A laboratory device to automatically measure growth of cell culture non-invasively

van der Helm, Eric; Redl, Stephanie Maria Anna

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(71) Applicant: **DANMARKS TEKNISKE UNIVERSITET** [DK/DK]; Anker Engelunds Vej 101 A, 2800 Kgs. Lyngby (DK).

(72) Inventors: **VAN DER HELM, Eric**; Istedgade 75, 1. tv., 1650 Copenhagen V (DK). **REDL, Stephanie**; Musvågevej 7, 3. th., 2400 Copenhagen NV (DK).

(74) Agent: **INSPICOS P/S**; Kogle Allé 2, 2970 Hørsholm (DK).

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(54) Title: A LABORATORY DEVICE TO AUTOMATICALLY MEASURE GROWTH OF CELL CULTURE NON-INVASIVELY

(57) Abstract: The invention relates to a system for non-invasive and automatic real-time cells culture growth measurements. The system comprises a vessel for holding a liquid sample with cell culture, an optoelectronic circuit system, an electronic system, and a holder. The optoelectronic circuit system comprises a light source configured to emit light radiation onto the liquid sample being held by the vessel, and a photodetector configured to detect the light radiation after the sample has been exposed to the light radiation. The electronic system is configured to receive signals generated by the photodetector and determine the cell growth based on the signals. The holder includes the light source and photodetector of the optoelectronic circuit and is configured to receive and secure the vessel.

A LABORATORY DEVICE TO AUTOMATICALLY MEASURE GROWTH OF CELL CULTURE NON-INVASIVELY

FIELD OF THE INVENTION

The present invention relates to a system for non-invasive and automatic real-time cell culture growth measurements.

The invention further relates to a method for non-invasive and automatic real-time cell culture growth measurements.

BACKGROUND OF THE INVENTION

Different cell cultures serve as production hosts in industrial biotechnology. A fundamental part of a research cycle is to keep track of the growth of the cell culture. This is necessary to compare growth of different, for instance, bacterial strains, compare cultivation conditions, or allow experiments with bacteria which are in a certain growth stage. To track the growth of cell cultures the cells are typically cultured in closed glass or plastic vessels of 5 – 500 ml and cultivated in an incubator set at a certain temperature. Then, the closed vessel is regularly taken out of the incubator, e.g. about every 20 minutes, and an optical density of the culture in the vessel is measured by a spectrophotometer. Finally, the measured value is documented and a growth curve is constructed from the measured values. Scientists typically carry out this work manually. Manually profiling the growth of a cell culture in a sample is a tedious and complicated process, as the density of the cells in the sample needs to be measured regularly over a long period to be able to precisely construct a growth curve. Furthermore, such manual sampling disturbs growing cells due to a drop in temperature and other environmental conditions such as pressure and gas concentration, and introduces noise in the measurements.

To date, there is no automated solution existing to replace the task of manual sampling and measurements in mid-sized cell cultures.

It is an object of embodiments of the invention to provide an automatic system that measures the growth of a cell culture in real time, without disturbing the conditions under which the sample is stored.

DESCRIPTION OF THE INVENTION

The above-mentioned objects are complied with by providing, in a first aspect, a system for non-invasive and automatic real-time cell culture growth measurements, the system comprising:

- a vessel for holding a liquid sample with cell culture;
- an optoelectronic circuit system comprising a light source configured to emit light radiation onto the liquid sample being held by the vessel, and a photodetector configured to detect the light radiation after the sample has been exposed to the light radiation,
- an electronic system configured to receive signals generated by the photodetector and determine the cell growth based on the signals; and
- a holder including the light source and photodetector of the optoelectronic circuit and configured to receive and secure the vessel.

The vessel may have a volume such that it accommodates liquid sample with a cell culture of an arbitrary size, such as 5 - 500 ml, such as 500 – 1000 ml, such as larger than 1 l. The vessel may be produced from a polymeric material, a glass material, or the like. The vessel may correspond to any bottle and tube that are used in industry and/or academia for the purpose of cell culture growth. It further allows the system to be easily integrated in an existing workflow.

A liquid sample is a culture medium designed to support or advance growth of cells living therein. Different types of liquid may be used for growing different types of cells. A liquid may be mixed with some other substances which will help cells to be properly cultured. Examples of media are, but not limited to, Luria-Bertani (LB) broth, M9 minimal media, YPD broth and derivatives thereof.

A cell culture to be analysed may, for instance, include a number of different cells such as: bacteria, archaea or Eukaryota. Escherichia coli, Saccharomyces cerevisiae, Bacillus subtilis, Lactococcus lactis, Corynebacterium glutamicum, Pseudomonas putida, Streptomyces coelicolor, Clostridium acetobutylicum, etc. Those organisms provide bioscience based natural ingredients to the biofuel, pharma, food, beverage dietary supplements, and agricultural products.

An optoelectronic circuit is a combination of electrical-to-optical and/or optical-to-electrical devices. The main optical device is a light source positioned in such a way that it directly illuminates the liquid sample inside the vessel when the vessel is in the holder. The opto-electronical component is a photodetector, collecting and detecting light after its

interaction with the liquid sample. The circuit may also comprise mirrors, lenses, fibres, and other similar optical components. These may be integrated on a single optical chip.

The light source is attached to or forms part of the holder. It may be a laser, an LED, a lamp, a fluorescent lamp, or similar. The light radiation generated by the light source may have an intensity in the range of 1-10 mW such that it can penetrate the liquid sample and be absorbed by the cells in the sample. For the light radiation to be directed to the liquid sample, mirrors, fibres, and/or lenses may be used, but the light source may also point directly to the sample without any additional guidance.

The photodetector is attached to or forms part of the holder. It detects the light radiation going through the liquid sample and converts the light radiation into an electric signal such as voltage or current. The photodetector may also detect the light radiation which is scattered or reflected from the liquid sample. The light detection may involve different mechanisms such as photoemission, photovoltaic, etc.

An electric signal from the photodetector is then sent to the electronic system to be further processed so that the growth of cell culture can be determined. The growth curve may be constructed by comparing two successive electric signals generated at two different points in time. Points at the growth curve may be determined using the Lambert-Beer law, using the formula for optical density, $OD = -log_{10}(t/t_0)$ where t is time of measurement taken and t_0 denotes the time point that serves as initial measurement. Namely, the light source continuously illuminates the sample while the photodetector detects the light after it passes the sample. The photodetector generates a current proportional to the illumination which is then converted to the adequate voltage value. Finally, the optical density is calculated by comparing the obtained luminance of a sample at a time point t when the current voltage has been measured, with the luminance at a time point t_0 when no cellular growth was present.

The electronic system, receiving the output of the photodetector may comprise various electronical devices, such as processors, logic units, comparators, and the like. Various electronical devices will process the electric signal from the photodetector and determine the cell growth. All the devices may be integrated in a single electronic chip.

The holder is designed such that it secures the vessel with the liquid sample in it. Particularly the holder is configured such that the vessel can easily be inserted in the holder and removed from the holder. In that way, the holder can be reused for multiple vessels. The holder may be shaped such that it matches one particular shape for fixing of vessels with this shape, or it may be made to match different shapes to thereby enable its use in combination with vessels of different shape. In other words, the holder may correspond to any bottle and tube that are

used in industry and/or academia for the purpose of cell culture growth. The holder may e.g. comprise a resilient gasket arranged to interact with an outer surface of the vessel to hold the vessel in the holder and to prevent external light to enter into the space between the holder and the vessel. This may improve the accuracy of the measurement.

Furthermore, the holder includes the light source and the photodetector, and optionally other optical elements that direct the light radiation onto the liquid sample. The holder may comprise a powering means to supply power to the light source and the photodetector.

Particularly, the holder may include independent powering means, e.g. including battery powering. Having such a configuration, the holder can be moved from one place to another without disturbing the measurements. The electronic system may optionally be included in the holder.

In one embodiment of the invention, the vessel may have a circular cross-section. The circular cross-sectional shape of the vessel allows for multiple orientations of the vessel inside the holder. Namely, the vessel does not need to take a predefined orientation, but it can rather be placed randomly inside the holder. This would ease handling of the vessel, in particular when a user is placing the vessel inside the holder.

Once the vessel is placed into the holder, the holder may comprise means for fixing the vessel to prevent rotation of the vessel in the holder. In one example, the vessel may be fixed to the holder by friction. For that purpose, at least one of the holder and vessel may include a high friction material like rubber, etc. In another example, the fixation may be established by various screws, clamps, pins, or the like.

The light source may include multiple light emitters. These light emitters may be positioned such that they illuminate the vessel from and in different directions, and/or at different points of the vessel.

Furthermore, the photodetector may include multiple photo sensors. The photo sensors may be photodiodes. The number of photo sensors may and may not be equal to the number of the light emitters. For instance, one light emitter may be followed by a beam splitter which splits the light beam into two, and then two photo sensors will be required to detect these two beams. In another example, light generated from one light emitter may be scattered by the vessel. The scattered beams may be detected by different photo sensors. In yet another example, one photo sensor may have a large sensing area, capable of sensing more than one beam.

The system may be configured to correct the determined cell growth based on multiple light emitters and photo sensors. Namely, the vessel surface may have non homogeneous characteristics due to which determination of the cell growth may be inaccurate if the determination is only based on a single light beam. Multiple light beams may mitigate this non-homogeneous characteristic, as the cell growth will be determined based on the multiple light beams illuminating the vessel at different points. Furthermore, the multiple light sources and photo sensors additionally allow for any orientation of the vessel inside the holder, at the beginning of the measurement.

In one particular embodiment of the invention, the system may include N light emitters and M photo sensors. It should be noted that the numbers N of light emitters and M of photo sensors can be any whole number equal or larger than two. Each light beam generated by the light emitters can be scattered and the scattered light can be detected by each of the M photo sensors. Such a configuration results in N×M measurements. All the recorded measurements are then averaged out to give a final value related to the cell growth. The light emitters and/or the photo sensors could be configured for cyclic activation. In one example, the measurement is performed such that only one light emitter is turned on at the time. The photo sensors simultaneously detect light emitted from the first emitter and scattered from the vessel. Subsequently, the second emitter is turned on and the scattered light is detected. This repeats until all the light emitters have been individually activated and the whole cycle starts again. The light emitters can be programed to be turned on in time slots of, e.g., 500 ms.

In one embodiment, all light emitters are turned on simultaneously.

In one embodiment, all light emitters have the same wave length.

In one embodiment, at least two light emitters have different wave length.

The system may further comprise a pressure sensor configured to measure pressure inside the holder or vessel. The pressure sensor may constantly or intermittently provide the pressure value inside the holder/vessel as changes in pressure may affect the cell growth.

The system may further comprise a temperature sensor configured to measure temperature inside the holder or vessel. Similarly as for the pressure sensor, the temperature sensor may constantly or intermittently provide the temperature value inside the holder/vessel as changes in temperature may affect the cell growth.

According to one embodiment of the invention, at least one of the pressure sensor or the temperature sensor is included on the holder. The effect of including the pressure sensor and/ or the temperature sensor in the holder is that the holder combines all the electronic devices which are to interact with the sample, and the use of the system becomes easy and entangling of cables can be avoided. Further, signals from the pressure and/or temperature sensor can be transmitted wirelessly in combination with signals from the optoelectronic system and prevention of signal interference between individually transmitting elements can be avoided.

As indicated above, the system may be configured to be movable during a measurement. In this case, the system may comprise independent powering such as, for instance, a battery. Reasons for ensuring mobility of the system may be various. For instance, there may be a need to take a small amount of the liquid sample from the vessel and this can only be performed under certain conditions. Furthermore, there may be a need for a change in the environmental conditions to which the sample is exposed.

The electronic system may comprise a first system and a second system. The first system may be configured to measure optical properties of the liquid sample with cell culture, based on data from the optoelectronic system. The data from the optoelectronic system may express absorption, reflection, and/or scattering of the liquid sample by means of the light source and photodetector. Namely, the photodetector generates an electrical signal which electrical signal comprises information about optical density of the liquid sample. The electronic system then receives the electrical signal, and creates data related to the optical density of the liquid sample. The second system may be configured to receive data from the first system and do further data processing.

In one example, the data from the first system, i.e. the optical properties relates merely to the data directly obtained from the optoelectronic system, e.g. just filtered and refined, and the second system process these data to provide therefrom an optical density (OD) which can express directly the cell growth.

In another example, the first system may refine the data from the optoelectronic system and provide OD data for the second system. I.e. in this case, the optical properties may be the OD, and the second system may be reduced to a data collection system, e.g. used for preparing presentation of data or for sampling of data from different first systems. I.e. one second system could communicate with several first systems.

The term "optical density" is to be understood as the cloudiness or haziness of the liquid sample caused by large numbers of individual cells. Optical density may be determined by

measuring absorption, reflection, and/or scattering of the liquid sample. In other words, the light radiation may be directed to the sample such that it further gets reflected, absorbed, or scattered and then this new light beam is detected by the photodetector, and further processed by the electronic system. By measuring, for instance, light absorption, a number of cells may be determined based on level of absorption. The same is valid for light scattering and reflection. Depending on the concentration of the cells in the liquid sample, the light radiation will experience different modification. For instance, the intensity of the light radiation may be decreased compared to the initial intensity before an interaction with the sample. The ratio between intensities before and after interaction with sample may provide optical density value.

In one embodiment, the first system may be local and the second system may be external. Namely, the first system may be in a direct connection with the holder and photodetector and may be included in the holder. The local system may automatically send the data online to a second system, which may be remotely located with respect to the holder and the first system. It, for instance, may be a cloud computing system. The cloud computing system may comprise a server or a computer configured to receive the data. As mentioned above, the second system may then determine optical density by comparing two values of successively measured data and create growth curve of cell culture or simply display, store, or otherwise enrich the data. By having such an electronic system, the data regarding the cell culture growth may be measured in real-time without a need to remove the sample from the holder at regular time intervals. The first system may include a data storage enabling sampling of data over time and transmission of data packages containing sampled data. This will reduce the need for data transmission.

In one embodiment of the invention, the electronic system may be configured to establish a wireless/wired connection with at least one portable device such as a mobile phone, cell phone, personal computer, laptop or the like and send the digital data over the wireless/wired connection. In case of a wireless connection, the system and a portable device may typically be connected via Internet. The wireless/wired connection between the system and a portable device enables availability of measured data any time during the day, outside the normal working hours. Furthermore, the wireless connection may enable storing of the measurements data in the cloud so that a number of users can access them. The connection between the system and a portable device may also be wired, via appropriate USB cable. The portable devices may be configured to visualize the digital data which comprises information about the cell culture growth.

The vessel may comprise a syringe access and the holder may be configured to secure the vessel such that the syringe access is accessible during measurement. The syringe access

may, for instance, be a rubber stopper. Such an access may be convenient when there is a need for taking out a small amount of the sample from the vessel without interrupting the ongoing measurement. If the holder comprises the aforementioned gasket, the gasket may particularly be located between the syringe access and the light source and photo detector, when the vessel is located in the holder.

The vessel may be made from a material having a refractive index in the range between 1.45 and 1.8. This is relevant in cases where measurements are based on light radiation reflected from the sample as well as from the vessel. Knowing the refractive index of the vessel may be used in an initial calibration of the measurement. Similarly, knowing an absorption coefficient of the vessel material, calibration may be performed for the cases when light radiation transmitted through the vessel and the sample is detected.

The holder may be configured such that it shields the vessel from external light when the vessel is in the holder. This further facilitate moving of the holder and vessel during measurements and prevents different light conditions, e.g. inside and outside an incubation chamber, from influencing the measurements.

Furthermore, the holder and the vessel may comprise a stirring mechanism which may be implemented via, e.g., a vibration effect. Vibrations may be simultaneous with the measurements, without disturbing the same.

The light source may be a high power light-emitting diode (LED). The LEDs may be advantageous as they have small size, long operating lifetime, and durability. LEDs emit light over a specific wavelength range, which may be suitable for a cell culture under test.

In one embodiment of the invention, the light emitter may be a laser configured to emit coherent light radiation. The laser may be directed towards the vessel illuminating the liquid sample with cell culture.

The laser may be a continuous wave (CW) laser, or a pulsed laser. A CW laser may emit a monochromatic light which wavelength may be tuned over a wide range. Wavelength to be used may be dependent on the cell culture which is to be analysed. A pulsed laser generates a spectrum of wavelengths which may also be tuned and selected depending on the cell culture.

The holder may be arranged in and incubator which is configured to maintain environmental conditions needed for the cell culture growth. The environmental conditions may include predetermined temperature, predetermined pressure, predetermined gas composition, and

predetermined humidity. Typically, there is a need for keeping both the temperature and pressure high. For instance, it is preferred that the temperature is kept above 37°C and at least 20 psi of gas overpressure. A cell culture to be analysed may be an anaerobic organism and therefore anaerobic conditions may be preferred for the analysis, i.e., the concentration of oxygen may be required to be as low as possible, close to no presence of oxygen at all. Usually, concentration of oxygen and a pressure value together, serve for evaluation of conditions which the cell culture is exposed to. In other cases, the cells to be analysed may need appropriate CO_2 or H_2 concentration.

In a second aspect, the present invention relates to a method for non-invasive and automatic real-time cell culture growth measurements, the method comprising the steps of:

- providing a liquid sample with cell culture into a vessel;
- placing the vessel into a holder, the holder being configured to receive and secure the vessel;
- directly illuminating the liquid sample to a light radiation from a light source;
- detecting the light radiation in real time after the sample exposure, the light radiation comprising information about optical density of the liquid sample;
- generating an electrical signal based on the detected light radiation;
- disregarding the electrical signal if it is taken while the measured pressure and temperature did not correspond to the predetermined values;
- sending the electrical signal to an electronic system;
- determining the cell culture growth by the electronic system by comparing at least two electrical signals generated successively;
- outputting the determined cell culture growth value by the electronic system.

Having an automatic method to measure the cell growth, measurement noise is significantly lower compared to manual measurements leading to measurements with high precision. Both high and low concentrations of the cells disposed in the liquid sample, is possible to identify. Furthermore, since no manual sampling is needed, the cost of measurements is drastically decreased. Additionally, the use of an automatic device makes a workspace safer, since no handling with syringes and needles is required.

The step of disregarding the electrical signal if it is taken while the measured pressure and temperature did not correspond to the predetermined values is to neglect measurements when the holder is, for instance, not in the incubator or when the incubator is open. Furthermore, any ambient light, i.e. light in range between 50 - 60 Hz, may be filtered out by disregarding measurements taken when the sample has been exposed to the ambient light. Additionally, outliers based on temperature may be disregarded. In some cases, an average

of, e.g., 20 measurements may be taken without disregarding any of the measurements taken.

In a yet another embodiment, an increase of an optical density of the cell culture is measured while an amount of cells in the liquid sample increases. The optical density of the cell culture represents the degree to which the culture modifies the light radiation used to illuminate the culture. The increase in optical density is measured in real-time with time intervals that can be set shorter than 1 minute. By having such a short time interval between two measurements, high temporal resolution of the measured growth is achieved.

The method may also comprise a step of providing a calibration means inside the holder, the calibration means providing calibration of the intensity of radiation generated by the light source. Calibration may be performed by determining a calibration number which shows a ratio between the intensities of input light and output light when there are no cells present in the liquid sample. By comparing the calibration number and, for instance, a ratio which has been obtained from two successive measurements, a cell culture growth may be obtained.

The light radiation from a light source may be reflected, transmitted and/or scattered from the vessel. The reflected, transmitted and scattered light may then be detected by three different photo sensors. Naturally, one beam can be reflected and scattered a number of times and therefore a plurality of photo sensors may be used.

The liquid sample may be illuminated from different directions while a position of the vessel in the holder is maintained fixed. Illumination from different directions can be achieved by having multiple light emitters illuminating the vessel.

The illumination from different directions may be simultaneous illumination. The multiple light emitters may illuminate the vessel simultaneously, and the light beams from multiple light emitters may be detected by multiple photo sensors. In this embodiment, the number of the light emitters may be equal to the number of the photo sensors. Finally, each detected signal may contribute to determination of the cell culture growth.

In another embodiment, the illumination from different directions may be successive illumination. The multiple light emitters may not illuminate the vessel at the same time, but rather one after another. In this embodiment, the number of the light emitters may typically be different from the number of the photo sensors involved.

The illumination from different directions may be with the same wave length or with different wavelength, and it may be with the same light intensity or with different light intensity.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in further details with reference to the accompanying drawings, in which:

Fig. 1 illustrates a system for non-invasive and automatic real-time cell cultures growth measurements;

Fig. 2 illustrates the measured growth rate as described in the section "Examples"; and

Fig. 3 illustrates an electronic circuit performing a cell growth measurement;

Fig. 4 illustrates one embodiment of the system with multiple light emitters and photo sensors;

Fig. 5 illustrates another embodiment of the system having multiple light emitters and photo sensors.

DETAILED DISCLOSURE OF THE INVENTION

In its most general aspect, the present invention relates to a system for non-invasive and automatic real-time cell culture growth measurements comprising a vessel for holding a liquid sample with cell culture, an optoelectronic circuit system comprising a light source, and a photodetector, an electronic system, and a holder configured to receive and secure the vessel and to include the photodetector and the light source of the optoelectronic circuit.

Fig. 1 illustrates a system 100 comprising a holder 101 with a vessel 102, a light source 103, a photodetector 104, and a block 105 representing a local system of an electronic system. The holder 101 secures the vessel 102 and includes the light source 103, the photodetector 104, and the local system 105. The vessel 102 holds a liquid sample with cell culture 106. The light source 103 is directed towards the vessel 102 such that the sample is illuminated all times during measurements. After passing through the sample 106, the light beam is directed to the photodetector 104. The photodetector 104 converts an optical signal into a digital one, which is then sent to the local system 105. The local system 105 establishes a wireless connection with a remote system 107 comprising a second system that may be a server configured to calculate the growth of the cell culture. Further, the remote system 107 establishes a connection with a number of portable devices 108 which may include a mobile

phone, personal computer, laptop or the like and which receives information related to the cell culture growth.

Fig. 2 will be described below, in EXAMPLES section.

Fig. 3 illustrates an electronic circuit 300 configured to perform a cell growth measurement. The light source 103 continuously illuminates the sample 106. In the physical line of sight behind the light source and sample, the photodetector 104 is placed. This photodetector generates a current proportional to the illumination, according to the equation:

$$I_{photodiode} = \frac{Luminance_{photodiode}}{Sensitivity_{photodiode}}$$

Using an operational amplifier 301 in a current-to-voltage mode and by wiring the photodetector 104 in a photovoltaic mode, the current generated by the photodetector 104 is converted into a voltage.

$$V_{opamp} = I_{photodiode} \times R_{feedback\ resistor}$$

$R_{feedback\ resistor}$ is indicated 302 in Fig. 3.

The voltage is measured using an analog-to-digital converter (ADC) 303. On the digital end, communication with the ADC 303 is established using a Serial Peripheral Interface (SPI) bus 304. By communicating over the SPI bus 304, a $Data_{ADC}$ number is obtained, which encodes the voltage, and thus the current through the photodetector and further the illuminance of the photodetector, using the following equations below.

$$V_{opamp} = \frac{Data_{ADC}}{2^{24} - 1} \times V_{ref}$$

V_{ref} is 2.5 V.

Finally the optical density (OD) is calculated by a microcontroller (MCU) 305 by comparing the obtained luminance of a sample at a time point $t=x$ with the luminance at a time point $t=0$ when no cellular growth was present.

$$OD = -log \frac{Luminance_{photodiode\ t=x}}{Luminance_{photodiode\ t=0}} \times Calibration\ factor$$

Calibration factor is empirically established prior to a set of experiments.

Fig. 4 illustrates one embodiment of the system comprising a vessel 102 and three pairs of light emitters and photo sensors, 103a and 104a, 103b and 104b, and 103c and 104c. The light emitters 103a, 103b, 103c, are positioned such that they illuminate the vessel 102 from different directions, and each of the light emitters 103a, 103b, 103c illuminate the vessel 102 at different points. Each light emitter 103a, 103b, 103c has a corresponding photo sensors, 104a, 1034, and 104c. Such a configuration is capable of correcting the determined cell growth.

Fig. 5 illustrates another embodiment of the system comprising a vessel 102, three light emitters 103-1, 103-2, and 103-3 and three photo sensors 104-1, 104-2, and 104-3. It sould be noted that the arrangement of emitters 103-1/3 and sensors 104-1/3 is different than in the embodiment illustrated in Fig. 4. Three light emitters 103-1/3 and three photo sensors 104-1/3 are arranged in such a way that each photo sensor 104 is positioned such that it can detect light from each of the light emitters 103. Each light beam generated by the light emitters 103 is transmitted through, scattered and reflected from the vessel 102 such that three beams are generated. These three light beams are then detected by each of the three photo sensors 104-1/3. This arrangement therefore results in $3\times3=9$ measurements. All the recorded measurements are then averaged out to give a final value related to the cell growth. The measurement is performed such that only one light emitter 103 is turned on at the time. At first, light emitter 103-1 is turned on and the photo sensors 104-1/3 simultaneously detect light emitted from the first emitter 103-1 and transmitted through, scattered and reflected from the vessel 102. Subsequently, e.g. 500 ms later, the second emitter 103-2 is turned on and the scattered light is again detected by the photo sensors 104-1/3. This repeats until all the light emitters 103 have been individually activated and the whole cycle then starts again. Number of the emitters and sensors is chosen arbitrary and it can, naturally, be different than three. It also may be that number of emitters and sensors is not the same.

In yet another exemplary embodiment, only scattered light emitted from the emitters can be detected by the detectors.

EXAMPLES

The system illustrated in Fig. 1 was used to measure the growth of the obligate anaerobic bacterium Clostridium acetobutylicum ATCC 824 on 2 different carbon sources without human intervention in real time. Clostridium acetobutylicum was cultured in 10-mL Hungate tubes. A

growth rate of 0.51 h^{-1} on glucose and 0.22 h^{-1} on xylose has been observed. These values are in agreement with the growth rates under comparable conditions described in literature.

Fig. 2a illustrates measurement of the cell growth performed for obligate anaerobic bacterium Clostridium acetobutylicum ATCC 824 cultured on glucose. The X axis shows time in hours while the Y axis shows optical density in a logarithmic scale, i.e., $\log_2$.

Fig. 2b illustrates the same measurements for the same bacterial cells cultured on xylose. As in Fig. 2a, the X axis shows time in hours while the Y axis shows optical density in a logarithmic scale, i.e., $\log_2$.

Fig. 2c illustrates growth rate per hour of the same bacterial cells. It can be seen that the growth rate Z representing hours is significantly higher when Clostridium acetobutylicum is cultured on glucose A than on xylose B.

CLAIMS

1. A system for non-invasive and automatic real-time cells culture growth measurements, the system comprising:
   - a vessel for holding a liquid sample with cell culture;
   - an optoelectronic circuit system comprising a light source configured to emit light radiation onto the liquid sample being held by the vessel, and a photodetector configured to detect the light radiation after the sample has been exposed to the light radiation,
   - an electronic system configured to receive signals generated by the photodetector and determine the cell growth based on the signals; and
   - a holder including the light source and photodetector of the optoelectronic circuit and configured to receive and secure the vessel.

2. A system according to claim 1, wherein the vessel has a circular cross-section.

3. A system according to claim 1 or 2, wherein the holder comprises means for fixing the vessel to prevent rotation of the vessel in the holder.

4. A system according to any of the preceding claims, wherein the light source includes multiple light emitters.

5. A system according to any of the preceding claims, wherein the photodetector includes multiple photo sensors.

6. A system according to claims 4 and 5, wherein the system is configured to correct the determined cell growth based on multiple light emitters and photo sensors.

7. A system according to any of the preceding claims, the system further comprising a pressure sensor configured to measure pressure inside the holder or vessel.

8. A system according to any of the preceding claims, the system further comprising a temperature sensor configured to measure a temperature inside the holder or vessel.

9. A system according to claims 7 or 8 wherein at least one of the pressure sensor and temperature sensor is included in the holder.

10. A system according to any of the preceding claims, wherein the holder is configured to be movable during a measurement.

11. A system according to any of the preceding claims, wherein the electronic system comprises a first system and a second system, the first system being configured to measure optical properties of the liquid sample with cell culture based on data from the optoelectronic system, the data expressing absorption, reflection, and/or scattering of the liquid sample by means of the light source and photodetector, and wherein the second system is configured to receive the optical properties from the first system.

12. A system according to claim 6, wherein the first system is local and the second system is external.

13. A system according to any of the preceding claims, wherein the electronic system is configured to establish a wireless connection with at least one portable device such as mobile phone, cell phone, personal computer, laptop or the like and send the digital data over the wireless connection.

14. A system according to any of the preceding claims, wherein the vessel comprises a syringe access, and where the holder is configured to secure the vessel such that the syringe access is accessible during measurement.

15. A system according to any of the preceding claims, wherein the vessel is made from a material having a refractive index in the range between 1.45 and 1.8.

16. A system according to any of the preceding claims, wherein at least one of the holder and the vessel further comprises a stirring mechanism.

17. A system according to any of the preceding claims 4-16, wherein the light emitter is a laser configured to emit light radiation, the laser being directed towards the vessel and illuminating the liquid sample with cell culture, the laser being configured to emit a monochromatic light continuously, or the laser is configured to emit pulsed light.

18. A system according to any of the preceding claims, wherein the holder is arranged in an incubator, the incubator being configured to maintain environmental conditions, the environmental conditions including predetermined temperature, predetermined pressure, predetermined gas composition, and predetermined humidity.

19. A method for non-invasive and automatic real-time cell culture growth measurements, the method comprising the steps of:
    - providing a liquid sample with cell culture into a vessel;
    - placing the vessel into a holder, the holder being configured to receive and secure the vessel;
    - directly illuminating the liquid sample to a light radiation from a light source;
    - detecting the light radiation in real time after the sample exposure, the light radiation comprising information about optical density of the liquid sample;
    - generating an electrical signal based on the detected light radiation;
    - disregarding the electrical signal if it is taken while the measured pressure and temperature did not correspond to the predetermined values;
    - sending the electrical signal to an electronic system;
    - determining the cell culture growth by the electronic system by comparing at least two electrical signals generated successively;
    - outputting the determined cell culture growth value by the electronic system.

20. A method according to claim 19, wherein an increase of an optical density of the cell culture is measured while an amount of culture cells in the liquid sample increases.

21. A method according to claims 19 or 20, wherein the light radiation from a light source is reflected, transmitted and/or scattered from the vessel.

22. A method according to any of the claims 19-21, wherein the liquid sample is illuminated from different directions while a position of the vessel in the holder is maintained fixed.

23. A method according to claim 22, wherein the illumination from different directions is simultaneous illumination.

24. A method according to claim 22, wherein the illumination from different directions is successive illumination.

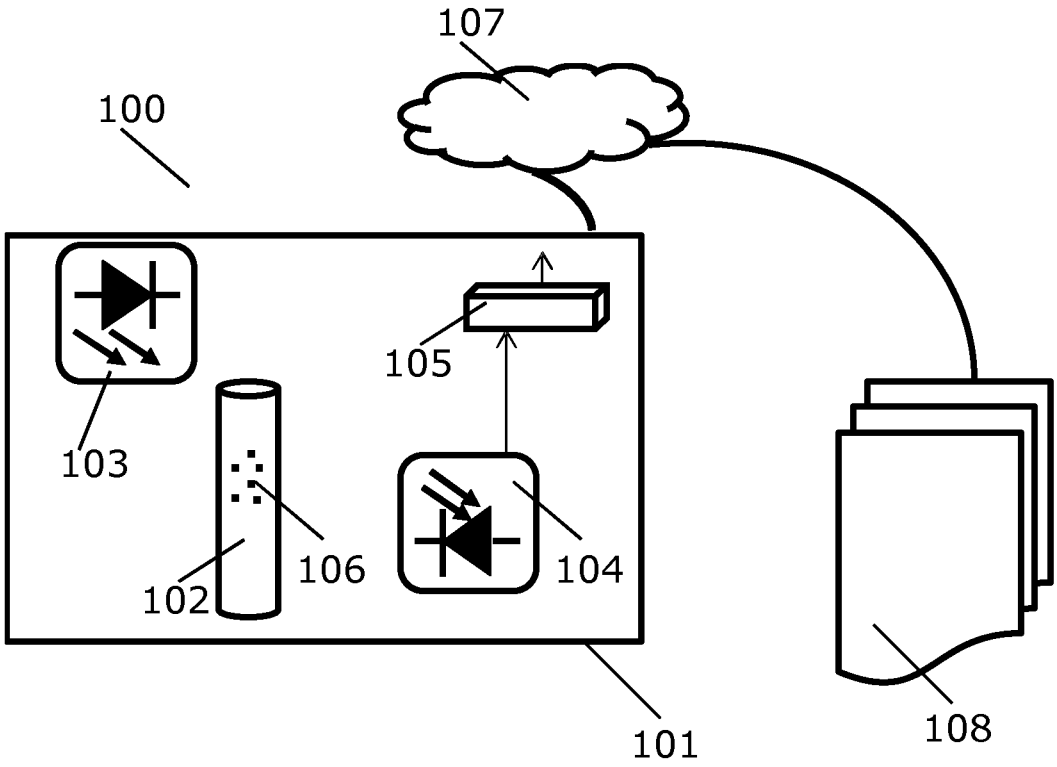
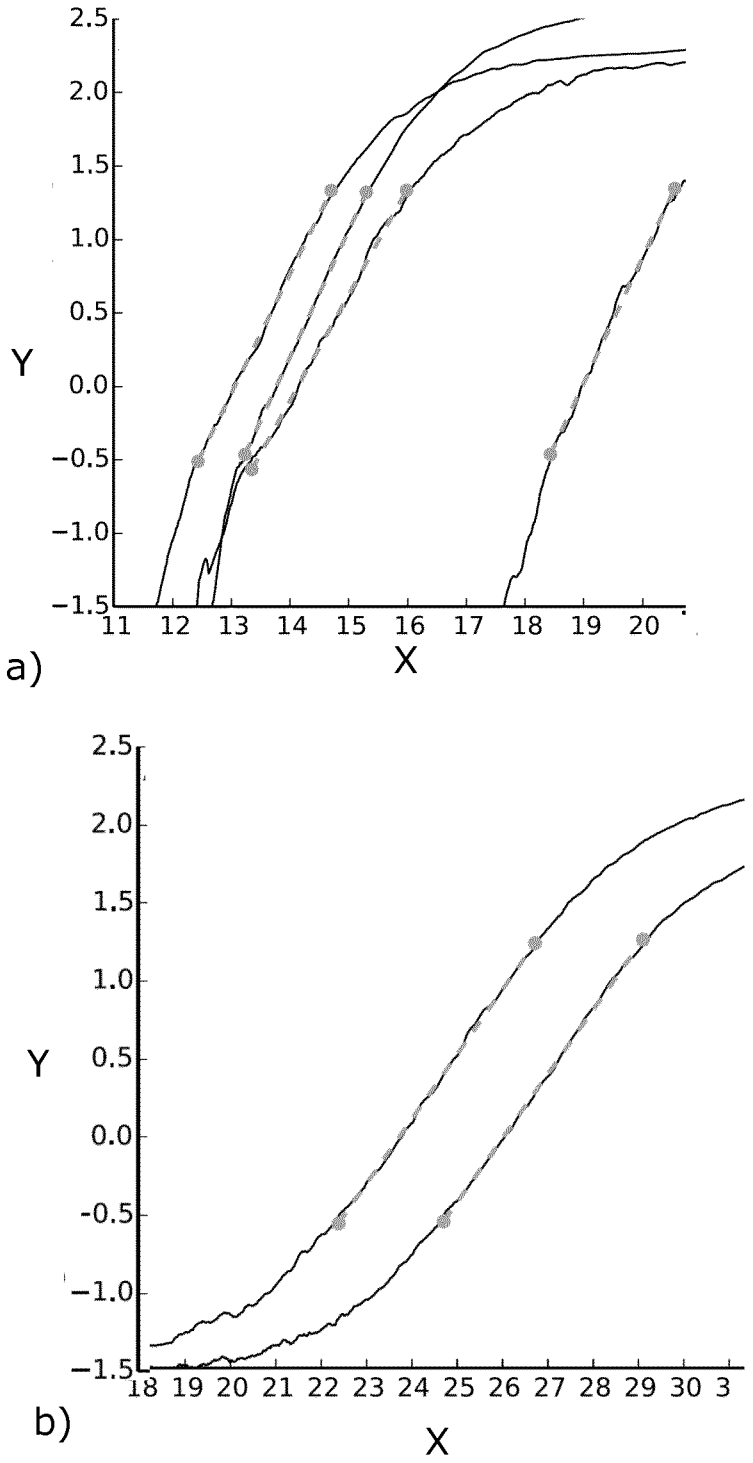


Fig. 1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Y
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X
a)
2.5
2.0
1.5
1.0
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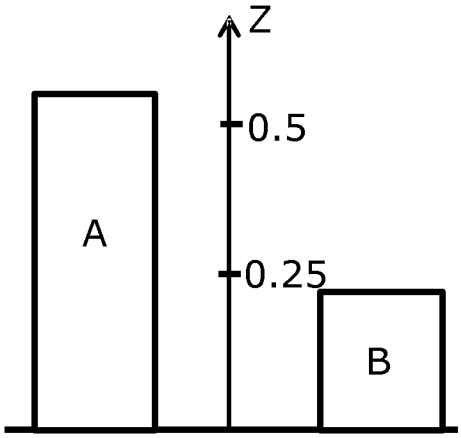
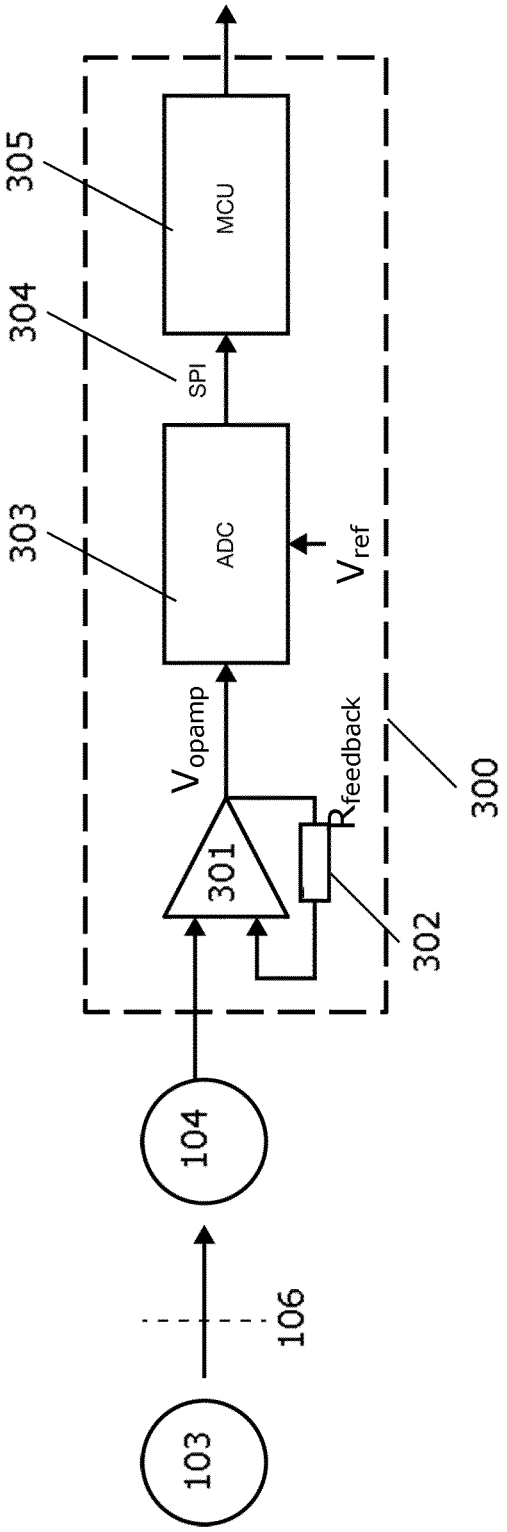
−1.5
Y
18 19 20 21 22 23 24 25 26 27 28 29 30 3
X
b)

Fig. 2



c)

Fig. 2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ADC
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V_{opamp}
$R_{feedback}$
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300
302
104
106
103

Fig. 3

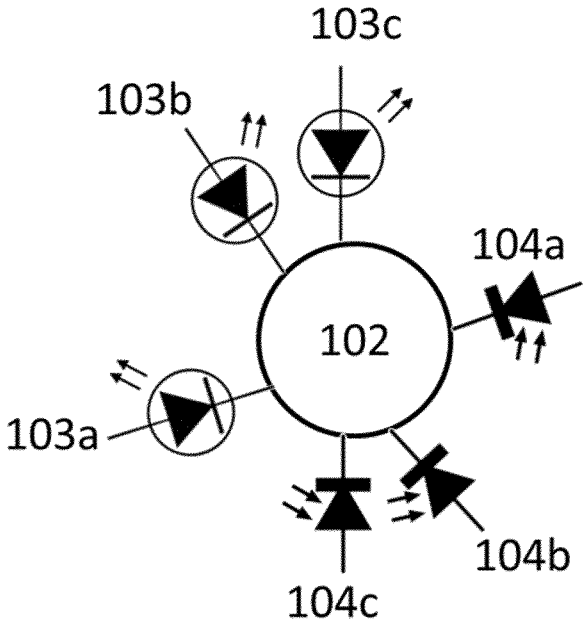

Fig. 4

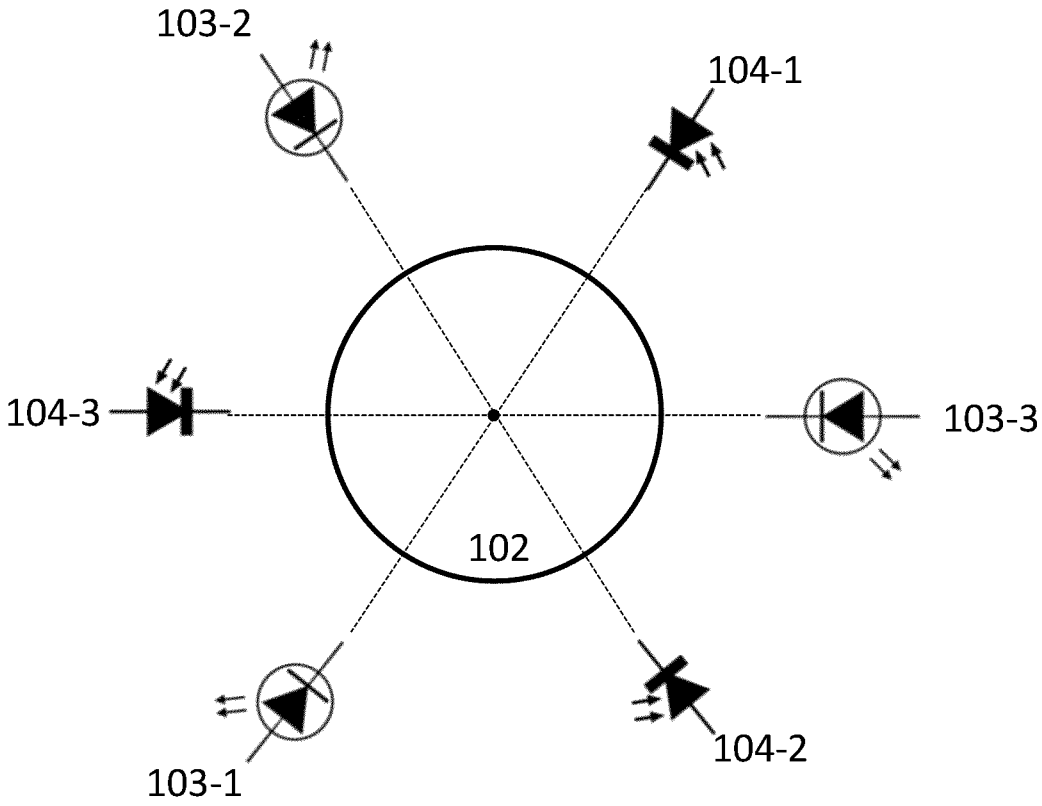

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/080522

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12M1/00 C12M1/34 C12M1/36
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12M

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

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 723 554 B1 (GAILLON LAURENT [FR] ET AL) 20 April 2004 (2004-04-20)	1-3, 10-12, 14,15, 17-20,22
Y	column 1, lines 5-10,25-41 column 2, paragraph 40-57 column 3, paragraph 7-45 column 4, paragraph 41-63 column 13, paragraph 4-30 figure 2	1-24

X	US 2008/293091 A1 (KANIPAYOR RAVI [CA] ET AL) 27 November 2008 (2008-11-27)	1,3, 8-10,15, 18-20,22
Y	figures 1-2 paragraphs [0007], [0008], [0011], [0022], [0024], [0025], [0040]	1-24

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

[X] See patent family annex.

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"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
20 February 2018	01/03/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Böhm, Ingo

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/080522

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/231242 A1 (SARASWAT MACK [NZ] ET AL) 11 August 2016 (2016-08-11)	1-3, 8-13, 16-20,22
Y	paragraphs [0001], [0007], [0008], [0017], [0021], [0057], [0059], [0066] figures 4B,5B	1-24
X	US 2005/254055 A1 (PENG HONG [US]) 17 November 2005 (2005-11-17)	1-3, 8-10,15, 16,19-22
Y	paragraphs [0008] - [0010], [0027]	1-24
Y	US 2014/200840 A1 (COX SHANE [AU] ET AL) 17 July 2014 (2014-07-17) paragraphs [0001], [0010], [0011], [0013] - [0019], [0030], [0044], [0052] figures	1-24
Y	US 5 310 658 A (BERNDT KLAUS W [US]) 10 May 1994 (1994-05-10) column 1, lines 6-6,27-34,63-68 column 2, lines 1-5,14-30,54-60 column 3, lines 7-9,51	1-24
Y	EP 2 371 942 A2 (UNIV MARYLAND [US]; UNIV MARYLAND BIOTECH INST [US]) 5 October 2011 (2011-10-05) paragraphs [0008], [0009], [0018], [0019], [0029], [0041], [0042], [0047], [0048]	1-24
A	US 2013/030715 A1 (TIXIER SEBASTIEN [CA] ET AL) 31 January 2013 (2013-01-31) claims 1-5	1,19
A	MAROSE S ET AL: "Optical sensor systems for bioprocess monitoring", TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 17, no. 1, 31 January 1999 (1999-01-31), pages 30-34, XP004155531, ISSN: 0167-7799, DOI: 10.1016/S0167-7799(98)01247-5 the whole document	19,20

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/080522

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6723554 B1	20-04-2004	AT 258679 T	15-02-2004
		DE 69821388 D1	04-03-2004
		DE 69821388 T2	25-11-2004
		DK 1034422 T3	24-05-2004
		EP 1034422 A1	13-09-2000
		EP 1398619 A2	17-03-2004
		ES 2212376 T3	16-07-2004
		FR 2771508 A1	28-05-1999
		PT 1034422 E	30-06-2004
		US 6723554 B1	20-04-2004
		WO 9927349 A1	03-06-1999
US 2008293091 A1	27-11-2008	US 2008293091 A1	27-11-2008
		WO 2008144899 A1	04-12-2008
US 2016231242 A1	11-08-2016	AU 2014330131 A1	05-05-2016
		CA 2925078 A1	09-04-2015
		CN 105593665 A	18-05-2016
		EP 3058346 A1	24-08-2016
		JP 2016537618 A	01-12-2016
		KR 20160143633 A	14-12-2016
		US 2016231242 A1	11-08-2016
		WO 2015050464 A1	09-04-2015
US 2005254055 A1	17-11-2005	NONE	
US 2014200840 A1	17-07-2014	NONE	
US 5310658 A	10-05-1994	AU 668414 B2	02-05-1996
		BR 9304162 A	07-06-1994
		CA 2107289 A1	10-04-1994
		DE 69319843 D1	27-08-1998
		DE 69319843 T2	11-02-1999
		EP 0597584 A1	18-05-1994
		ES 2119864 T3	16-10-1998
		JP 2604539 B2	30-04-1997
		JP H06197752 A	19-07-1994
		US 5310658 A	10-05-1994
EP 2371942 A2	05-10-2011	AU 8290801 A	25-02-2002
		CA 2419474 A1	21-02-2002
		DE 01961662 T1	03-05-2007
		EP 1309719 A1	14-05-2003
		EP 2371942 A2	05-10-2011
		US 2002025547 A1	28-02-2002
		US 2004121453 A1	24-06-2004
		WO 0214539 A1	21-02-2002
US 2013030715 A1	31-01-2013	CA 2842261 A1	07-02-2013
		CN 103814138 A	21-05-2014
		EP 2737075 A1	04-06-2014
		JP 2014521321 A	28-08-2014
		US 2013030715 A1	31-01-2013
		WO 2013016799 A1	07-02-2013