supplied to a microprocessor 10, in which the signals are processed into objectively quantified fluorescence values, which are supplied to a display 11 for visual display.

In a preferred implementation of the apparatus the light source 1 is controlled and operated from the controlled power supply 2 in a square pulse mode, for which as mentioned above the pulse frequency is selected to be equal to or to constitute a whole fraction or multiple of the normal 50 or 60 HZ operating AC current for ambient light sources in the environment, in which the apparatus is used. As a typical example the pulse frequency could be 300 Hz with a duty cycle of 30 %.

By use of this feature the fluorescence detector 8 and the microprocessor 10 may be controlled to measure the intensity of the light input to the detector 8 both in light-on and light-off periods of the pulsed

light. In connection therewith the microprocessor 10 is preferably structured, controlled or programmed to calculate quantified output values to be displayed by subtraction of detector signals measured by the detector in the light-off periods from the detector signals measured in the light-on periods.

By these measures it is ensured that undesired influence from ambient light sources are effectively suppressed in the quantified values obtained from the fluorescence emission with the result that reliable fluorescence detection can be accomplished without any requirement for darkened environmental conditions for the measurement.

In the practical implementation the measuring signals supplied from the detector 8 to the microprocessor 10 in the light-on and light-off periods, respectively, are sampled into digital values and an average over several samples is calculated before the subtraction of the light-off signals from the light-on signals.

The microprocessor 10 is further connected with the controlled power supply 2 and receives input from the feed-back control detector 6 for control of the light emission from the light source 1 via the controlled power supply 2.

In figures 4 and 5 a preferred design of the apparatus as a hand-held applicator adapted for attachment to the optics of a standard digital camera is shown in perspective views from the front and rear sides, respectively.

In a front surface portion 12 of the applicator housing 13 six exit openings L1 to L6 are located for light emission from six LED's positioned inside the housing 13 in a substantially circular arrangement with substantially equal spacing between them, the exit openings L1-L6 being positioned with substantially uniform separation on a circle surrounding a central opening 14 serving as image entry for a digital camera (not shown) that may be attached to the rear side of the housing 13 shown in figure 5. Equally distributed between the light exit openings L1 to L6 are six entry openings F1 to F6 for input of fluorescence light to fluorescence detector components positioned in the housing 13 behind the front surface portion 12. Spectral limitation filters such as the filters 3 and 9 in

figure 3 may be provided for each of the light exit openings L1-L6 and each of the fluorescence entry openings F1-F6, respectively, preferably by being arranged in an exchangeable filter holder (not shown) located in the housing 13 with the filters positioned in alignment with their respective exit or entry openings.

As indicated in dashed lines the individual LED light sources are configured and positioned for emission of light towards the circular illumination area 4 in light beams converging towards the common plane of the skin region, within which the illumination area 4 is located, thereby providing a substantially uniform illumination of the illumination area.

In the rear side of the applicator housing 13 the numerical display 11 is positioned in an upper part outside the portion of the rear side that will be covered by the housing of a camera attached to the applicator.

The common plane, towards which the light beams from the exit openings L1 to L6 in the front surface portion 12 converge, can conveniently be about 70 mm in front of the front surface portion 12. To enable the handheld applicator to be held in a stable position, in which the common plane for the light beams will coincide with the skin surface, the applicator is preferably equipped with support and spacer means extending from the front surface portion 12 to the common plane for the converging light beams.

In the illustrated embodiment such support and spacer means is formed as shown in figure 5 by a funnel-like frustro-conical light screen 15 connected with the front surface portion 12.

In use the illustrated applicator may thus be positioned with the free end of the light screen 15 in contact with the skin region under investigation outside the illumination area 4 and the opposite end of the screen 15 in engagement with a recess or track 16 surrounding the front surface portion 12.

As an illustrative example of the feasibility of the method and apparatus of the invention to diagnosing of dermatologic conditions figure 6 shows a graphic representation of fluorescence intensity levels measured from target regions of normal skin and a skin region having a high metabolic activity caused by actinic keratosis. The pronounced difference

in fluorescence intensity is caused by the ability of a photosensitizing agent introduced into the skin region to accumulate in areas having a high metabolism.

As a further example, the method and apparatus of the invention may find useful application in investigation of skin desquamation and stratum corneum renewal.

The outer layer of the skin, the epidermis, is composed of a series of overlying layers of cells, all of which are produced in the lower basal layer. During their continuous production the cells are pushed upwards towards the skin surface while undergoing a maturation process, in which the epidermal cells (the keratinocytes) change their metabolism and morphological shape and turn into flat sheets of keratin forming the outermost stratum corneum layer, these cells being bound to each other by lipids and waxes to from a waterproof outer protective sheath for the body.

The rate of cell production and hence the shedding or desquamation of the outermost cells is important both in connection with skin diseases and for skin health in general. Thus, a too low desquamation rate may result in a leaky protective layer and a too high rate in visible scaled, a dull skin appearance or dandruff.

Many different drugs including systemic drugs like Roaccutane, topical drugs as Differin, topical cosmetics as Vitamin A containing creams and cosmetic products as Alpha Hydroxy Acid containing preparations, scrub creams with mechanically abrading particles etc. have been developed to regulate the rate of desquamation of the stratum corneum.

As the desquamation rate of the epidermis is the measure of efficacy for such drugs and products this parameter has always been of interest for the purpose of documentation.

Whereas determination of fluorescence as a measure for desquamation rate has previously been applied by use of the fluorescent dye dansyl chloride, this method has been abandoned due to suspicion regarding a harmful cardiogenic potential of the dye, the possibilities for finding a valid substitution for dansyl chloride has been investigated as a

potential application of the method and apparatus of the invention, whereby dihydroxyacetone (DHA) has been found to be a promising chemical for use as a fluorescence generating component in implementation of the method of the invention. DHA has thus been approved by health authorities and dermatologic and medical scientific associations in a number of countries.

As DHA is an ingredient of many commercial skin tanning compositions with temporary effect the use can be considered very safe.

By implementation of the method of the invention the stratum corneum turnover rate and the barrier function may be determined in vivo by objective and quantified assessment.

Whereas the method and apparatus of the invention has been explained with reference to a few examples of useful applications and embodiments, these are intended as illustrative examples only, which are not limiting to the scope of the invention as defined by the appended claims.

# PATENT CLAIMS

1. A method for determination of in vivo fluorescence emitted by a fluorescence generating component introduced into a skin region of a patient, comprising the steps of

illuminating an illumination area (4) within the skin region with non-focussed pulsed light including a fluorescence excitation wavelength for the fluorescence generating component,

measuring light input to detector means (8) arranged to receive light emitted by fluorescence from the illumination area (4), the detector means (8) having a light-sensitivity range including at least one fluorescence emission wavelength of the fluorescence generating component, and

processing light input measured by the detector means (8) to obtain an averaged quantity of fluorescence from the illumination area (4) and provide an objective quantification thereof with correction for influences from light sources other than the illumination area.

2. A method as claimed in claim 1, characterized in that the fluorescence generating component comprises a photosensitizing agent introduced into the skin region by supply of a photosensitizer fluid comprising the photosensitizing agent or a precursor for generation of the photosensitizing agent in the skin region.

3. A method as claimed in claim 2, characterized in that the photosensitizing agent comprises a xanthene.

4. A method as claimed in claim 2, characterized in that the xanthene is rose bengal.

5. A method as claimed in claim 2, characterized in that the photosensitizing agent comprises a porphyrin.

6. A method as claimed in claim 2, characterized in that the photosensitizer fluid comprises ALA or an ALA derivative as precursor for the production of protoporphyrin IX as photosensitizing agent.

7. A method as claimed in claim 1, characterized in that the fluorescence generating component comprises a skin tanning composition supplied to the skin region.

8. A method as claimed in claim 7, characterized in

that the skin tanning composition comprises dihydroxyacetone.

9. A method as claimed in any of claims 1 to 8, c h a r a c t e r i z e d in that the illumination area is uniformly illuminated.

10. A method as claimed in any of claims 1 to 9, c h a r a c t e r i z e d in that the non-focussed pulsed light is controlled to provide an illumination area (4) having a size between about 2 and about 20 $cm^2$, preferably between 10 and 18 $cm^2$.

11. A method as claimed in any of claims 1 to 10, c h a r a c t e r i z e d in that the pulsed light is emitted with a pulse frequency equal to or constituting a whole fraction or multiple of the frequency of an operating AC voltage for ambient light sources with respect to a light source used for the non-focussed pulsed light.

12. A method as claimed in any of claims 1 to 11, c h a r a c t e r i z e d in. that the photo-sensitivity range of the detector means (8) includes the wavelength range from about 580 to about 800 nm, preferably from about 610 nm to about 720 nm.

13. A method as claimed in any of claims 1 to 10, c h a r a c t e r i z e d in that the light input to the detector means (8) is measured in light-on as well as light-off periods of the pulsed light.

14. A method as claimed in any of claims 1 to 13, c h a r a c t e r i z e d in that the light input measured by the detector means (8) is sampled and supplied to processor means (10) for calculation of an average light input over a plurality of samples.

15. A method as claimed in claims 13 and 14, c h a r a c t e r i z e d in that in said calculation the light input measured by the detector means (8) in said light-off periods is subtracted from the light input measured in said light-on periods.

16. An apparatus for determination of in vivo fluorescence from a fluorescence generating component introduced into a skin region by the method of any of claims 1 to 12, comprising a housing (13) including

a light source (1) for illumination of an illumination area (4) within the skin region by emission of non-focussed pulsed light including a fluorescence excitation wavelength for the fluorescence generating component,

controlled power supply means (2) for supplying and controlling operating power to the light source (1),

first detector means (8) arranged to receive light emitted by fluorescence from the illumination area (4) and having a light-sensitivity range including at least one fluorescence emission wavelength of the fluorescence generating component, and

processor means (10) for processing light input measured by the first detector means (8) to obtain an averaged quantity of fluorescence from the illumination area and provide an objective quantification thereof with correction for influences from light sources other than the illumination area.

17. An apparatus as claimed in claim 16, c h a r a c t e r i z e d in that feed-back control means provided for the emission of non-focussed, pulsed light from the light source includes second detector means (6) arranged to receive light input to the illumination area (4) by emission from the light source (1).

18. An apparatus as claimed in claims 16 or 17, c h a r a c t e r i z e d in that the light-sensitivity range of the first detector means (8) includes the wavelength range from about 580 to about 800 nm, preferably from about 610 nm to about 720 nm.

19. An apparatus as claimed in any of claims 16 to 18, c h a r a c t e r i z e d in that the controlled power supply means (2) is programmed to set the pulse frequency for emission of the pulsed light to be equal to or constitute a whole fraction or multiple of the frequency of an operating AC voltage for ambient light sources with respect to the illumination light source.

20. An apparatus as claimed in any of claims 16 to 19, c h a r a c t e r i z e d in that the first detector means (8) is controlled for measuring the light input thereto in light-on as well as light-off periods of the pulsed light from the illumination light source.

21. An apparatus as claimed in any of claims 16 to 20, c h a r a c t e r i z e d in that the processor means (10) is adapted for sampling of the light input measured by the first detector means (8) and calculation of an averaged quantity thereof over a plurality of samples.

22. An apparatus as claimed in claim 20 and 21, characterized in that the processor means (10) is controlled in said calculation to subtract the light input measured by the first detector means in said light-off periods from the light input measured in said light-on periods to provide an averaged quantity of fluorescence emitted from the illumination area (4).

23. An apparatus as claimed in any of claims 16 to 22, characterized in that the illumination light source (1) comprises an arrangement of individual light sources (L1-L6) positioned within the housing (13) with substantially equal spacing along the perimeter of a circle for light emission through exit openings (L1-L6) in a front surface part (12) of the housing (13) in the form of beams converging towards a common plane to produce substantially uniform illumination of a substantially circular illumination area (4).

24. An apparatus as claimed in claim 23, characterized in that entry openings (F1-F6) for fluorescence light emitted from the illumination area (4) are positioned with substantially uniform spacing in said front surface portion (12).

25. An apparatus as claimed in claim 23 or 24, characterized in that spectral limitation filters (3, 9) are positioned in an exchangeable filter holder in the housing (13) in alignment with said exit and entry openings (L1-L6, F1-F6), respectively.

26 An apparatus as claimed in any of claims 23 to 25, characterized in that the illumination area (4) has a diameter between about 16 and about 50 mm, preferably between 36 and 48 mm.

27. An apparatus as claimed in claims 16 to 26, characterized in that the housing (13) is provided with a display (11) for indication of the averaged quantity of fluorescence emitted from the illumination area (4).

28. An apparatus as claimed in any of claims 16 to 27, characterized in that the housing (13) is adapted for attachment of a digital still or video camera such that a focal plane of the camera is positioned in the illumination area (4) for imaging the fluorescence emitted therefrom.

29. An apparatus as claimed in any of claims 16 to 28, c h a r a c t e r i z e d in that the housing (13) is adapted to be handheld.

30. An apparatus as claimed in any of claims 23 to 26, c h a r a c t e r i z e d in that support and spacer means is connected with said front surface part (12) of the housing (13) for positioning of the illumination light source (L1-L6) in a prescribed distance from the illumination area (4) with the housing (13) supported by the support and spacer means resting against the skin region outside the illumination area (4).

31. An apparatus as claimed in claims 28 and 30, c h a r a c t e r i z e d in that said support and spacer means comprises a substantially conical screen (15) of light screening material connected with said front surface part (12) of the housing (13) to surround the illumination light source (L1-L6).

32. Use of the method as claimed in any of claims 1 to 15 in a photodynamic treatment process for monitoring accumulation of a photosensitizing agent in a skin region.

33. Use of the method as claimed in any of claims 1 to 15 in a dermatologic procedure for diagnosing a skin condition implying accumulation of a photosensitizing agent in a skin region.

34. Use of the method as claimed in claim 7 or 8 in an investigation procedure for determination of skin desquamation and stratum corneum renewal.

35. Use of the apparatus as claimed in any of claims 16 to 31 in a photodynamic treatment process for monitoring the accumulation of a photosensitizing agent in a skin region.

36. Use of the apparatus as claimed in any of claims 16 to 31 in a dermatologic procedure for diagnosing a skin condition implying accumulation of a photosensitizing agent in a skin region.

37. Use of the apparatus as claimed in any of claims 16 to 31 in an investigation procedure for determination of skin desquamation and stratum corneum renewal.

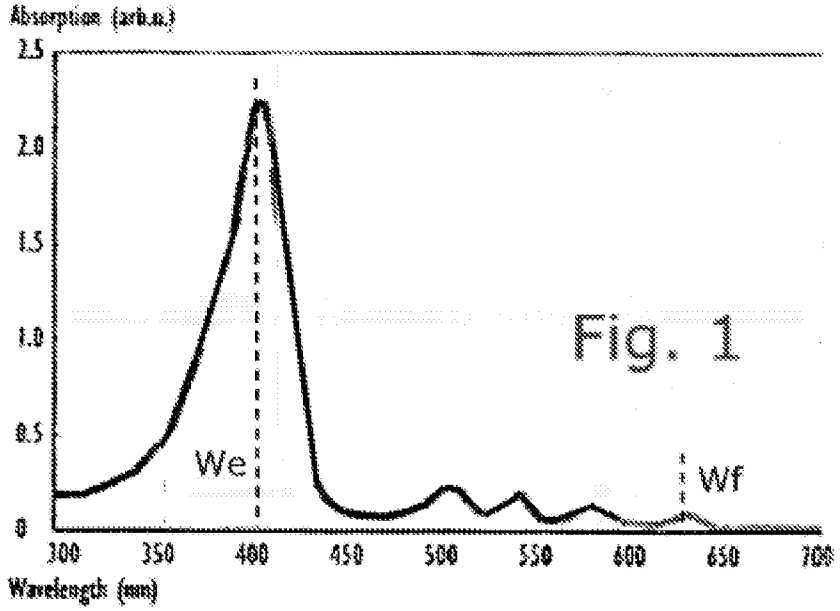

Absorption (arb.u.)
2.5
2.0
1.5
1.0
0.5
0
We
Wf
300
350
400
450
500
550
600
650
700
Wavelength (nm)

Fig. 1

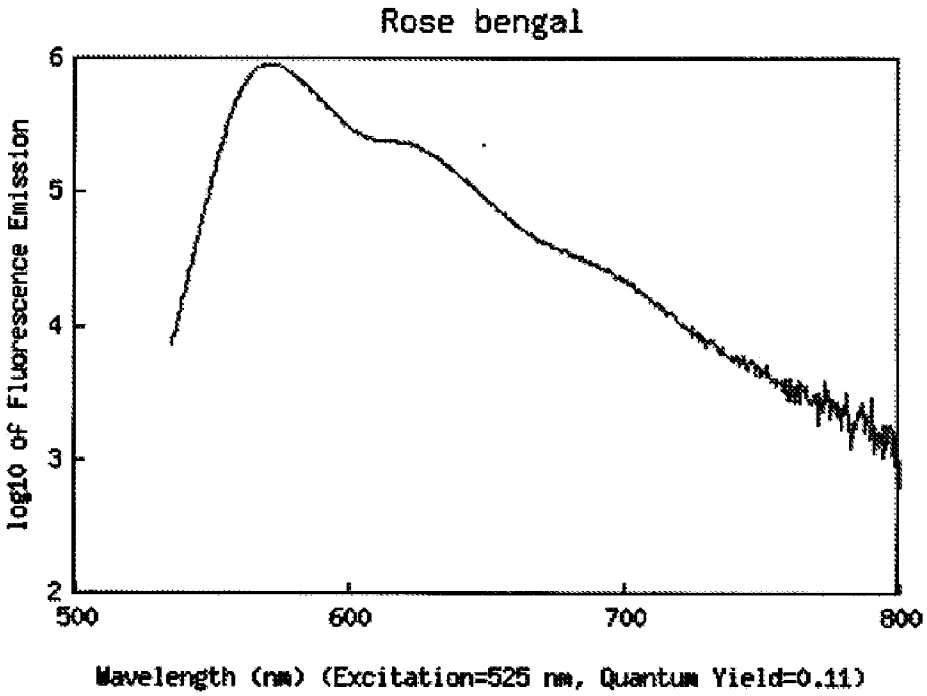

Rose bengal
log10 of Fluorescence Emission
6
5
4
3
2
500
600
700
800
Wavelength (nm) (Excitation=525 nm, Quantum Yield=0.11)

Fig. 2

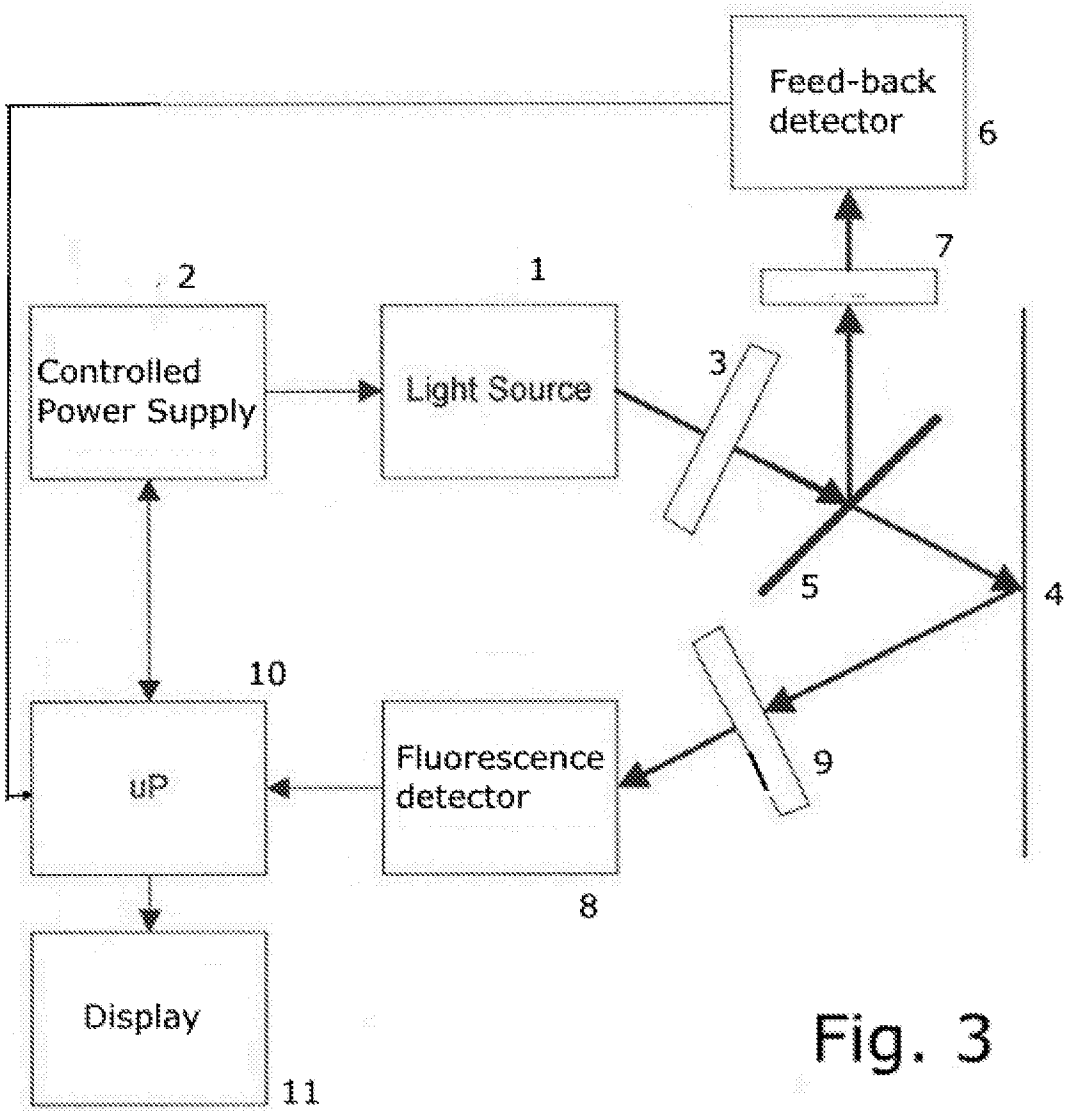

Feed-back detector
6
7
2
1
Controlled Power Supply
Light Source
3
5
4
10
uP
Fluorescence detector
9
8
Display
11

Fig. 3

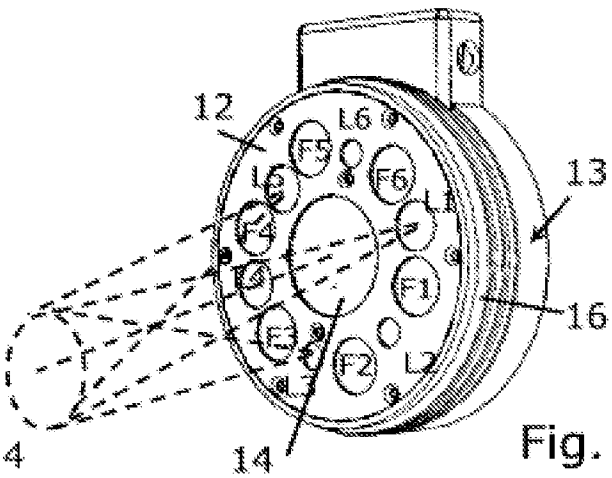



Fig. 4

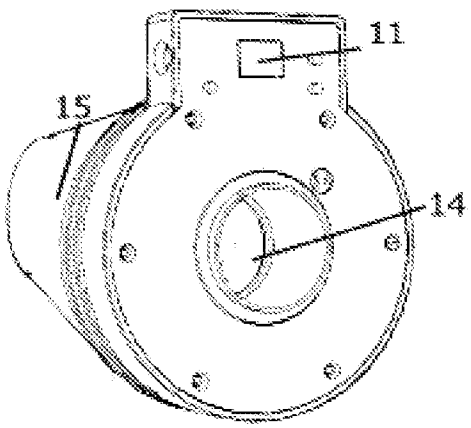



Fig. 5

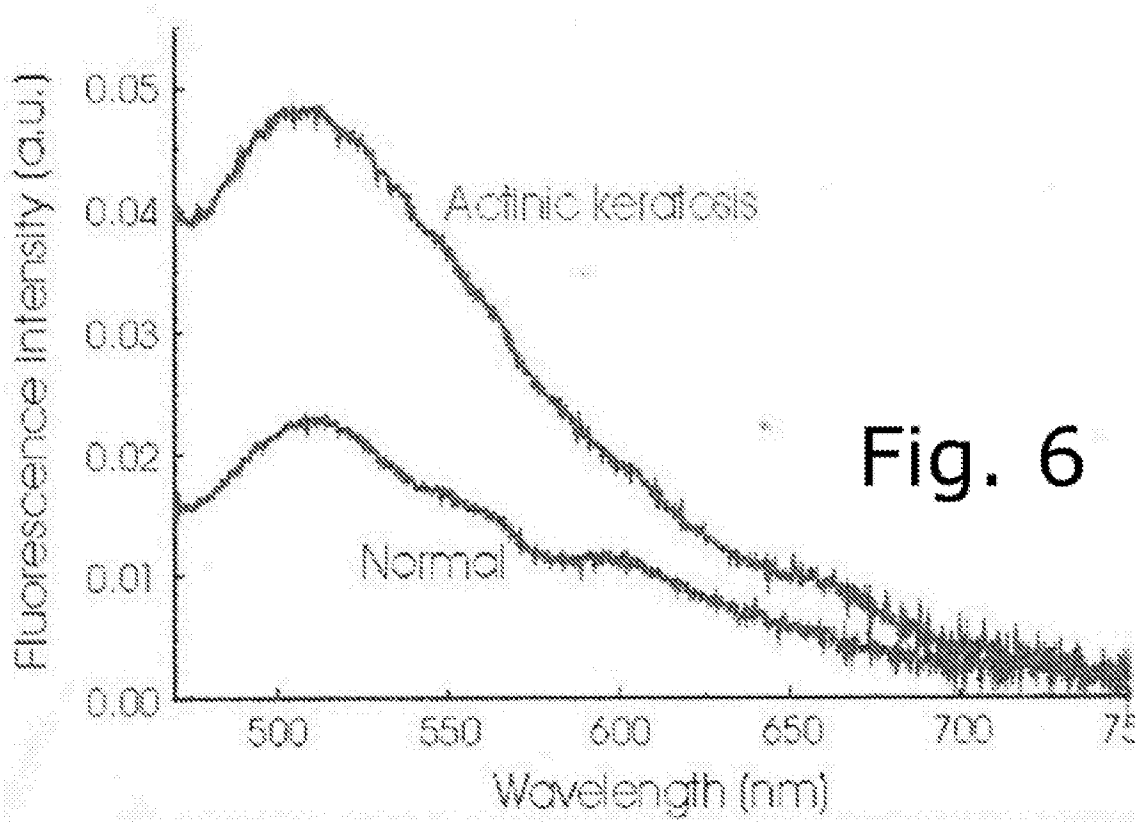

Fluorescence Intensity (a.u.)
0.05
0.04
0.03
0.02
0.01
0.00
Actinic keratosis
Normal
500
550
600
650
700
Wavelength (nm)

Fig. 6

# INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2007/050028

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61N5/06 A61B5/103 A61B18/20

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)
A61N A61B

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 practical, search terms used)
EPO-Internal, 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 | US 6 748 259 B1 (BENARON DAVID A [US] ET AL) 8 June 2004 (2004-06-08)<br><br>column 9, line 29 - column 10, line 11<br>column 10, line 35 - column 11, line 16<br>column 14, line 24 - line 67<br>column 24, line 53 - line 66; figures | 1,2,5,6,<br>9,10,<br>12-16,<br>18,<br>20-24,<br>26-29 |
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-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* 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 document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 21 December 2007 | 11/01/2008 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Strubel, Christine |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2007/050028

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
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| A | US 7 135 033 B2 (ALTSHULER GREGORY B [US] ET AL ALTSHULER GREGORY B [US] ET AL) 14 November 2006 (2006-11-14) column 10, line 19 - line 41 column 13, line 4 - line 17 column 37, line 39 - line 54 | 1-3,7,16 |
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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2007/050028

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 32, 35
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2007/050028

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