# Optical controlling of a chemical reaction

van der Zaag, Pieter; Pierik, Anke

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 March 2016 (31.03.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/046408 A1

(51) International Patent Classification:
*C12Q 1/68* (2006.01)

(21) International Application Number:
PCT/EP2015/072184

(22) International Filing Date:
25 September 2015 (25.09.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
14186554.3 26 September 2014 (26.09.2014) EP

(71) Applicant: **KONINKLIJKE PHILIPS N.V.** [NL/NL]; High Tech Campus 5, NL-5656 AE Eindhoven (NL).

(72) Inventors: **VAN DER ZAAG, Pieter Jan**; c/o High Tech Campus 5, NL-5656 AE Eindhoven (NL). **PIERIK, Anke**; c/o High Tech Campus 5, NL-5656 AE Eindhoven (NL).

(74) Agents: **SCHUDELARO, Antonius Adrianus Petrus** et al.; High Tech Campus 5, NL-5656 AE Eindhoven (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17**:

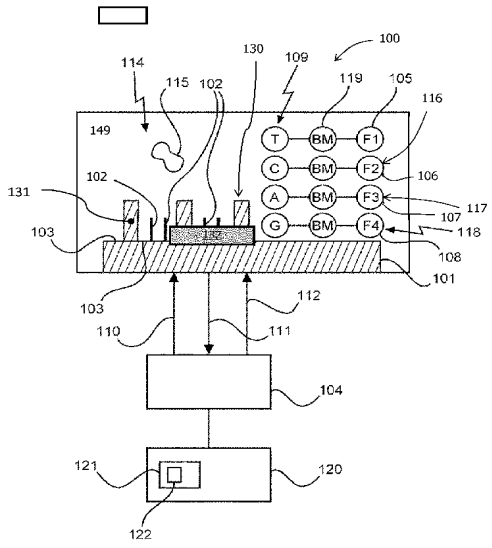
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

**Published**:

— *with international search report (Art. 21(3))*

(54) **Title**: OPTICAL CONTROLLING OF A CHEMICAL REACTION

Fig. 1




(57) **Abstract**: The present invention relates to a device for enabling an optically controlled chemical reaction in a reaction chamber; it comprises a substrate on a removable cartridge for binding at least one molecule on a first surface of the substrate, an optical arrangement configured to direct cleavage light to the substrate to optically induce a photochemical cleavage reaction, and a wiregrid further thermally coupled to a heat conductive element. Such a device can be used for example to carry out an optically controlled chemical reaction, particularly a nucleic sequencing reaction. It can be used to carry out reactions with an increased throughput without overheating the device itself or the reagents used.

WO 2016/046408 A1

# Optical controlling of a chemical reaction

## FIELD OF THE INVENTION

The present invention relates to a device and a method for enabling an optically controlled chemical reaction in a reaction chamber comprising a reagent fluid. In particular, the present invention relates to a device for enabling an optically controlled iterative stepwise reaction to determine a sequence of a nucleic acid, and a method for enabling an optically controlled iterative stepwise reaction to determine a sequence of nucleic acid. The device comprises a removable cartridge comprising a substrate for binding at least one molecule on a first surface of the substrate, and an optical arrangement configured to direct cleavage light to the substrate to optically induce a photochemical cleavage reaction.

## BACKGROUND OF THE INVENTION

WO 2013/105025 A1, which is incorporated into the present text by reference, describes a device and a method for optically controlling the iterative incorporation of fluorescently labeled nucleic acids into a molecule attached to the surface of a wiregrid substrate. Based on a strong optical confinement of excitation light and of cleavage light by evanescent waves, the sequencing reaction can be read-out without washing the surface. Stepwise sequencing is achieved by using nucleotides with optically cleavable blocking moieties. After read-out the built-in nucleotide is deblocked by cleavage light through the same substrate. This ensures that only bound nucleotides will be unblocked.

Such device and method yield the best results at an optimal temperature range. The cleavage light required for this method must be a high intensity UV light. V.A. Litosch et al. in Nucl. Acid Research 36 (6) (2010) e39) estimate that an intensity of up to 1 $W/cm^2$is required, although recent improvements lowered such threshold to about $4mW/cm^2$.

## SUMMARY OF THE INVENTION

It would be advantageous to have a procedure that allows for an optically controlled chemical reaction, particularly a nucleic sequencing reaction, with an increased throughput.

It is therefore an object of the present invention to provide a device and a method according to the opening paragraph, which address such concern.

In a first aspect of the invention the object is achieved by a device for enabling an optically controlled chemical reaction in a reaction chamber comprising a reagent fluid, said device comprising: a removable cartridge comprising a substrate for binding at least one molecule on a first surface of the substrate; an optical arrangement configured to direct cleavage light to the substrate to optically induce a photochemical cleavage reaction; wherein the substrate further comprises a wiregrid, and wherein the wiregrid is further thermally coupled to a heat conductive element.

According to the invention, the reagent fluid may be cooled. Cooling may for example be achieved by connecting the wiregrid to a conductive element having a higher thermal mass than the substrate, through which excess heat can be transferred to a cooling medium (e.g. the surrounding atmosphere). Additionally or alternatively, means for actively cooling the reagent fluid may be provided, too, for example a Peltier element.

By thermal mass it is intended the ability of a body to store thermal energy. Therefore, in the context of the present invention, the higher the thermal mass of an element is, the larger the amount of heat generated by the cleavage light that can be conducted and thus dissipated by such element.

The "reaction chamber" is typically an open cavity, a closed cavity, or a cavity connected to other cavities by fluid connection channels.

According to a second aspect, the object is achieved by a method for optically controlling a chemical reaction in a reaction chamber comprising a reagent fluid, said method comprising the following steps: providing a substrate with a molecule bound on a first surface of the substrate , wherein said first surface is a wall of the reaction chamber; irradiating the substrate with cleavage light of a cleavage wavelength $\lambda_{CL}$, preferably UV light, by an optical arrangement and thereby optically inducing a photochemical cleaving reaction; and thermally coupling the substrate to a heat conductive element

It shall be noted that all embodiments of the present invention concerning a method, might be carried out with the order of the steps as described, nevertheless this has not to be the only and essential order of the steps of the method. All different orders and combinations of the method steps are herewith described.

The described device and method are based on the same basic idea, i.e. to control the temperature of the substrate that is irradiated with cleavage light, so that overheating of the wiregrid substrate and of the buffers containing the reagents caused by

overheating is prevented. Since overheating would reduce the sustainable throughput rate, the invention has a substantial positive effect on the performance of the system, meaning that the reaction can be carried out at a higher throughput rate. Explanations and embodiments described for the device are therefore analogously valid for the method, too, and vice versa.

The device and the method allow for (photo-) chemical reactions taking place with a high throughput at the surface of the substrate. This is because the heat conductive element coupled to the substrate allows the excessive heat which is produced by the irradiation of cleavage light to be carried away. Hence high intensities of cleavage light can be applied without damaging material at the surface, which enables higher reaction rates.

On the other hand, it might be necessary to increase the temperature of the substrate, should this be lowered below the temperature required for an optimal reaction inside the reaction chamber; should this be the case, the heat conductive element allows the substrate to be heated by an external heat source.

In the following various preferred embodiments will be described in more detail that can be realized both in combination with the device as well as with the method (even if they are only explained for one of the device and the method). Synergistic effects may arise from different combinations of the embodiments although they might not be described in detail.

In one embodiment, the heat conductive element is capable of actively cooling and/or heating the substrate. An advantage of this embodiment is that the reaction temperature can be maintained within a close range to the optimal temperature.
In the context of the present invention, the reaction temperature is the temperature of the fluid while the reaction takes place; the reaction temperature is raised by the cleavage light, which heats up both the substrate and the reaction fluid.

In an embodiment of the device according to the first aspect, the heat conductive element is further connected to a device for controlling the temperature. An advantage of this embodiment is that the reaction temperature can be more finely controlled by such device. The reaction temperature may for example be controlled in a feedback loop based on a sensed temperature (e.g. of the reagent fluid or of the substrate) such that the temperature at the reaction surface is always kept at an optimal level.

It was already mentioned that the coupling of the wiregrid to a heat conductive element can prevent overheating of the volume at the surface of the substrate. Accordingly, comparatively higher intensities of the cleavage light can be applied. In a preferred

embodiment, intensity of the cleavage light is larger than about 0.1 $mW/cm^2$, larger than about 0.5 $mW/cm^2$, larger than about 1 $mW/cm^2$, or larger than about 5 $mW/cm^2$.

In the following, further embodiments of the invention will be explained.

## BRIEF DESCRIPTION OF THE DRAWINGS

Exemplary embodiments of the invention will be described in the following drawings.

Fig. 1 is a schematic cross section of a device according to a first exemplary embodiment of the invention.

Fig. 2 is a schematic cross section of a device according to a second exemplary embodiment of the invention.

Fig. 3 is a schematic top view of a close-up of the wiregrid and the heat conductive element comprised in the device according to an exemplary embodiment of the invention.

Fig. 4 is a schematic top view of a close-up of the wiregrid and the heat conductive element comprised in the device according to another exemplary embodiment of the invention.

## DETAILED DESCRIPTION OF EMBODIMENTS

In the context of the present invention, the term "blocking moiety" is to be understood as a moiety which blocks a synthesizing activity of an enzyme in the case where the blocking moiety is incorporated into a molecule at which the enzyme performs a synthesizing process. A blocking moiety may be e.g. a blocking molecule.

In the context of the present invention, the term "cleavable" should be understood as allowing to be cleaved away by absorbing cleavage light of wavelength $\lambda_{CL}$.

In the context of the present invention it should be understood, that every embodiment of the optical arrangement disclosed herein may be configured to emit polarized excitation light and polarized cleavage light. Thus, a polarizer or already polarized light sources may be used. Details will be described later on.

Furthermore, the term "excitation light" in the context of the present invention applies to the wavelength $\lambda_{Ex1}$, $\lambda_{Ex2}$, $\lambda_{Ex3}$ and $\lambda_{Ex4}$, respectively.

According to an exemplary embodiment of the invention, a device of the kind defined above for optically controlling a nucleic acid sequence is presented. In particular, the device is configured to optically control an iterative stepwise reaction to determine a sequence of a nucleic acid by synthesis. Alternatively, instead of sequencing by synthesis, a

synthesis by ligation is also to be understood in the scope of the present invention. The presented device comprises a substrate for binding at least one molecule on a first surface of the substrate. The device further comprises an optical arrangement which is configured to direct excitation light of at least a first excitation wavelength $\lambda_{Ex1}$ to the substrate to excite a fluorescent label of a first nucleotide which is incorporated into the molecule that is bound on the first surface of the substrate. The optical arrangement is further configured to receive and detect fluorescent light emitted by the fluorescent label of the first nucleotide which is incorporated into the bound molecule. The bound molecule might be a nucleic acid fragment and can be understood as the nucleic acid whose sequence of nucleotides is determined by the present invention and which can be DNA fragment, DNA, RNA, mRNA or any other nucleic acid.

Furthermore, the optical arrangement is configured to direct cleavage light of a cleavage wavelength $\lambda_{CL}$, preferably UV light, to the substrate to optically induce a photochemical cleavage reaction at the first incorporated nucleotide to cleave a blocking moiety and the fluorescent label away from the first incorporated nucleotide. Furthermore, the substrate is configured to confine the excitation light and is configured to provide thus for an evanescent wave of the excitation light at the first surface of the substrate. Furthermore, the substrate is configured to confine the cleavage light, preferably UV light, and is further configured to provide for an evanescent wave of cleavage light at the first surface of the substrate. The device allows for ensemble based easy read out but no or a reduced number of washing steps are required any more, meaning a single reagent filling for all reads.

Stepwise sequencing is achieved by using nucleotides with optically cleavable blocking groups. After read-out, the built-in nucleotide is unblocked by cleavage light like for example UV radiation through the same nano-photonic substrate. This ensures that only bound nucleotides will be unblocked.

As it will be explained in detail in the following, the optical arrangement may also be configured to direct to the substrate excitation light of a first, and a second, and a third and a fourth excitation wavelength $\lambda_{Ex1}$, $\lambda_{Ex2}$, $\lambda_{Ex3}$ and $\lambda_{Ex4}$, each capable to excite a different fluorescent label, wherein each different fluorescent label is bound to a specific nucleotides, for example Adenine (A) and Guanine (G) and Thymine (T) or Uracil (U) and Cytosine (C).

In other words, the wavelengths are chosen such that the used fluorescent labels can be optically excited by the respected excitation light.

In addition, the substrate provides for spots which may be covered with clones of identical molecules, in order to increase the optical signal, which is received by detecting the fluorescence. Therefore, a substrate may be provided as an array of such spots with respectively different clones, such that throughput of sequencing is increased.

The evanescent wave of cleavage light and the evanescent wave of excitation light can be generated by the substrate of the presented device by means of a wiregrid. This may allow for using a focused beam of high intensity such that the photo-optical reaction occurs at a high rate in a very limited area very close to the surface.

Furthermore the substrate may be out of a polymer e.g. poly-(cyclo-)olefin, poly-carbonate, polyester or PMMA. Also metal and semiconductors may be used.

According to another exemplary embodiment of the invention, the device further comprises the molecule which is bound to the first surface of the substrate. The device further comprises a solution ("reagent fluid") with a plurality of nucleotides and an enzyme. Therein, the nucleotides respectively comprise the blocking moiety. The blocking moiety is configured to block a synthesizing activity of the enzyme when the respective moiety is incorporated into the molecule bound to the first surface of the device.

As exemplary embodiments, the blocking moieties may be embodied as 3'-blocked reversible terminator or as 3'-unblocked reversible terminator as described and defined in *"Sequencing technologies, the next generation" by Michael L. Metzker, Nature Review Genetics 11 (2010) 31-46.*

The wiregrid may comprise a pattern of metal wires on, for example, a glass substrate. The spacing between the wires acts as a metal-clad slab waveguide, in which the major contribution comes to two fundamental modes. For example, a wiregrid may comprise aluminium wires which reflect excitation light with polarization parallel to the wires (TE polarization) and which transmit polarization orthogonal to the wires (TM polarization). For TE polarized excitation light incident on the wiregrid the resulting mode in between the wires is the evanescent mode, For example, with a wire height of 60 nanometres the TM polarized mode is transmitted with a loss of light in the order of 10% or less, while the TE polarized mode is evanescently decaying. The maximum transmission of TM polarized light may be higher than 95%. The evanescent field in the case of incident TE excitation light is depicted in both Figs. 3a and 3b of WO 2013/105025 A1. The excitation light and the cleavage light irradiated by the optical arrangement of the present invention may be of TE polarization in this and every other embodiment of the present invention. Although aluminium has been mentioned as the metal constituting the wiregrid, it is to be understood that any other material

suitable for making a wiregrid is comprised within the scope of the present application, for example gold. Moreover, the wiregrid may also be coated by other material or substances in order to confer additional features to it, for example to make it more resistant to corrosion or to increase its hydrophobicity/hydrophilicity.

The use of the wiregrid has the additional advantage of being largely independent on the angle in incidence. Therefore, it can be used in combination with focussed beams to achieve a high intensity locally while keeping the rest in the dark. In other words, the wiregrid allows to excite and be sensitive to only those molecules, for example DNA fragments, that are very close to the surface in the evanescent field and thus no detection or effect on any label nucleotide outside the evanescent field is caused. For example, the evanescent field may elongate about 20 nanometres from the first surface of the substrate. This may be the case for both the excitation light and the cleavage light.

The term "excitation light" in the context of the present invention applies to the wavelength $\lambda_{Ex1}$, $\lambda_{Ex2}$, $\lambda_{Ex3}$ and $\lambda_{Ex4}$, respectively. Consequently, for all four excitation wavelengths the substrate ensures that confinement and a creation of an evanescent wave of the respective light are generated. If desired, more or less light sources and/or fluorescent labels can be used without departing from the present invention.

Working principle of the enzyme and the blocking moiety has been already described above. That disclosure applies within the herein described exemplary embodiment. The presented embodiments allow for synchronizing incorporation of the next nucleotides and ensure that the detected fluorescent signal is highly reliable.

The fact that the cleavage light is in an evanescent mode with respect to the substrate provides for the advantage that a repeated exposure does not lead to fluorescent labels in the solution which are bleached and which lose their function. In other words, the presented embodiment avoids such a bleaching and function-losing of fluorescent labels in solution.

Fig. 1 depicts an embodiment of a device 100 for optically controlling a chemical reaction according to the invention, in this case particularly an iterative stepwise reaction to determine a sequence of a nucleic acid by synthesis. The device comprises a substrate 101 for binding at least one molecule 102 on the first surface 103 of the substrate. The molecule 102 which is bound on the first or front surface 103 of the substrate 101 can for example be a fragment of a DNA. The first surface 103 constitutes a wall or border of a reaction chamber 149 in which a fluid to be processed (here a reagent fluid 114 that is

described in more detail below) can be accommodated. The reaction chamber is typically a part of a larger (micro) fluidic device or cartridge that is not shown in more detail.

Furthermore, the optical arrangement 104 is shown in Fig. 1. Fig. 1 schematically shows that the optical arrangement is configured to direct excitation light 110 of for example the first excitation wavelength $\lambda_{Ex1}$ to the substrate. Furthermore, four different nucleotides are schematically shown and are depicted with reference signs 109, 116, 117 and 118. For example, a first nucleotide 109 is shown as Thymine, T. The nucleotide 109 comprises a blocking moiety 119. Furthermore, the blocking moiety 119 comprises the first fluorescent label 105. In an analog way, second nucleotide 116 is schematically depicted in Fig. 1, from which can be gathered that also a blocking moiety 119 and the second fluorescent label 106 is comprised. The third nucleotide 117 comprises also a blocking moiety and a third fluorescent label 107. Additionally, the fourth nucleotide 118 is schematically depicted which comprises also a blocking moiety and a fourth fluorescent label 108. However, solution 114 may comprise a much larger plurality of such nucleotides, and nucleotides 109, 116, 117 and 118 are shown here merely as a symbolic depiction.

Furthermore, Fig. 1 shows a solution 114 which fills the reaction chamber 149 and in which the nucleotides and the enzyme 115 are comprised. In case one of the shown four nucleotides is incorporated in the bound molecule 102, the presented device 100 provides for the following advantages. The optical arrangement is configured to receive and detect fluorescence light emitted by the fluorescent label of the first nucleotide incorporated into the bound molecule 102.

As can further be gathered from Fig. 1, the optical arrangement is configured to direct cleavage light 112 of cleavage wavelength $\lambda_{CL}$ to the substrate. This allows for optically inducing a photochemical cleavage reaction at the first incorporated nucleotide to cleave the respective fluorescence label from the first incorporated nucleotide. Furthermore, the substrate 101 is configured to confine excitation light such that an evanescent wave of the excitation light at the first surface of the substrate is created. Moreover, the substrate is configured to confine also the cleavage light such that an evanescent wave of the cleavage light at the first surface of the substrate is created.

In the embodiment of Fig. 1, the substrate 101 is configured as a wiregrid 130 for the excitation light 110 and for the cleavage light 112. Therefore, the wiregrid 130 comprises a regular pattern, like for example a regular structure made of a plurality of metal wires 131. As can be gathered from Fig. 1, slit-like openings are provided between the wires

131, in which openings the bound molecules 102 are immobilized at the first surface 103 of the substrate 101.

Furthermore, Fig. 1 depicts a processing unit 120 which comprises a computer-readable medium 121 on which a computer program element 122 is stored. Said program element 122 is adapted to instruct the processing unit 120 to further instruct the device 100 to perform the above and below described method for optically controlling an iterative stepwise reaction to determine a sequence of a nucleic acid by synthesis. The device 100 of Fig. 1 is configured to stepwise and optically induce the incorporation of nucleotides 109, 116, 117, 119 with a sequence, which corresponds to the sequence of nucleotides of the bound molecule 102. In case the molecule 102 is a DNA fragment, the nucleotides comprised by the solution 114 are incorporated into molecule 102 in a sequence that corresponds to the nucleotide sequence of molecule 102.

The device is further configured to base the determination of the sequence of the incorporated nucleotides on the received and detected response fluorescence light emitted by the fluorescent label of the respective incorporated nucleotide. Therefore, the presented device 100 of Fig. 1 firstly ensures that only nucleotides are read-out by the excitation light 110, which nucleotides are incorporated into a bound molecule 102 by the use of an evanescent wave of the excitation light. Secondly, the device 100 of Fig. 1 ensures that only bound nucleotides will be unblocked by the cleavage light which avoids a bleaching and loss of function of nucleotides that are not yet contained i.e. incorporated by the molecule 102. Consequently, the detected fluorescence signal 100 may be seen as the light 111, is highly reliable for the determination of the sequence of the nucleic acids.

Consequently, the cost and speed of the nucleic acid sequencing, like for example the DNA sequencing performed with the device 100 of Fig. 1 are both improved. Less reagents and enzymes are necessary as no washing step is needed. The device of Fig. 1 shows a simplification and cost reduction of sequencing. The presented device 100 of Fig. 1 allows for a new process combination by allowing an assemble-based easy read-out without any washing step, meaning a single reagent filling for all reads. The blocking moieties used within the exemplary nucleotides 109, 116, 117, 118 may for example be a photo-cleavable 3'-unblocked reversible terminator. However, also other blocking moieties, using for example steric hindering, may be used to reach the desired and above described effects.

Furthermore, the optical arrangement 104 as shown in Fig. 1 may be configured to provide the irradiated cleavage light with an intensity such that the cleaving reaction time $t_{cleavage}$ is smaller than the time it takes to incorporate the second nucleotide into

the molecule 102. As the cleaving reaction time $t_{cleavage}$ depends on the intensity of the irradiated cleavage light, Fig. 1 may provide for a selected combination of nucleotides with a specific blocking moiety and a configuration of the optical arrangement regarding the intensity of the cleavage light. In other words, the intensity of the cleavage light of the device of Fig.1 is adapted such that for the used combination of nucleotides and blocking moieties the cleaving reaction time $t_{cleavage}$ is smaller than $t_{incorporation}$.

If desired, additionally or alternatively, the following set-up of device 100 may be provided to the user. If the reagent fluid is stationary and movement of molecules driven by diffusion, then the residence may be seen as an average residence time in the spot of cleavage light of a non-incorporated nucleotide. An optical arrangement may further be configured to provide the irradiated cleavage light with an intensity such that $t_{cleavage}$ is smaller than $t_{residence}$. Consequently, no degradation of free and unbound nucleotides due to an undesired cleavage reaction happens. Thus, by configuring the device such that $t_{cleavage}$ is smaller than $t_{residence}$ the probability that a non-incorporated nucleotide is affected by cleaving is reduced or eliminated. In other words, to avoid cleavage reactions in the bulk the average residence time of the molecules in the evanescent field of the wiregrid should be smaller or much smaller than the reaction time required for cleavage at the pertinent intensity. With a depth of the evanescent field of the order of 25 nm or less and a diffusion coefficient of the nucleotide of the order of $1x10^{-10}$ $m^2/s$ the time it takes for the molecule to diffuse in and out the evanescent field can be estimated as: $(5x10^{-8}\ m)^2 / 1x10^{-10} = 25\ \mu s$. Depending on the illumination time required for unblocking the bound molecules the probability of damage can be derived. Assume an illumination time of 0.1 s this would be 1:4000, with an illumination time of 10 ms it would be 1:400, etc.

Likewise the total damage is proportional to the volume fraction in the evanescent field over the total volume of reagent solution. With a chamber height of 100 μm the ratio is 1:4000. This means that in the worst case of damaging all molecules in the evanescent field only 0.025% of the molecules will be damaged. With a read length of 100 finally 2.5 % of the molecules in solution would be damaged (worst case) which is still acceptable from a sequencing point of view.

In the following, information for using the device of Fig. 1 (and Fig. 2) is provided.

For an improved synchronization the unblocking step should be carried out as fast as possible, i.e. with the highest intensity possible. This can be achieved by focusing the cleavage light, preferably the UV light with a lens and scanning the surface by moving the

lens or the substrate. The unblocking step is carried out after reading the sequencing step. This reading can be carried out by scanning a focused beam or step-and-scan with field illumination. It may also be possible to embody cleavage light as a single flash of, for example, UV light for the total surface. In view of the reaction rate for the base incorporation for the sequencing reaction, the local cleavage light illumination time should be, for example, below 1 minute. In a preferred embodiment the read scanning can be coupled to the unblocking scanning by integrating both light beams in a single actuator, possibly even in a single lens by aligning the light beams. Alternatively, two lenses can be integrated in a single stage or two separate stages can operate synchronously. This can also be implemented in the step and scan read approach, in which the UV-step is also carried out in a step and scan mode by illuminating the same field as the reader. The preferred embodiment will depend on the available UV light source and its power. One can also envision a single flash of UV for the total surface if enough power is available and/or the area of the sequencing surface is limited. In view of the reaction rate for the base incorporation for the sequencing reaction the local UV illumination time should be well below 1 minute.

A single fluid sequencing using a wiregrid as well as single molecule sequencing has been described above. The approaches may use so called 3'-unblocked reversible terminators in which a flash of UV light is needed to de-block the nucleotide so that the next labeled nucleotide with a fluorophore attached can be incorporated by the polymerase. Reading out the color of the incorporated nucleotide allows the base incorporated to be determined and hence sequencing to be done.

In the described procedures high intensity UV light is needed. Typical intensity values range from about 4 $mW/cm^2$ to about 1 $W/cm^2$. This corresponds to a considerable amount of energy that may cause heating in the wiregrid and the buffers containing the reagents.

In order to improve the system performance and avoid overheating of the wiregrid 130 and of the solution 114 and even of the substrate 101, it is proposed to thermally couple the wiregrid to a heat conductive element, for example a metallic element with a high thermal capacity; this will produce a cooling effect by dispersing the heat away from the reaction chamber and helping to avoid local overheating.

The aforementioned proposal is realized in the device 100 of Fig. 1 by a heat conductive element 132. In the illustrated embodiment, wiregrid 130 is coupled to at least one heat conductive element 132, wherein said heat conductive element 132 is in physical contact with at least one of the metal wires 131, more preferably with a plurality or even all

wires 131. Although heat conductive element 132 is depicted in Fig. 1 as being in contact with the lower portion of two wires 131, it is understood that heat conductive element 132 can also contact wiregrid 130 in any other position, for example on the top of it, and it can be in contact with any number of wires. Heat conductive element 132 can be a single metallic bar, but it can also comprise two or more metallic and/or non-metallic elements attached to different sides of wiregrid 130, and it can have any shape.

Heat conductive element 132 acts as a heat sink, attracting the heat away from the wires 131, thanks to the high thermal capacity, in turn lowering the temperature of the wiregrid 130 and hence of the solution 114 and of the reaction chamber 149. Molecules 102 are always surrounded by the chemicals they need while excess heat, particularly heat generated by cleavage light, is carried away from the surface to avoid overheating.

Accordingly a sequencing system is provided in which sequencing is done using a wiregrid connected to a heat sink to avoid overheating the system while de-blocking using UV light.

Fig. 2 shows a device 200 which is configured to optically control an iterative stepwise reaction to determine a sequence of a nucleic acid by synthesis. Similar to Fig. 1, a substrate 201 is shown on which a plurality of molecules 202 are immobilized, i.e. are bound. As can be seen from Fig. 2, the metallic wires 231 of a wiregrid 230 similar to that of Fig. 1 provides for slit-like openings 215 in which the molecules 202 are bound on the first surface 203. The substrate comprises several adjacent binding positions 209, 210, 211 and 212 for binding molecules to the first surface along a first direction 213. Said binding positions may be seen as spots which can be covered with clones of identical molecules, such that the optical signal, which is generated, can be increased. The substrate 201 then provides for an array of such spots, i.e. of such binding positions, with respectively different clones. This may enhance the throughput. Both devices 100 and 200 of Figs. 1 and 2 allow a nucleic acid sequencing with only one liquid, thereby avoiding the need to provide for washing steps in which the solution liquid is changed.

Furthermore, the optical arrangement 204 comprises five different light sources 201 to 205. The light sources 201 to 204 may be seen as excitation light sources in order to provide for four different excitation wavelength $\lambda_{Ex1}$ to $\lambda_{Ex4}$ as described previously. The light source 205 provides for cleavage light with a wavelength $\lambda_{CL}$. For example, the light source 205 may emit UV light. Reference numeral 206 symbolically depicts a switching device which allows the optical arrangement 204 to switch between the five wavelengths $\lambda_{Ex1}$ to $\lambda_{Ex4}$ and $\lambda_{CL}$. Furthermore, the light emitted by at least one of said light sources 201 to 205

is directed towards the polarization filter 200. Furthermore, a dichroic mirror 207 is shown which transmits the emitted light of the light sources 201 to 205 towards the substrate 201. After a fluorescent label has been excited by an evanescent wave of excitation light (at least one of the wavelengths $\lambda_{Ex1}$ to $\lambda_{Ex4}$), the fluorescence photons emitted by the fluorescent label or labels are directed towards the dichroic mirror 207 and are directed towards fluorescence detector 208. As can be seen from Fig. 2, the optical arrangement 204 may be scanned along the direction 213. Consequently, the device 200 of Fig. 2 is configured to perform an optical scan by moving the substrate 201 and the optical arrangement 204 relative to each other along the first direction 213. Consequently, the device allows to perform the optical scan such that each binding position is firstly irradiated with the excitation light and subsequently and secondly is irradiated the cleavage light of the cleavage wavelength in a movement along the first direction 213. The unblocking step, using the cleavage light, can thus be carried out after reading the fluorescence of the excited incorporated nucleotides.

Fig. 2 further shows a heat conductive element 232 as described above with respect to Fig. 1 which allows for a dispersion of excess heat generated by the UV light.

Fig. 3 schematically shows a top view close-up of substrate 301, wherein wiregrid 330 is coupled to a heat conductive element 332. Element 332 comprises in turn a first bar 332a and a second bar 332b, attached parallel to each other to opposite sides of wires 331. In this figure, each of the metal wires 331 is in contact with both bar 332a and bar 332b, so that the heat sink function of heat conductive element 332 is doubled. However, it is to be understood that heat conductive element 332 can have any shape or size, and it can be coupled to wiregrid 330 in any position and with any configuration which are possible to imagine.

In another embodiment, the heat conductive element is capable of actively controlling the temperature of the wiregrid.

Fig. 4 schematically shows a top view of a close-up of substrate 401 according to the latter embodiment. In this Figure 4 substrate 401 is shown as comprised within reaction chamber 449, containing reagent fluid 414, similarly to the previous embodiments. A temperature detector 437 measures the temperature of the reaction chamber 449. Alternatively or additionally, temperature detector 437 can also measure the temperature of reagent fluid 414, and/or of substrate 401. Temperature detector 437 is coupled to processing unit 438, which in turn is connected to temperature control unit 436. Processing unit 438 can correspond to processing unit 120 of Fig. 1, but it can also be an independent processing unit, or it can be integrated in temperature control unit (436). Bars 432a and 432b are each

connected to temperature control unit 436, such as a Peltier element. According to this embodiment, it is possible not only to disperse the excess heat generated by the UV light, but to maintain a constant temperature in the wiregrid 430, and hence in the reaction chamber 449. It is possible to set processing unit 438 so that it maintain a predetermined temperature; based on the temperature detected and communicated to the processing unit 438 by temperature detector 437, processing unit 438 can control temperature control unit 436 so that it will either heat or cool substrate 401 through bars 432a and 432b until the temperature in the reaction chamber 449 corresponds to the desired temperature. It is thus possible to set temperature control unit 436 so that the sequencing reaction can be optimized and made more efficient; for example, the temperature of the reaction chamber 449 can be set at different amount depending on the specific protocol used.

In one embodiment, heat conductive element 132 can be part of the removable cartridge that also comprises substrate 101 and wiregrid 130. According to this embodiment, device 100 is designed in a way that when said cartridge is inserted into device 100, heat conductive element 132 is in physical contact with the other features it interact with, for example temperature control unit (436).

Alternatively, in another embodiment, heat conductive element 132 is integrated into device 100. According to this embodiment, device 100 is designed so that heat conductive element 132 will be in physical contact with wiregrid 130, once the removable cartridge is inserted into device 100.

Further details and other exemplary devices and methods may be found in the WO 2013/105025 A1 which is entirely incorporated into the present text by reference.

While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive; the invention is not limited to the disclosed embodiments. Other variations to the disclosed embodiments can be understood and effected by those skilled in the art in practicing the claimed invention, from a study of the drawings, the disclosure, and the appended claims. In the claims, the word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. A single processor or other unit may fulfill the functions of several items recited in the claims. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage. A computer program may be stored/distributed on a suitable medium, such as an optical storage medium or a solid-state medium supplied together with or as part of other hardware, but may also be

distributed in other forms, such as via the Internet or other wired or wireless telecommunication systems. Any reference signs in the claims should not be construed as limiting the scope.

CLAIMS:

1. A device (100) for enabling an optically controlled chemical reaction in a reaction chamber (149) comprising a reagent fluid (114), said device comprising:
- a removable cartridge comprising a substrate (101) for binding at least one molecule (102) on a first surface (103) of the substrate;
- an optical arrangement (104) configured to direct cleavage light (112) to the substrate to optically induce a photochemical cleavage reaction;
wherein the substrate further comprises a wiregrid (130),
and wherein the wiregrid (130) is further thermally coupled to a heat conductive element (132).

2. The device (100) according to claim 1,
wherein the heat conductive element (132) is capable of actively cooling and/or heating the substrate

3. The device (100) according to claim 1,
wherein the heat conductive element (132) is further connected to a temperature control unit (436)

4. The device (100) according to claim 1,
wherein the heat conductive element (132) is part of the cartridge

5. The device (100) according to claim 1,
wherein the device further comprises a temperature detector (437) for detecting the temperature of the reaction chamber (449)/reagent fluid (414)and wherein the temperature control unit (436) is arranged to regulate the temperature within the reaction chamber (449)/reagent fluid (414) in dependence on the detected temperature.

6. The device (100) according to claim 1,

wherein the optical arrangement is configured to direct excitation light (110) of at least a first excitation wavelength $\lambda_{Ex1}$ to the substrate to excite a fluorescent label (105, 106, 107, 108) of a first nucleotide (109, 116, 117, 118) incorporated into the molecule (102) bound on the first surface (103) of the substrate,

wherein the optical arrangement is further configured to receive and detect fluorescent light (111) emitted by the fluorescent label of the first nucleotide incorporated into the bound molecule (102).

7. The device (100) according to claim 1 or 2,

wherein the substrate (101) is configured to confine the excitation light (110) and is configured to provide for an evanescent wave of the excitation light at the first surface (103) of the substrate, and/or

wherein the substrate is configured to confine the cleavage light (112) and is configured to provide for an evanescent wave of cleavage light at the first surface of the substrate.

8. The device (100) according to claim 1,

wherein the heat conductive element (132) is coupled to at least one wire of wiregrid (130)

9. The device (100) according to claim 1,

wherein the heat conductive element (132) is a metallic rod.

10. A method for optically controlling a chemical reaction in a reaction chamber (149) comprising a reagent fluid (114), said method comprising the following steps:

- providing a substrate (101) with a molecule (102) bound on a first surface (103) of the substrate , wherein said first surface is a wall of the reaction chamber (149),
- irradiating the substrate with cleavage light (112) of a cleavage wavelength $\lambda_{CL}$, preferably UV light, by an optical arrangement and thereby optically inducing a photochemical cleaving reaction,
- thermally coupling the substrate to a heat conductive element

11. The method according to claim 9, further comprising the steps:

- irradiating the substrate (101) with excitation light (110) of at least a first excitation wavelength $\lambda_{Ex1}$ by an optical arrangement and thereby optically exciting a

fluorescent label of a first nucleotide which is incorporated in the bound molecule on the substrate,

- confining the excitation light by the substrate thereby providing for an evanescent wave of the excitation light by the substrate at the first surface of the substrate,
- receiving and detecting fluorescence of the excited fluorescent label of the first incorporated nucleotide by the optical arrangement,
- irradiating the substrate with the cleavage light and thereby optically inducing a photochemical cleaving reaction at the first incorporated nucleotide, and
- confining the cleavage light of the cleavage wavelength $\lambda_{CL}$ by the substrate thereby providing for an evanescent wave of the cleavage light by the substrate at the first surface of the substrate.

12. The method according to claim 9,
wherein the substrate is actively cooled and/or heated by means of the heat conductive element (132)

13. The method according to claim 9,
wherein the heat conductive element (132) is further connected to a temperature control unit (436)

14. The method according to claim 9,
wherein the temperature of reaction chamber (449) is determined by temperature detector (437), and wherein said temperature of reaction chamber (449) is controlled by temperature control unit (436) in dependence on the determined temperature.

15. The method according to claim 9,
wherein the used heat conductive element (132) is part of the cartridge

Fig. 1

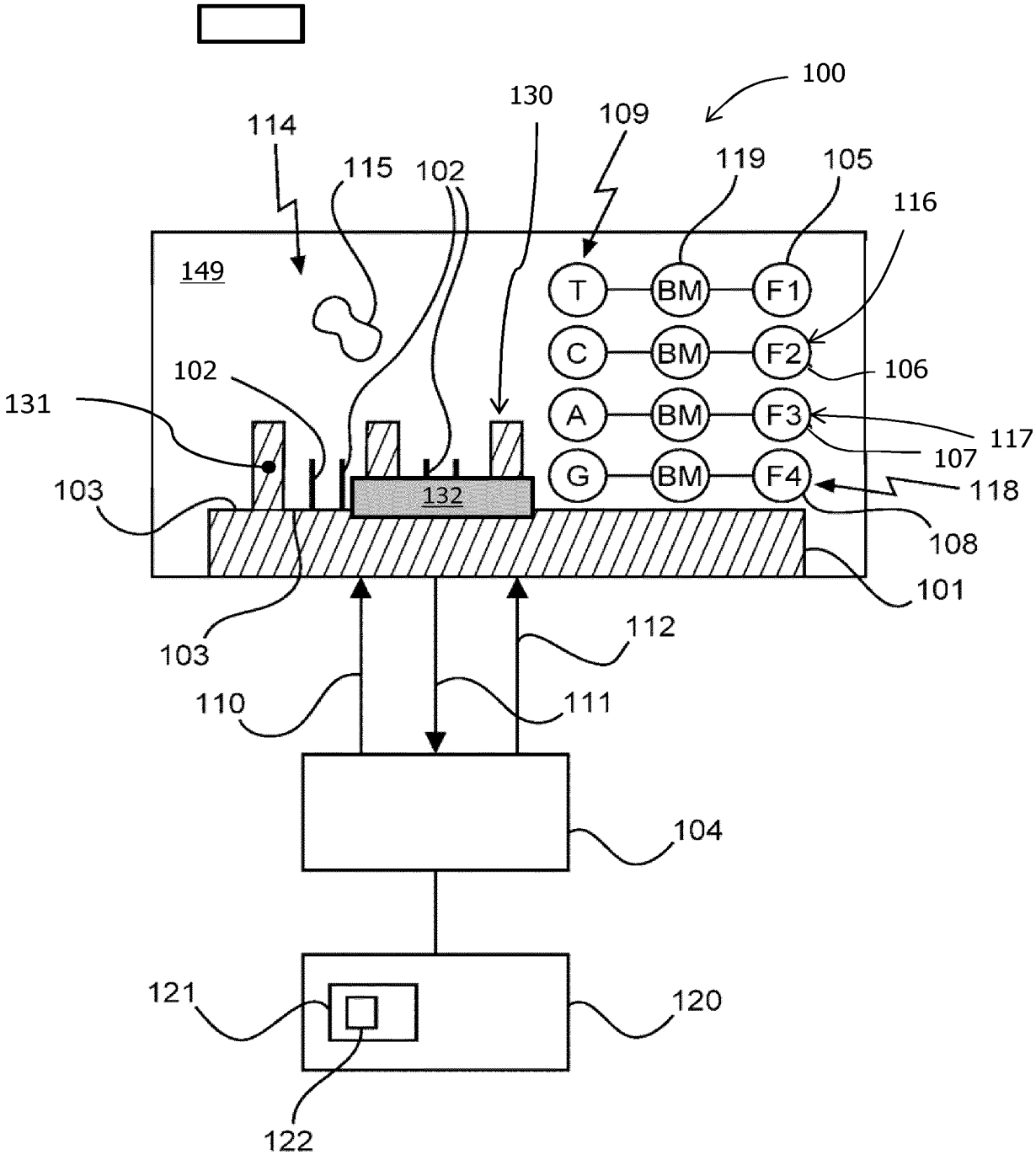

100
130
109
114
115
102
119
105
116
149
T
BM
F1
C
BM
F2
106
102
131
A
BM
F3
117
107
G
BM
F4
118
103
132
108
101
103
112
110
111
104
121
120
122

Fig. 2

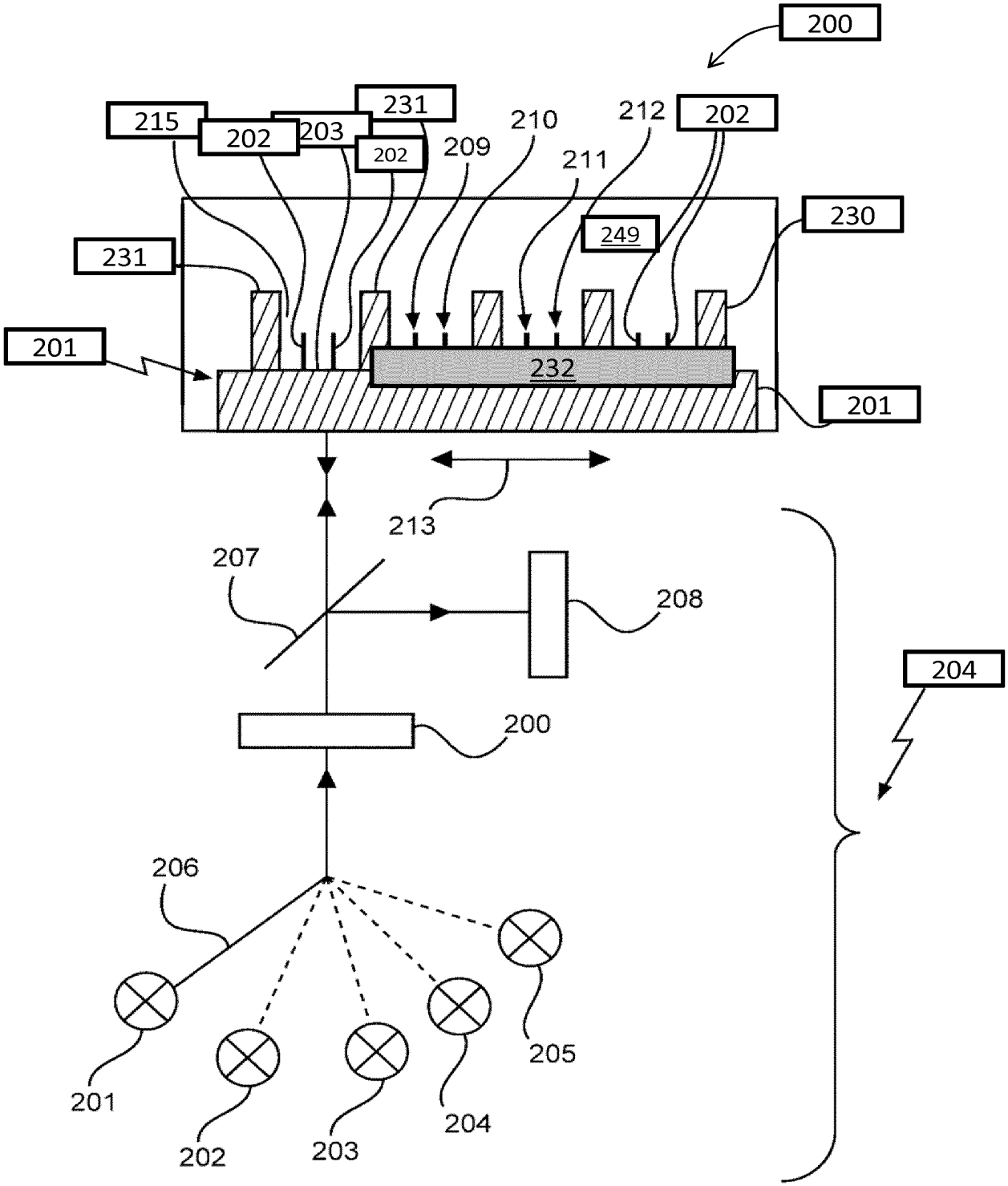

200
231
215
202
203
202
209
210
212
202
211
230
249
231
201
232
201
213
207
208
204
200
206
201
202
203
204
205

Fig. 2

Fig. 3

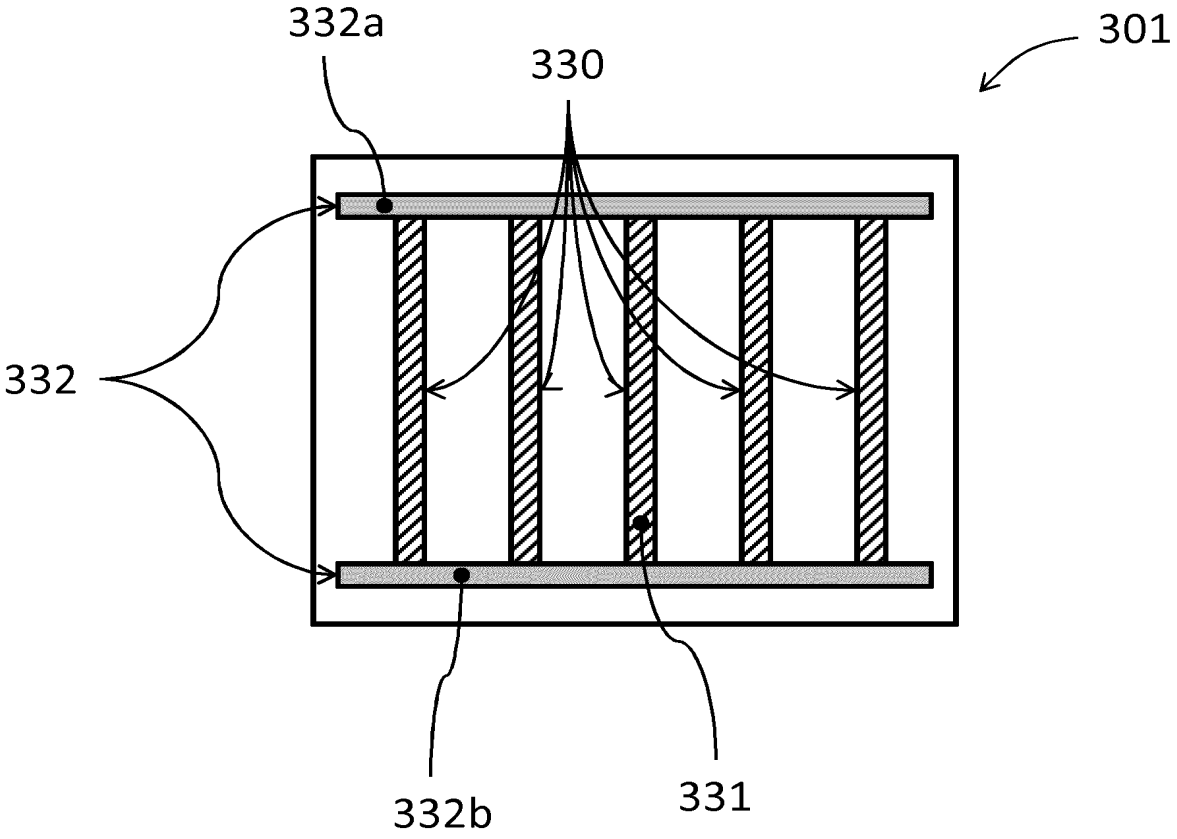
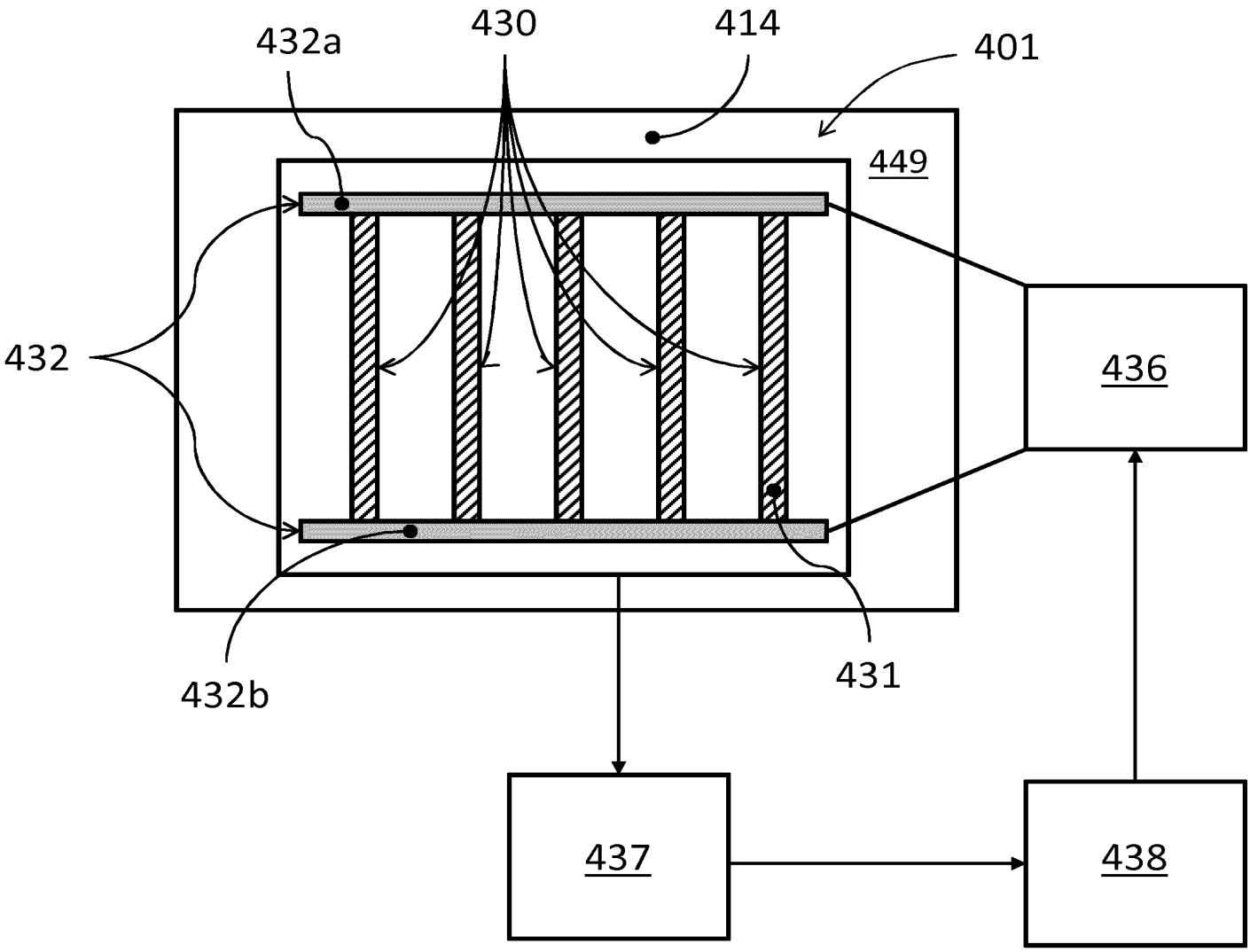


Fig. 4



# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/072184

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
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)
C12Q

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, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>Y | US 2009/312188 A1 (DUER REUVEN [US] ET AL) 17 December 2009 (2009-12-17)<br>abstract; figure 3d<br>paragraphs [0093], [0094]<br>claims 1,2 | 10-15<br>1-15 |
| Y | WO 2013/105025 A1 (KONINKL PHILIPS NV [NL]) 18 July 2013 (2013-07-18)<br>cited in the application<br>abstract; claims 1, 9; figure 1 | 1-15 |
| A | WO 2006/105360 A1 (APPLERA CORP [US]; SUN HONGYE [US]; FUNG STEVEN [US]; WOO SAM LEE [US]) 5 October 2006 (2006-10-05)<br>abstract; claims 16,20; figure 6 | 1-15 |

-/--

[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 application or patent 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 |
|---|---|
| 23 November 2015 | 01/12/2015 |

| 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,<br>Fax: (+31-70) 340-3016 | Barz, Wolfgang |

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | CARL W FULLER ET AL: "The challenges of sequencing by synthesis", NATURE BIOTECHNOLOGY, vol. 27, no. 11, 1 November 2009 (2009-11-01), pages 1013-1023, XP055013694, ISSN: 1087-0156, DOI: 10.1038/nbt.1585 the whole document ----- | 1-15 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/072184

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 2009312188 A1 | 17-12-2009 | AU 2009260321 A1 | 23-12-2009 |
| | | CA 2727158 A1 | 23-12-2009 |
| | | CN 102124128 A | 13-07-2011 |
| | | EP 2297356 A1 | 23-03-2011 |
| | | GB 2461026 A | 23-12-2009 |
| | | JP 2011525111 A | 15-09-2011 |
| | | KR 20110036581 A | 07-04-2011 |
| | | US 2009312188 A1 | 17-12-2009 |
| | | WO 2009155181 A1 | 23-12-2009 |
| WO 2013105025 A1 | 18-07-2013 | CN 104053498 A | 17-09-2014 |
| | | EP 2802411 A1 | 19-11-2014 |
| | | JP 2015503357 A | 02-02-2015 |
| | | US 2015079585 A1 | 19-03-2015 |
| | | WO 2013105025 A1 | 18-07-2013 |
| WO 2006105360 A1 | 05-10-2006 | CN 101184853 A | 21-05-2008 |
| | | EP 1902143 A1 | 26-03-2008 |
| | | JP 2008534018 A | 28-08-2008 |
| | | US 2007238186 A1 | 11-10-2007 |
| | | US 2009226927 A1 | 10-09-2009 |
| | | US 2012244537 A1 | 27-09-2012 |
| | | US 2014193820 A1 | 10-07-2014 |
| | | WO 2006105360 A1 | 05-10-2006 |