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

(43) International Publication Date
2 May 2008 (02.05.2008)

(10) International Publication Number
WO 2008/049795 A1

(51) International Patent Classification:
- B01J 19/00 (2006.01)
- C04B 60/04 (2006.01)
- C04B 60/14 (2006.01)

(21) International Application Number:
PCT/EP2007/061235

(22) International Filing Date: 19 October 2007 (19.10.2007)

(57) Abstract: The invention pertains generally to the field of biology and particularly to techniques and apparatus for the synthesis and analysis of biopolymers using micro-arrays. The present invention provides improved apparatus and methods in defining the active array locations for each nucleotide-adding operation, enhancing accuracy, flexibility, speed and performance. The apparatus comprises a process chamber, a light source (e.g., laser), a scanning device and at least one sensor unit for detecting the light received. The sensor unit functions as a reference position and may be used for calibrating the light beam. The information for the sensor units is used to compensate for offset drift, gain drift or loss of laser power. In addition, the present invention relates to the synthesis of biopolymers, e.g., DNA-sequences, peptides, carbohydrates and lipids, carried out using a patterning process on an activated or chemically inert surface of a substrate. Non-grid patterns are claimed as a non-trivial extension of laser based pattern generation in arrays. Substrates comprising at least one channel and at least one biopolymer at a spatially selected region, which extends over the sides of the channel, are also claimed.

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(54) Title: METHOD AND SYSTEM FOR CALIBRATING LASER FOCUS AND POSITION IN MICRO-ARRAYS

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WO 2008/049795 A1
Method and system for calibrating laser focus and position in micro-arrays

FIELD OF THE INVENTION

This invention pertains generally to the field of biology and particularly to techniques and apparatus for the synthesis and analysis of biopolymers using micro-arrays.

Specifically, the present invention provides improved apparatus and methods in defining the active array locations for each nucleotide-adding operation, enhancing accuracy, flexibility, speed and performance.

In addition, the present invention relates to the synthesis of biopolymers, e.g. DNA-sequences, peptides, carbohydrates and lipids, carried out using a patterning process on an activated or chemically inert surface of a substrate. Non-grid patterns are claimed as a non-trivial extension of laser based pattern generation in arrays.

BACKGROUND OF THE INVENTION

The analysis of biopolymers is a fundamental tool in modern biology and is conventionally carried out by processes based on analysis of individual molecules. Most methods do not allow extensive testing of many molecules at the same time.

In order to address this problem, arrays have been synthesized, enabling the interrogation of a multitude of ligands or probes at the same time. For instance, an array containing probes can be used to search for complementary sequences on a target strand of DNA, with detection of the target that has hybridized to particular probes accomplished by the use of fluorescent or other markers coupled to the targets and inspection by an appropriate fluorescence scanning microscope.

For the production of arrays, several alternatives have been proposed.

One such alternative, utilizing an array of oligonucleotide probes synthesized by photolithographic techniques is described in Pease, et al., "Light-Generated Oligonucleotide Arrays for Rapid DNA Sequence Analysis," Proc. Natl. Acad. Sci. USA, Vol. 91, pp. 5022-5026, May 1994. In this approach, the surface of a solid support modified with photolabile protecting
groups is illuminated through a photolithographic mask, yielding reactive hydroxyl groups in the illuminated regions. Next, a fluid containing an appropriate activated deoxynucleoside base, is provided to the substrate to couple the base to the activated sites. The selective photo-de-protection and coupling cycle is repeated to build up levels of bases until the desired set of probes is obtained. By controlling the sequencing of activation patterns projected on the micro-array in conjunction with a DNA synthesizer providing the reagents to the substrate, the sequence of the oligonucleotide probe at each site in the array is known. A disadvantage of the lithographic mask approach is that four different lithographic masks are needed for each monomeric base, and the total number of different masks required is thus four times the length of the DNA probe sequences to be synthesized.

In a different alternative, oligonucleotide micro-arrays are made by depositing small droplets containing oligonucleotides directly on glass substrate using inkjet printing technology. The number of different DNA probes in the array is limited by the number of fluid containers in the system.

In-situ DNA synthesis techniques construct micro-arrays containing an arrangement of many different DNA probes, by creating the probes from a limited set of oligonucleotide building blocks. Various techniques use direct deposition of the oligonucleotide building blocks on to the substrate, e.g. the same inkjet technology that can also used for deposition of complete oligonucleotides.

In the so-called maskless DNA micro-array approach described in WO99/42813, the projection pattern is made without a mask, but by a digital micro-array mirror device.

In WO 2003/072240 and WO 2004/065635, a substrate provided with a layer of DNA synthesis linkers, with the link sites and protected by a photolabile group, is prepared to support the DNA probes to be fabricated. To activate the substrate in specific places to couple with the first level of nucleoside bases, a pattern of spots is projected using a laser beam and scanning optics, yielding reactive hydroxyl groups in the illuminated regions. Next, a fluid containing an appropriate activated base, is provided to the substrate to couple at the activated sites. The selective photo-de-protection and coupling cycle is repeated to build up levels of bases until the desired set of probes is obtained.
In all methods, it is necessary to control with very high precision the position where the photolabile protecting groups are illuminated or the building blocks are deposited. However, the laser can have difficulties to target the exact same position to create a homogenous spot of oligonucleotides, in particular when repeating the process various times. Specifically, the laser may be subject to offset drift (the movement of the complete grid) and gain drift (expansion or contraction of the grid). Also, imperfections in the lenses, tolerance build up in all the optical components and non-linearities in the galvano mirror control signals create distortions in the field.

Accordingly, there is a need for an improved method and apparatus that provides accuracy, flexibility and speed/performance in defining the active array locations for each nucleotide-adding operation. Specifically, a means to accurately calibrate the system is required.

SUMMARY OF THE INVENTION

In accordance with the present invention, a light beam is calibrated between each cycle of synthesizing biopolymers, e.g. oligonucleotides. Calibration is performed by at least one sensor unit in a fixed position relative to the substrate. This sensor unit functions as a reference position measured at the onset of the synthesis of an array. Preferably, three sensor units are used, detecting the beam profile and correcting for a disposition relative to the reference position(s) measured at the onset of the synthesis of a array, now covering both the X- and the Y-axis. Information from the sensor units is used to compensate for offset drift and gain drift, and can also be used to compensate for loss of power of the laser.

In general terms, the apparatus comprises one or more sensor units which are placed around a target. A transmitter unit (light source) directs at least one light beam via a scanning device towards the target, repeatedly scanning the light beam over the sensor units and the target area. Via the control module, a controller process determines the position of the beam from the intensity and/or position of the light measured at sensor unit and information about the designated beam direction.

It is an object of the present invention to provide an apparatus for use in synthesis of an array of light-sensitive compounds for the synthesis of biopolymers, comprising
(a) a process chamber comprising a substrate with an active or chemically inert surface on which the array(s) may be formed;
(b) a light source; and
(c) a scanning device having position coordinates for directing the light from the light source to a selected position on the substrate; characterized in that the process chamber and/or the substrate comprises at least one sensor unit or at least one sensor unit alongside the process chamber for detecting when the light is received in or alongside the process chamber and/or at the substrate.

It is a further object of the present invention to provide a method of synthesizing arrays of oligonucleotides comprising the steps of:

(a) providing an apparatus according to the invention, wherein said substrate comprises active sites for DNA synthesis;

(b) directing the light from the light source to a selected position on the substrate by the scanning device, e.g. through projection optics, to illuminate those positions in the array on the substrate which are to be activated to de-protect reactive sites thereon to make them available for binding to bases;

(c) providing a fluid containing an appropriate base to the substrate and binding the selected base to the illuminated positions;

(d) providing new position coordinates to the scanning device to select new selected positions on the substrate to illuminate those positions in the array on the substrate which are to be activated to de-protect OH groups thereon to make them available for binding to bases; and

(e) repeating steps (c) through (d).

It is a further object of the present invention to provide a method of synthesizing arrays of peptides comprising the steps of:

(a) providing an apparatus according to the invention, wherein said substrate comprises active sites for peptide synthesis;

(b) directing the light from the light source to a selected position on the substrate by the scanning device, e.g. through projection optics, to illuminate those positions in the array on the substrate which are to be activated to de-protect reactive sites thereon to make them available for binding to amino acids;

(c) providing a fluid containing an appropriate amino acid to the substrate and binding the selected amino acid to the illuminated positions;

(d) providing new position coordinates to the scanning device to select new selected positions on the substrate to illuminate those positions in the array on the substrate which are to be activated to de-protect OH groups thereon to make them available for binding to amino acid; and
(e) repeating steps (c) through (d).

Preferably, the methods as described above comprises the steps of:
(i) directing the light to selected positions at the substrate and/or the process chamber;
(ii) detecting the light received at a sensor unit, wherein a location of the detected light produces light position coordinates;
(iii) forming scanning device position data versus light position data by creating associations between light position coordinates and scanning device position coordinates corresponding to the position of the scanning device when the light is detected;
(iv) determining light position coordinates for a selected position of the light; and
(v) calculating selected scanning device position coordinates that correspond with the selected position based on the scanning device position versus light position data.

In addition, it is an object of the present invention to provide an array comprising biopolymers bound to selected positions, wherein said biopolymers are synthesized by the method according to the invention, wherein said biopolymers form patterns.

BRIEF DESCRIPTION OF THE FIGURES
Figure 1 depicts schematically the apparatus according to the invention, comprising (1) process chamber; (2) substrate; (3) sensor unit; (4) light source; (5) scanning device; (6) light beam, and (20) control module.

Figure 2 provides examples of possible patterns synthesized according to the methods of the invention.

Figure 3: The effect of calibration. Figure 3A: probes synthesized on micro-array with calibration; Figure 3B: probes synthesized on micro-array without calibration.

DETAILED DESCRIPTION OF THE INVENTION
As used herein, the singular forms "a", "an", and "the" include both singular and plural referents unless the context clearly dictates otherwise. By way of example, "a sensor unit" refers to one or more than one sensor units.

The disclosures of all patents and publications (including published patent applications) referenced in this specification are specifically incorporated herein by reference in their entirety.
to the same extent as if each such individual patent and publication were specifically and individually indicated to be incorporated by reference.

With reference to the drawings, an exemplary apparatus that may be used for synthesis of biopolymers from light-sensitive compounds is shown generally in Figure 1, and includes a process chamber (1) comprising a substrate (2) with an active surface on which the array(s) may be formed; a light source (4); and a scanning device (5) having position coordinates for directing the light (6) from the light source (4) to a selected position on the substrate; wherein the process chamber (1) comprises three sensor units (3) for detecting when the light is received in the process chamber.

In particular, the present invention relates to an apparatus for use in synthesis of an array of biopolymers, comprising
(a) a process chamber comprising a substrate with an active surface on which the array(s) may be formed;
(b) a light source; and
(c) a scanning device having position coordinates for directing the light from the light source to a selected position on the substrate;
characterized in that the process chamber and/or the substrate comprises at least one sensor unit or at least one sensor unit alongside the process chamber for detecting when the light is received in or alongside the process chamber and/or at the substrate.

Opto-mechanical System: light source
The light source may be an ultraviolet or near ultraviolet source, such as a mercury arc lamp, an optional filter to receive the output beam (6) from the light source (4) and selectively pass only the desired wave lengths (e.g. between 370-375 nm) and a condenser lens for forming a collimated beam.

In a preferred embodiment, the light source is a laser beam, more preferably an UV laser (e.g. from Coherent). Preferably, the laser is focused. Even more preferably, the laser beam has a wavelength between 365 - 380 nm and more preferably between 370 - 375 nm, such as 372 nm. The person skilled in the art will appreciate that the UV wavelength may be selected as a compromise between maximum absorption of the photo labile protection group, e.g. NPOOC, and avoiding damage to the biological material that has been synthesized in previous cycles.
The main requirement being that the wavelength of light used in the imaging process be long
enough not to excite transitions (chemical changes) in the nucleotide bases (which are particularly sensitive at 280 nm). Hence, wavelengths longer than 300 nm should be used. The term ultraviolet (UV) laser beam as used in this specification means lasers that emit in the UV at wavelengths preferable above 300 nm. 365 nm is the 1-line of mercury, which is the one used most commonly in wafer lithography. Examples of lasers that emit in this region are Cadmium lasers (325 nm), Nitrogen lasers (337 nm), and Xenon Fluoride lasers (352 nm). Additionally, wavelengths longer than 500 nm have to be avoided as they can cause heating of the components in the optical path.

Any type of laser emitting the above mentioned wavelengths may be used, such as for instance Q-switched (pulsed) lasers or Continuous Wave (CW) lasers.

The beam profile that hits the array aims to have steep slopes - on the flanks - and the constant intensity across a large image plane with some margin on the exact location of the focal plane (depth of field). Accordingly, the apparatus preferably comprises a telecentric F-Theta lens (e.g. from Sill optics) to create a straight focal plane instead of a curved one like regular lenses. The telecentric function of the lens ensures that the laser beam is projected under 90 degrees on the target surface. The telecentric function also helps to keep the spots round. In addition, the apparatus preferably comprises a beam expander (e.g. from Sill optics), which enlarges the laser beam to an optimal diameter for the scanhead. The beam expander may further help ensuring that the laser beam remains a parallel beam. In a still more preferred embodiment, the apparatus preferably comprises an aperture, which in combination with the beam expander and F-Theta lens, may help creating sharp round spots with high contrasts. The aperture helps in shaping the beam and cutting off side lobes in the beam.

Other devices for filtering or monochromating the source light, e.g., diffraction gratings, dichroic mirrors, and prisms, may also be used rather than a transmission filter, and are generically referred to as "filters" herein.

Sensor units

As mentioned above, the light beam can have difficulty to light the exact same position to create a homogenous spot of biopolymers. Therefore, the position of the light beam, e.g. laser position, has to be calibrated between each projection of the array. Calibration can be performed by using the sensor units of the invention.
The sensor units detect the beam profile and correct for a disposition relative to a reference position measured at the onset of the synthesis of the array to cover both the X- and the Y-axis. Information from the sensors is used to compensate for offset drift and/or gain drift by adjusting the scanning device (5). The sensors are located in or alongside the process chamber, in a fixed position. Nevertheless, the sensor units may also be located on the substrate, also at a fixed position. A single sensor unit enables measurement and calibration of the beam intensity, focus length and zero position offset. Multiple sensor units placed around the target enable the measurement and calibration of beam direction. The apparatus comprises at least one, but preferably more than one, e.g. 2, 3 or more sensor units. It will be appreciated that more than one sensor unit increases the accuracy of determining the drift. The said sensor unit is preferably a photosensor, preferably chosen from the group consisting of photoresistors, photovoltaic cells and photodiodes. The sensor unit for detecting the light is preferably an array of photosensors, each photosensor having a plurality of pixels, and the position determining means comprises: means for calculating an intensity weighted centroid of pixels in the photosensors illuminated by the light and using the intensity weighted centroid as the scanning device position coordinates when forming the scanning device position versus light position data. As such, functionally the sensors are part of the scanning device.

The sensor unit is used for calibrating the position of the light beam. Accordingly, the present invention relates to an apparatus as described herein, wherein said sensor unit for detecting the light produces light position coordinates when the light is received. In an even more preferred embodiment, apparatus according to the invention comprises data forming means for forming scanning device position data versus light position data by creating associations between light position coordinates and scanning device position coordinates corresponding to the position of the light when the light is detected at the at least one sensor unit.

Accordingly, the present invention relates to an apparatus as defined herein, in which the light position coordinates are defined by the intensity weighted centroid of pixels at the photosensor.

In a further embodiment, the present invention relates to an apparatus as defined herein, in which the light position coordinates are defined by an incremental intensity increase measures by the photosensor at the flanks of the laser beam.
Scanning device

The scanning device (5) according to the present invention receives position coordinates from a control module (20) for directing the light (6) from the light source (4) to a selected position on the substrate (2).

Preferably, the scanning device comprises at least one motor-driven mirror, the mirror may be mounted on a rotatable spool arranged between poles of a device generating a magnetic field, and that the scanning device has an adjustable source of electricity connected with the spool for controlling the orientation of the mirror. This has been shown to provide a fast and accurately working scanning device of relatively little complexity, that in addition can be realised at low costs, and that can be widely used in the above mentioned field. For example, the apparatus according to the invention makes it possible to simply carry out a flow synthesis in a number of consecutive synthesis steps, with the area to be illuminated being moved on slightly for each subsequent synthesis step. Preferably, the scanning device comprises a galvano-mirror or any other means to actively change the direction of the light, e.g. laser, beam to change the position of the laser beam on the substrate.

The apparatus, i.e. the scanning device, may further comprise a scanhead (e.g. from Scan Labs), comprising a set of two galvanometer (motorized) mirrors in a fixed mount under 90 degrees that together aims a bundle to project the beam onto a programmable position on the target surface. The mirror surfaces may be covered with wavelength specific (di)-electric coating to ensure very high reflectivity. The motor windings are preferably kept at constant temperature. The mirrors are directed by the control module (20).

Alternative formats

The person skilled in the art will appreciate that the invention can be used in many different formats without departing from the crux of the invention. For instance, the light beam is held in a fixed position, while the process chamber can be repositioned, or the light beam can be re-located along the x-direction, and the process chamber can be re-directed along the y-direction.

Accordingly, in an alternative embodiment, the light beam (6) is held in a fixed position, and the process chamber (1) comprising the substrate (2) receives position coordinates from a control module (20), to direct a selected position of the substrate under the focus of the light beam.
Typically, the process chamber comprising the substrate is moved by a precision motion stage with a resolution on the order of 1 μm. The control module (20) is responsible for moving the process chamber, possibly mounted on a carrier, in x-direction and y-direction. The control module commands the drivers.

In a further embodiment, the present invention relates to a scanning device in which the light beam can be moved along the x-axis, while the substrate can be moved along the y-axis.

In an even further embodiment, the process chamber is mounted on a rotational carrier, i.e. the carrier comprising the process chamber can rotate along a center. In general, a spindle motor may drive the rotational motion of the carrier. Revolving may be at a variable speed. The scanning device may be mounted on a movable sled, e.g. in x-direction and/or y-direction, mounted above the process chamber.

Hence, the present invention relates to an apparatus as defined herein, wherein said scanning device comprises displacement of the process chamber on an X-Y stage as a means to change the position of the light, e.g. laser, beam on the substrate.

In a further embodiment, the present invention relates to an apparatus as defined herein, wherein said scanning device comprises displacement of the process chamber in a rotational movement as a means to change the position of the light, e.g. laser, beam on the substrate.

In a preferred embodiment, the present invention relates to an apparatus as defined herein, wherein said scanning device comprises displacement of the process chamber in one direction and actively positioning the light, e.g. laser, beam in a direction perpendicular to the first direction as a means to change the position of the light, e.g. laser, beam on the substrate in the X-Y plane.

In an even further embodiment, the present invention relates to an apparatus as defined herein, wherein said scanning device comprises displacement of the process chamber as a means to change the position of the light, e.g. laser, beam on the substrate.

Any driver known in the art, able to precisely moving the components may be used. For instance, linear motors, stepper motors, brushless DC motors or servo motors may be used. It is preferred that the drivers maneuver a threaded spindle connected to the process chamber.
The apparatus according to any of the preceding claims, wherein said apparatus further comprises a control module (20). In addition to controlling the relative position of the optics and the solid support, the control module can also be used to regulate the light source. As described above, the intensity and wavelength of the light can be regulated. Furthermore, whether the light is striking the surface of the solid support can also be regulated. Control of the spatial coordinates of the light beam at a discrete location of the solid support for deprotection of the chemical units in the photoactive sector can be achieved by employing a shutter to block the light beam from striking the surface of the solid support or by causing a pulse of light through electronic control. Any means for pulsating the light beam can be used in an invention apparatus. Control of pulsation of the light can be conveniently regulated by a control module interfaced with a light source including, for example, a laser controller.

In a further embodiment, the apparatus may comprise an interrupter, to suspend the operation it is currently doing in favor of the operation that produced the interrupt signal. Preferably, the interrupter interrupts the light beam physically, when re-positioning the scanning device to a new location. The interrupter may be controlled by a control module.

The light beam may be attenuated when positioned at or around the sensor units to reduce the energy density to avoid damaging the sensor unit, e.g. the photodetector.

Scanning - calibration
The sensor unit (3) positioned in the substrate or at or alongside the process chamber is used to define the exact location reference of the light beam. The method is particularly useful in applications where the light beam has to scan repeatedly and with high accuracy over the target during long periods of time.

The beam (6) is directed to the process chamber (1) while the control module (20) monitors the sensor units (3). Signals passed from the sensor units to the control module are used to control the beam by determining a profile of detector signal versus scanner position. A first scan by the scanning device (5) across the sensor unit in the x-direction is made to determine the middle, i.e. x-center, of the sensor unit. A second scan of the light beam across the sensor unit is made in a perpendicular direction to determine the scan position that corresponds to the y-center of the sensor unit. From these two perpendicular scans of the light beam, the x and y scanner coordinates to precisely place the beam at the known location of the sensor unit are calculated.
By setting coordinates for the scanning device (5) at these calculated coordinates, the light beam can be placed at the same position that the sensor unit was in during previous scans. The terms "scanning" and "directing the light" are used interchangeably throughout the present invention. Hence, in the present invention, the position of the light beam is calibrated, e.g. per scan. The term "calibration" or "calibrating" refers to the act of checking and/or adjusting the accuracy of the position of the light beam by comparison with the position of the sensor units. The sensor units function as a reference position and may be used for calibrating the light beam.

An alternative method for finding the center of a sensor unit is to scan the light beam across the sensor unit to locate multiple points (scanner X, Y coordinates are recorded for each point) on the circumference of the sensor unit, or along the perimeter of the sensor unit if the sensor unit is not circular, and then to use formulas, well known to those skilled in the art, to calculate the center of the sensor unit.

Alternatively, the flanks of the beam profile at the position, where the beam has the maximum diameter in the x-direction or alternatively the y-direction can also be used to determine the light position of the laser beam.

After locating the scan position that corresponds to the center of the sensor unit, the light beam is moved to a different location by the scanning device. Typically the light beam is moved by a precision motion stage, e.g. the galvano mirrors, with a resolution on the order of 1 µm. In case the new position is a further sensor unit, the light beam locating process (discussed above) is repeated to determine the position of the scanning device that corresponds to the new location. The scanner coordinates for any point at the substrate can be calculated using well known interpolation or extrapolation methods. The step of directing the light includes the step of using pre-selected scanning device position coordinates to direct and detect the light. The resolution and accuracy of the technique is only limited by the resolution of the scanning mechanism and the mirror positioning equipment.

As a further alternative, instead of a single detector, an array of photodetectors (e.g. a CCD) is used to detect the light beam. The center position of the light beam may be determined by calculating the centroid of the illuminated pixels, weighted by the intensity in each pixel. The calculation may be performed at periodic intervals (of time or increments of movement) or at preset scanner coordinates. In a further embodiment, the present invention relates to a method
as described herein, wherein the light is detected by the sensor units at predetermined intervals, wherein the predetermined intervals are intervals of time, and/or wherein the predetermined intervals are intervals of space.

The present invention thus provides a method as described herein, wherein the sensor unit for detecting the light is an array of photodetectors, each photodetector having a plurality of pixels, and the method further includes the steps of: calculating an intensity weighted centroid of pixels in the photodetectors illuminated by the light; and using the intensity weighted centroid as the light position coordinates when forming the scanning device position versus light position data.

The position of the scanning device, e.g. the galvano mirror, is commanded by a control signal from the control module. The control module is responsible for moving the light beam panel in the X-Y plane, e.g. commanding the galvano mirror that positions the light beam in the X-Y plane and turning the laser on and off as required during movement.

The light beam is scanned over the substrate including the sensor units, where it is detected. The position of the light beam, when it is detected by the sensor unit, is correlated with the position of the scanning device. This correlated data is used to determine the scanner device position coordinates that correspond with a desired position of the light beam.

The system also allows operators to request an automatic calibration or automatic test to be initiated as required.

Accordingly, the present invention relates to a method for synthesizing biopolymers as described herein, comprising a step (f) of providing position coordinates to the scanning device to direct light to the at least one sensor unit. Preferably, step (f) is repeated after each cycle of steps (c) through (d), or complete scan of the substrate.

In one embodiment, the apparatus and method of the present invention provides an automated process to accurately and automatically calibrate the position of the laser beams in X-Y fields. Hence, the present invention relates to a method as described herein, wherein the light beam is directed to the at least one sensor unit is correlated with the position coordinates of the light.

The present invention thus provides a method for synthesizing biopolymers as described herein comprising the steps of:
(i) directing the light to selected positions at the substrate and/or the process chamber;
(ii) detecting the light received at a sensor unit, wherein a location of the detected light produces light position coordinates;
(iii) forming scanning device position data versus light position data by creating associations between light position coordinates and scanning device position coordinates corresponding to the position of the scanning device when the light is detected;
(iv) determining light position coordinates for a selected position of the light; and
(v) calculating selected scanning device position coordinates that correspond with the selected position based on the scanning device position versus light position data.

Preferably, the method includes a further step of using the selected scanning device position coordinates to position the light at the selected position. The step of calculating the selected scanning device position coordinates may comprise the steps of:

- determining if coordinates of the selected position match light position coordinates in the scanning device position versus light position data; and
- if the coordinates of the selected position match light position coordinates, using scanning device position coordinates that correlate with matching light position coordinates for the selected scanning device position coordinates.

Alternatively, the step of calculating the desired scanning device position coordinates may comprise the steps of:

- determining if coordinates of the selected position match light position coordinates in the scanning device position versus beam position data; and
- if the coordinates of the selected position do not match light position coordinates, using interpolation techniques to determine the selected scanning device position coordinates based on light position coordinates in close proximity to the selected position based on the scanning device position versus light position data.

**Flow cell - process chamber**

The process chamber (1) is suitable for receiving a substrate (2) on which the biopolymers are synthesized. The chemicals necessary for the biopolymer, e.g. oligonucleotide, synthesis need to be distributed over the substrate. This process occurs in the process chamber.

In a preferred embodiment, the process chamber is a flow cell, comprising an inner space which can be sealed from the outside environment. Preferably, the flow cell comprises the substrate, a
cover slide and sealing material. The cover slide must be transparent for the desired wave lengths. Preferably, the cover slide is a glass-device (e.g. quartz) that is put on top of the substrate, i.e. the cover slide covers the array. The cover slide creates a cavity that covers the area on the substrate, on which the biopolymers are synthesized. Preferably, the inner space of the flow cell has a small volume (e.g. 50µl) to reduce the amount of the expensive chemicals that are used. A specific internal feature of the flow cell is a triangular cavity with diamond shaped obstacles to create an even flow of solutions into the cavity that covers the substrate. This may help to avoid trapping of gaseous bubbles or cavities in the flow cell during synthesis. On the outside of the flow cell an inlet is situated that is connected via tubing to an oligosynthesizer. Within the flow cell, sealing material (e.g. Kalrez; a perfluor-elastomer from DuPont) can be applied to seal the connection between the cover slide and the substrate to avoid leakage. Any appropriate sealing material may be used.

In a further preferred embodiment, the cover slide and the substrate slide are assembled in a flow cell holder. Preferably, the sealing between cover slide and substrate is compressed evenly with a predefined force. The force may be applied by several springs. The cover slide with sealing is fixed within the flow cell holder. The substrate may be inserted into the holder, with a sliding mechanism pressing the substrate onto the cover slide. The flow cell holder preferably contains a fluid detection sensor on the outlet tube of the flow cell. This sensor detects if the flow cell chamber is filled with fluid.

Substrate
A number of materials suitable for use as substrates in the instant invention have been described in the art. Exemplary suitable materials include, for example, acrylic, styrene-methyl methacrylate copolymers, ethylene/ acrylic acid, acrylonitrile-butadiene-styrene (ABS), ABS/polycarbonate, ABS/polysulfone, ABS/polyvinyl chloride, ethylene propylene, ethylene vinyl acetate (EVA), nitrocellulose,nylons (including nylon 6, nylon 6/6, nylon 6/6-6, nylon 6/9, nylon 6/10, nylon 6/12, nylon 11 and nylon 12), polycarbononitrile (PAN), polyacrylate, polycarbonate, polybutylene terephthalate (PBT), polyethylene terephthalate (PET), polyethylene (including low density, linear low density, high density, cross-linked and ultra-high molecular weight grades), polypropylene homopolymer, polypropylene copolymers, polyethylene (including general purpose and high impact grades), polytetrafluoroethylene (PTFE), fluorinated ethylene-propylene (FEP), ethylene-tetrafluoroethylene (ETFE), perfluroalkoxyethylene (PFA), polyvinyl fluoride (PVF), polyvinylidene fluoride (PVDF), polychlorotrifluoroethylene (PCTFE), polyethylene-chlorotrifluoroethylene (ECTFE), polyvinyl alcohol (PVA), silicon
styreneacrylonitrile (SAN), styrene maleic anhydride (SMA), glass and metal oxides. The substrate may be covered with a further material, e.g. a gel-like matrix, amino silane or hydroxysilane. The substrate can be coated with an organic polymer, to ensure the most optimal coupling of either the first building block, such as a nucleotide, an oligonucleotide or a spacer/linker molecule. Preferred coatings are chosen from isothiocyanate, 'superaldehyde', 3D-Link™ or CodeLink™, mercaptosilane, EZ-RAYS™ and poly(dimethyl-siloxane), as described in Lindroos et al. (2001 Nucl Acids Res. Vol:29; No:13, e69; and Moorcroft et al. (2005 Nucl. Acids Res. Vol 33, No.8, e75), which are particularly incorporated herein in their entirety by reference.

**Arrays**

A wide variety of different molecules can be immobilized on the substrate of the present arrays. Similarly, the present methods are applicable to a wide variety of different molecules or receptors that may be placed on the substrate of the arrays. The methods and arrays are particularly exemplified herein in terms of polynucleotides immobilized on a substrate, but they are equally applicable to other types of molecules. For example, one of skilled in the art could easily adapt the present methods and arrays to apply to other nucleic acids (both DNA and RNA), peptides, polypeptides, proteins, antibodies, carbohydrates, small biomolecules (e.g. drug candidates), or any other types of molecule that can be immobilized on a substrate by any method.

The terms "selected region", e.g. the selected position intended for biopolymer synthesis, or "spot" are used interchangeably throughout the present invention. The latter terms relate to individually, spatially addressable positions on the substrate. The selected position is a localized area, typically on the surface of the substrate which is, was, or is intended to be used for biopolymer synthesis. The synthesis of the biopolymers may be on the surface, e.g. of a substantially flat plane (external) or on the surface of depressions or recessions in a substrate (channels) or on both.

In the present invention, a biopolymer is synthesized at a spatially selected region, i.e. at a particular spot. Synthesis of biopolymers at different spatial addresses yields an array of biopolymers whose sequences are identifiable by their spatial addresses.

The arrays of the present invention may be of any desired size, from two spots to $10^6$ spots or even more. The upper and lower limits on the size of the substrate are determined solely by the practical considerations of working with extremely small or large substrates. For a given
substrate size, the upper limit is determined only by the ability to synthesize and detect the biopolymers in a selected region in the array. The preferred number of spots on an array generally depends on the particular use to which the array is to be put. For example, sequencing by hybridization will generally require large arrays, while mutation detection may require only a small array. In general, arrays contain from 2 to about 10⁶ spots, or from about 4 to about 10⁵ spots, or from about 8 to about 10⁴ spots, or between about 10 and about 2000 spots, or from about 20 to about 200 spots.

Furthermore, not all spots on the array need to be unique. Indeed, in many applications, redundancies in the spots are desirable for the purposes of acting as internal controls.

Nevertheless, each spot contains preferably an assembly of identical biopolymers. The density of biopolymers per spot may vary, however.

A selected position, region or spot may have any convenient shape or geometry, e.g., circular, rectangular, elliptical, oval, wedge-shaped, spherical, etc. A selected region as determined from the planar, two dimensional (2D) top surface of the substrate may be smaller than about 1 cm² or less than 1 mm². Usually, the regions have an area of less than 50,000 µm², more usually less than 10,000 µm² and may be less than 100 µm² or may be less than 10µm².

Biopolymers
The composition of the synthesized biopolymers, e.g. receptors, is not critical. The only requirement is that they be capable of binding to a ligand, if any. The biopolymers may include for example RNA and amino acids (peptides, proteins) that may or may not be modified, thus pure DNA, RNA and protein building blocks (nucleotides, amino acids), as well as those with particular modifications (fluorochromes, sugar group-glycoproteins, phosphate, methyl), and also those of which the building blocks themselves are already modified (DNA, LNA, synthetic amino acids).

For example, the polynucleotides may be composed of all natural or all synthetic nucleotide bases, or a combination of both. Non-limiting examples of modified bases suitable for use with the instant invention are described, for example, in Practical Handbook of Biochemistry and Molecular Biology, G. Fasman, Ed., CRC Press, 1989, pp. 385-392. While in most instances the polynucleotides will be composed entirely of the natural bases (A, C, G, T or U), in certain circumstances the use of synthetic bases may be preferred.
Preferably, the biopolymers are nucleic acids. The term "nucleic acid" as used herein means a polymer composed of nucleotides (oligonucleotide), e.g. deoxyribonucleotides or ribonucleotides. The terms "ribonucleic acid" and "RNA" as used herein means a polymer composed of ribonucleotides. The terms "deoxyribonucleic acid" and "DNA" as used herein means a polymer composed of deoxyribonucleotides. The term "oligonucleotide" as used herein denotes single stranded nucleotide multimers of from about 10 to about 100 nucleotides in length. The term "polynucleotide" as used herein refers to single or double stranded polymer composed of nucleotide monomers of from about 10 to about 100 nucleotides in length, usually of greater than about 100 nucleotides in length up to about 1000 nucleotides in length.

The length of each biopolymer may be varied in order to match the binding characteristics of the ligand, e.g. the melting temperature of DNA probes. For instance, the calculated melting temperature for all oligonucleotides may be set approximately equal. This allows choosing or optimizing the hybridization conditions more precisely. Furthermore, the chances for suboptimal hybridization conditions are decreased and more precise signals are obtained.

Synthesis
A variety of techniques have been described for synthesizing and/or immobilizing arrays of polynucleotides, including in situ synthesis, where the polynucleotides are synthesized directly on the surface of the substrate (see, e.g., U.S. Pat. No. 5,744,305 to Fodor, et a/.,) and attachment of pre-synthesized polynucleotides to the surface of a substrate at discrete locations (see, e.g., WO 98/31836). Additional methods are described in WO 98/31836 at pages 41-45 and 47-48, among other places. Further examples of suitable chemistries and reagents are described, for example in Oligonucleotide Synthesis: A Practical Approach, M. J. Gait, Ed., IRL Press, Oxford, England, 1985. The present invention is suitable for use with any of these currently available, or later developed, techniques for synthesizing light-sensitive biopolymers.

In a preferred embodiment, the present invention relates to a method of synthesizing arrays of oligonucleotides comprising the steps of:
(a) providing an apparatus as mentioned herein, wherein said substrate comprises DNA synthesis linkers;
(b) directing the light from the light source to a selected position on the substrate by the scanning device, e.g. through projection optics, to illuminate those positions in the array on
the substrate which are to be activated to deprotect OH groups thereon to make them available for binding to chemically activated nucleosides or nucleotides;

(c) providing a fluid containing an appropriate chemically activated nucleosides or nucleotides to the substrate and binding the selected chemically activated nucleosides or nucleotides to the illuminated positions;

(d) providing new position coordinates to the scanning device to select new selected positions on the substrate to illuminate those positions in the array on the substrate which are to be activated to deprotect OH groups thereon to make them available for binding to chemically activated nucleosides or nucleotides; and

(e) repeating steps (c) through (d).

In an even more preferred embodiment, the present invention relates to the method of as described above, wherein steps (c) through (d) are repeated a selected number of times to build up a selected number of levels of bases in an oligonucleotide array on the substrate. Preferably, a selected nucleotide base is flowed over the substrate in step (c) to bind to selected positions utilizing phosphoramidite nucleotide synthesis.

In another preferred embodiment, the present invention relates to a method of synthesizing arrays of peptides or proteins comprising the steps of:

(a) providing an apparatus as mentioned supra, wherein said substrate comprises active sites for peptide or protein synthesis;

(b) directing the light from the light source to a selected position on the substrate by the scanning device, e.g. through projection optics, to illuminate those positions in the array on the substrate which are to be activated to deprotect reactive groups thereon to make them available for binding to chemically activated amino acids;

(c) providing a fluid containing an appropriate amino acid to the substrate and binding the selected amino acid to the illuminated positions;

(d) providing new position coordinates to the scanning device to select new selected positions on the substrate to illuminate those positions in the array on the substrate which are to be activated to deprotect reactive groups thereon to make them available for binding to chemically activated amino acids; and

(e) repeating steps (c) through (d).

In an even more preferred embodiment, the present invention relates to the method of as described above, wherein steps (c) through (d) are repeated a selected number of times to build
up a selected number of levels of amino acids in a peptide or protein array on the substrate. Preferably, a selected amino acid is flowed over the substrate in step (c) to bind to selected positions utilizing phosphoramidite synthesis.

A variety of techniques have been described for synthesizing and/or immobilizing arrays of peptides, including in situ synthesis, where the peptides can be synthesized on the surface of the substrate. Preferably solid phase peptide synthesis (SPPS) is utilized, which is well known in the art, including, Boc-SPPS, Fmoc-SPPS and BOP-SPPS (Benzotriazole-1-yl-oxy-tris-(dimethyl-amino)-phosphonium hexafluorophosphate), as described by Atherton and Sheppard (1989, Solid Phase peptide synthesis: a practical approach. Oxford, England: IRL Press), Stewart and Young (1984, Solid phase peptide synthesis, 2nd edition, Rockford: Pierce Chemical Company, 91), and Carpino (1993, "1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive". J. Am. Chem. Soc. 115 (10): 4397-4398) all of which are incorporated by reference in their entirety.

Synthesizer
The apparatus according to the invention, and in particular the process chamber, may be coupled to a DNA or amino acid synthesizer in order to control the flow of reaction liquids, i.e. suitable for directing a flow of appropriate compounds for the synthesis of biopolymers, such as oligonucleotides or peptides on the substrate. The present invention provides an apparatus as described herein, further comprising a synthesizer, e.g. a DNA or amino acid synthesizer.

The DNA oligosynthesizer may be a standard machine (e.g. Biosset) that handles a number of fluids essential to support a sequence of write operations: DIPEA (N-diisopropylethylamine), individual nucleotide base fluids (A,C,G,T) derivatized with NPPOC 2-(2-nitrophenyl)-propoxy-carbonyl), acetonitril solvent, all under inert gas (Argon) pressure. The oligosynthesizer is controlled by a computer, e.g. a PC or the control module, to allow full control of the various washing steps.

Characterization
Characterization of the interaction between a receptor located at the array and the corresponding ligand are well known in the art. In the next reference is made to nucleotide micro-arrays as a non-limiting example. Nucleotide arrays can be used e.g. to characterize a DNA sample. The DNA (ligand) in the sample is first labeled, e.g. with a fluorochrome (fluorescent moiety; examples of such moieties are: Cy3, Cy5 etc.) and is subsequently added to
the array where it hybridizes (synonym: binds) to the complementary DNA probe (or receptor) on an array; the coupled chains can subsequently be detected through the fluorochrome label, leading to characterization of the DNA sample based on its location on the array.

Patterns

All known DNA micro-arrays are based on regular grid patterns, and use substantially flat substrates.

In standard micro-array experiments the outcome of the experiment is only known after image and data analysis. Visual evaluation of a micro-array slide after scanning the slide turns out to be very tricky. The present invention provides a method to check the quality of the micro-array experiment right after scanning. Specifically, a pattern of dense biopolymers is generated, which provides an indication of the quality of the binding experiment. With laser-directed synthesis of biopolymers, the biopolymers can be assembled in any pattern. The molecules of the biopolymers, e.g. the sequences of the DNA, in the pattern are all identical and the ligand, e.g. reverse complementary sequence, is added to the sample. After binding, e.g. hybridization, the patterns are discernable with the naked eye on an image of the scanned surface and indicate that the binding, e.g. hybridization, has succeeded or failed.

It will be appreciated by the person skilled in the art, that the patterns may provide an identification tag of the particular substrate, e.g. a sequence of numbers.

Generating patterns of biopolymers using a mask-less light beam has several advantages over other technologies that can be applied. Firstly, a continuous pattern can be generated. Neither Ink-jet technology nor mask-less lithography will offer the possibility to generate a continuous pattern. Secondly, using a light beam offers the flexibility to create any pattern designed for any biopolymer-sequence that is required in short term. Other platforms have various drawbacks. Using lithography in combination with masks to create a pattern of light is time-consuming, laborious and expensive. For each pattern and/or each new sequence new masks have to be manufactured before they are used to synthesize a biopolymer of specific sequence.

Most of all, none of these technologies is available at the site of the research. Surfaces with patterns have to be ordered, obstructing research and diagnostics. Using a mask-less light beam according to the invention can easily be build into a device that can be made available to researchers on-site.
Accordingly, the present invention provides a method as described supra, wherein at least part of the oligonucleotides bound to said selected positions form a pattern. In an alternative embodiment, the present invention provides a method as described supra, wherein at least part of the peptides or proteins bound to said selected positions form a pattern. Preferably, the selected regions on the substrate are spatially arranged and laid out in precise patterns, such as rows of dots, or rows of squares, or lines to form distinct arrays.

Preferably, said patterns comprise non-square shapes, preferably selected from the group consisting of circles, lines, symbols, such as characters, fonts and icons, graphic images, such as logos, and patterns of irregular shapes. Alternatively, said patterns comprise patterns of shapes on non-rectangular grids, preferably selected from the group of circles in hexagonal dense packing, regular patterns of non-square shapes, and patterns made up of subgroups of shapes where the pattern of the shapes or the pattern of the subgroups is superimposed on a flat or non-flat pattern on the substrate, such as grooves, e.g. channels, or wells.

In tissue-array technology, tissue samples are organized in a regular pattern for parallel or simultaneous treatment. Most treatments involve hybridization of a probe of known sequence to the tissue sample for detection of genotype (DNA) or phenotype (RNA). For Fluorescent In Situ Hybridization (FISH) an RNA probe is used to detect the expression of its RNA counterpart in cells and tissues. After treatment, the slides containing the tissue have to be scrutinized if any signal can be seen on the sample itself. The present invention provides an easy and direct positive control for each tissue and each probe that is used. A pattern of biopolymers, e.g. DNA, is synthesized around or next to the appropriate sample that will detect the probe that is being applied to the sample. When the probe is properly labeled and hybridizes to the DNA on the slide the positive control will appear as a fluorescent pattern, e.g. a circle that circumvents the sample.

In addition, the present invention provides an array comprising oligonucleotides bound to selected positions, wherein said oligonucleotides are synthesized by the method described supra. Preferably, said oligonucleotides form patterns. In an even more preferred embodiment, said pattern is used for detecting quality of binding between receptor and ligand, e.g. hybridization.
It will be appreciated that the present invention provides an array comprising peptides or proteins bound to selected positions, wherein said peptides or proteins are synthesized by the method described supra. Preferably, said peptides or proteins form patterns. In an even more preferred embodiment, said pattern is used for detecting quality of binding between receptor and ligand.

Alternatively or in addition, the pattern is used for identifying individual arrays.

In a further embodiment, the present invention relates to (micro)arrays comprising channels. For instance the support has a surface with depressions or recessions, for instance one channel, and particularly preferable with a large number of channels. The channels are preferably microchannels with a cross section of, for instance, 10 to 1000 µm. The channels may be, depending on the surface properties, capillary channels, but also channels without capillary properties (for instance with a coating such as Teflon). A two-layer structure is possible, and the layers can be joined together for example by gluing or bonding, or not. The structure of the channels may in this case be introduced either only into one or else into both sides or halves. Methods which can be used for production are, for example, laser, milling, etching techniques or injection molding. Injection molding is particularly cost-effective and allows adequate quality of fabrication. Other methods are the LIGA technique or hot molding. A particularly preferred technique is the generation of channels using standard soft lithography (micro-moulding) as described by Moorcroft et al. (2005 Nucl. Acids Res. Vol 33, No.8, e75), which is specifically incorporated herein by reference. Various methods can be employed to produce the channels in the support. Account must be taken on the influence of the cross-sectional geometry of the channels, which has a great influence on the resulting hydrodynamic forces and the possibility of cleaning the channels. The following aspects should be taken into account in the arrangement of the channels in the plane: if a large number of parallel channels is used, it is possible to minimize the synthesis times, but the wetting or filling of the individual channel is correspondingly complex. If, at the other extreme, there is only a single long channel, the synthesis is correspondingly slow because the multiplexing of channels to bases or whole oligomers cannot be used, and all processes can take place only serially one after the other. The advantage of only one or a few channels is for the analysis, where the sample flows past each measurement point in all the channels.

The use of channels very greatly reduces the amount of fluid required and, at the same time, increases the quality in the subsequent detection of a sample compared with the use of a single
area. The uniform wetting of channels is hydrodynamically very simple, consumes little fluid and therefore can be miniaturized and automated very easily. This applies in particular also to the need for adequate quality of the channel washing processes. The fluid required is already reduced by 50% by the walls of the channels which, in principle, cover the space between two reaction regions in the support array. This applies both to the coating of the support during production, the synthesis of the biopolymers and to the "sample loading" for the analysis. A further reduction in the amounts of fluid results from the good wetting of the channel walls by a fluid flowing through and, in particular, by the effective washing processes which can, for example, be greatly improved by "cleansing" gas bubbles in the channels. On the other hand, good, statistically adequate distribution of the sample on a surface can be achieved only with a very large amount of sample. A further advantage of the channels is that the cycle times are shorter, resulting from the smaller volumes of fluid and, associated with this, the faster chemical reactions and operations. This results in shorter synthesis and binding or hybridization times. This additionally results in a distinct reduction in errors both in production and in detection, which further increases the number of measurements which can be evaluated per usage of material and time, and forms the basis for quality assurance based on accurately definable and reproducible flow processes.

A large number of structures and microchannel courses is possible as arrangement of the channels on the detector area. Parallel, meandering or "snake-shaped" structures, for example, are obvious for high parallelity of the fluidic processes. Preferably, the division of the channels could in this case take place according to the duality principle, where two new channels arise from each channel, and all of them are of equal length. Thus, 10 divisions result in $2^{10} = 2,048$ channels. Spiral arrangements have the advantage that their flow processes are less turbulent and their cleaning is better. Their great disadvantage is the feeding in and/or out, which must take place in the third dimension upward or downward. It is possible by use of a single, multiply coiled or spiral channel to establish a hybridization in the (slow) flow-through, which also makes it possible to detect rare events (for example rarely expressed genes). This introduces a chromatographic principle into DNA array technology.

The advantages of (micro)arrays comprising channels are evident. As further detailed above, production of the array of biopolymers consists of producing a substrate, which is preferably provided with (micro)channels, and of the biochemical coating process, preferably on the walls of the individual (micro)channels, so that subsequent synthesis of the biopolymers, for example oligonucleotides, in the channels is possible. This entails site-specific attachment of individual
biopolymer building blocks, e.g. oligomeric synthons (for example di-, tri-, tetra- or penta-
nucleotides or whole base sequences oligos) in the individual channels in the support by means
of photo-activation by a suitable light source. The positions (selected positions) intended for
synthesis of biopolymers in the channels of a substrate are filled with one or more fluids from
containers via feed lines, valves and fittings. After completion of the reaction, the reaction
regions are rinsed and refilled, after which another activation cycle follows. The progress of
biopolymer synthesis can be followed and controlled by means of suitable detection units. As
soon as the synthesis of the biopolymers is completed, the reaction regions are cleaned and are
then available for an analyte determination method. This results in a large number of
biopolymer-charged regions (specific binding or hybridization sites) in each channel, and each
region serves, because of its individual biopolymer, for the binding and subsequent detection of
a specific analyte, for example a DNA fragment. The regions are separated from one another in
one dimension of the planar support by the walls of the channels, and with photo-activated
binding a corresponding free space is left between two adjacent regions along the individual
channels. The result is a highly parallel, highly integrated array of specific biopolymers.
Specifically, the number, amount, and/or density of the synthesized biopolymers is preferably
known and/or identical per spot in a particular location in the channel, e.g. biopolymer
information per spot (BIS) should be identical and/or known in the channels. However, in situ
synthesis of biopolymers in channels is particularly sensitive to drift and imperfections of the
optical (e.g. lenses and mirrors), and mechanical (e.g. drivers, motors and scanners) systems.
In this case it is very important that the light beam is calibrated. Accordingly, the present
invention relates particularly to an apparatus for use in synthesis of an array of biopolymers as
described above, comprising: a process chamber comprising a substrate with an active surface
on which the array(s) may be formed, wherein said active surface is formed by channels. Thus,
the present invention relates also to (micro)arrays comprising channels.

It has been found that satisfying to very good BIS results are obtained when either the (surface)
area, e.g. diameter, of the light beam contacting the substrate overlaps and is larger than the
cross section of (micro)channels, or when the light beam moves (and thus illuminates and
photo-activates) perpendicular to or substantially perpendicular to the length axis or direction of
the channels. The total area illuminated thus extends to, and preferably over the sides, e.g. over
the periphery, of the channel. As mentioned above, in a two-layer structure, the structure of the
channels may be introduced only into one side or halve (A). The biopolymers can be
synthesized on the other side or halve (B), after which the sides or halves (A) and (B) are
aligned and joined together. In this case, the spot of biopolymers synthesized on (B) is
preferably larger than the cross section of channel on (A). Hence, the present invention relates preferably to a method of synthesizing arrays of biopolymers as described above, on a substrate comprising at least one (micro)channel, wherein the selected position on the substrate is larger than the cross section of said (micro)channel. Preferably, the selected position extends to and preferably extends over the sides of the channel.

In a further preferred embodiment, the present invention relates to a substrate comprising at least one channel and at last one biopolymer at a spot or spatially selected region, characterized in that said spot or spatially selected region overlaps a channel. In the present invention, the term "overlaps" refers to an area covering part of said channel and extending to and preferably over the sides or periphery of said channel. As such it will be understood that the diameter of said spot or spatially selected region (in the length axis as opposed to the latitude) may be larger than the cross section of said channel. Preferably, the said spot or spatially selected region intersects with the channel.

In a preferred embodiment, the invention relates to methods as described herein, wherein at least one selected position is located in a channel. Preferably, said selected position extends to the sides of said channel. Even more preferably, said selected position on the substrate overlaps a channel. Preferably, said selected position extends over the sides of said channel. Even more preferably, said selected position is larger than the cross section of said channel.

In a further preferred embodiment, the invention relates to substrate comprising at least one channel and at least one biopolymer at a spatially selected region, characterized in that said spatially selected region overlaps a channel. It is further preferred that said substrate comprises at least one channel and at least one biopolymer at a selected position, wherein said at least one selected position is located in a channel, characterized in that the diameter of said at least selected position is larger than the cross section of said channel. Even more preferably, said selected position extends to the sides of said channel. Even more preferably, said selected position on the overlaps said channel. Even more preferably, said selected position extends over the sides of said channel.
EXAMPLES

EXAMPLE 1
UV light from a laser as described above hits the substrate on a selected region, and a photo labile group (NPPOC, 2-(2-nitrophenyl)-propoxycarbonyl) or a structurally related NPPOC compound was activated and removed from the last nucleotide or a spacer (the nucleotide is de-capped). The substrate was covered with a liquid (DIPEA), which allows a more efficient removal of the NPPOC protective caps. DIPEA (N-diisopropylethylamine; EDIPA) is added to create an alkaline (basic) environment. Removal of the NPPOC results in a free -OH group. In the next processing step, acetonitril containing a single nucleotide base (A, C, G or T) was flushed over the microscope slide surface. The nucleotides that have to bind have a phosphoamidite moiety (a specific side chain of the nucleotide) at one site and a NPPOC moiety at another site of the molecule. In the presence of an activator (4,5 dicyanoimidazole, DCl), the phosphoamidite group was removed and the remaining part of the nucleotide reacts with any -OH group. The excess fluid is washed off with acetonitril. Cap A (containing tetrahydrofurane (THF) and acetic anhydride) and Cap B (containing THF, pyridine and 1-methylimidazole) are added to block remaining hydroxyl groups for being elongated in consecutive steps. Oxidizer (THF, iodine, pyridine and water) is added create a stronger bond from a phosphorous di-ester group to a phosphate group with the previous nucleotide. All reagents for synthesis are bought through Sigma-Proligo. All fluids are displaced with an inert gas, argon.

EXAMPLE 2
The micro-array of example 1 is subjected to a hybridization experiment with a fluorescently labeled probe.

To show the effect of calibration resequencing of two oligonucleotides of 60 nucleotides that differ by only two nucleotides has been performed with and without calibration. Resequencing entails the synthesis of 25-mers to determine the sequence of a stretch of DNA. Each 25-mer is complementary to the sequence, that is interrogated, but at one position. At this position all four bases are tested, resulting in four oligonucleotides of identical sequence but at one position. The target sequence, the stretch of 60 nucleotides, interacts most strongly with the one of the four oligonucleotides that is the most similar (In effect most of the times it is identical). This oligonucleotide gives the highest signal. To determine the next position on the target sequence the synthesized oligonucleotides are shifted by one nucleotide upstream or down stream.
In this example, the two 60 nt oligonucleotide were labeled differently, one with a red fluorescent label and one with a green fluorescent label.

In Figure 3(A) these labeled oligonucleotides were hybridized to the microarray that has been synthesized using calibration during synthesis. The probes that are reverse complementary to the red fluorescent 60 nt oligonucleotide are in column 5-8 and the probes that are reverse complementary to the green fluorescent 60 nt oligonucleotide are in column 13-16. The difference between the two 60nt oligonucleotides is located on one side of the oligonucleotide. Therefore, the lower part of 5-8 is red fluorescent and the lower part of column 12-16. On the other end of the 60nt oligonucleotides both of them are identical. Thus, the upper parts of column 5-8 and column 13-16 are all yellow. Column 1-4 and 9-12 hold the identical sequence to the 60 nt oligonucleotide and therefore do not interact with the labeled 60nt oligonucleotide since they are not the "mirror-image".

In Figure 3B the identical experiment as detailed above is shown, except that the probes on this microarray have been synthesized without calibration. Clearly the signal intensities are much lower. This is also demonstrated by the difference in intensity between the spots that should interact with the 60nt oligonucleotides in column 5-8 and 13-16 and the spots that should not interact with the 60nt oligonucleotides in column 1-4 and 9-12. The difference is much higher in Figure 3A (with calibration), than in Figure 3B (without calibration). For this demonstration it should be noticed that in both pictures the negative signal in column 1-4 and 9-12 are similar. For display purposes signal intensities of the negative spots in column 1-4 and 9-12 in the right picture are shown a bit clearer. It is noted that the positive signals in the right picture are still lower than in the left picture even though the intensities are increased a bit for visualization purposes.

It should also be noted that the scale of correction can be told from the correction factor that is used to do the calibration. In most of the experiment performed so far correction factors have been applied that have more impact when spots get smaller.

**EXAMPLE 3**

In tissue-array technology, tissue samples are organized in a regular pattern for parallel or simultaneous treatment.
A pattern of DNA is synthesized around or next to an appropriate tissue sample. An RNA probe is fluorescently labeled for Fluorescent In Situ Hybridization (FISH). The probe is hybridized to the tissue sample and the DNA pattern.

With fluorescence microscopy the pattern of DNA is detected confirming a successful experiment.
CLAIMS

1. Apparatus for use in synthesis of an array of biopolymers, comprising:
   (a) a process chamber comprising a substrate with an active surface on which the array(s) may be formed;
   (b) a light source; and
   (c) a scanning device having position coordinates for directing the light from the light source to a selected position on the substrate;
characterized in that the process chamber and/or the substrate comprises at least one sensor unit or at least one sensor unit alongside the process chamber for detecting when the light is received in or alongside the process chamber and/or at the substrate, wherein the light directed to the at least one sensor unit is correlated with the position coordinates of the light.

2. The apparatus according to claim 1, said sensor unit for detecting the light produces light position coordinates when the light is received.

3. The apparatus according to claim 1 or 2, wherein the sensor unit for detecting the light is an array of photosensors, each photosensor having a plurality of pixels, and the position determining means comprises: means for calculating an intensity weighted centroid of pixels in the photo-sensors illuminated by the light and using the intensity weighted centroid as the scanning device position coordinates when forming the scanning device position versus light position data.

4. The apparatus according to claim 3, wherein said sensor unit is a photosensor, preferably chosen from the group consisting of photoresistors, photovoltaic cells and photodiodes.

5. The apparatus according to any of claims 1 - 4, wherein said process chamber and/or the substrate comprises at least 3 sensor units.

6. The apparatus according to any of the preceding claims, further comprising data forming means for forming scanning device position data versus light position data by creating associations between light position coordinates and scanning device position.
coordinates corresponding to the position of the light when the light is detected at the at least one sensor unit.

7. The apparatus according to any of the preceding claims, in which the light position coordinates are defined by the intensity weighted centroid of pixels at the photosensor

8. The apparatus according to any of the preceding claims, in which the light position coordinates are defined by an incremental intensity increase measured by the photosensor at the flanks of the laser beam.

9. The apparatus according to any of the preceding claims, wherein said apparatus further comprises a control module (20).

10. The apparatus according to any of the preceding claims, wherein said scanning device comprises a galvano-mirror or any other means to actively change the direction of the laser beam to change the position of the laser beam on the substrate.

11. The apparatus according to any of the preceding claims, wherein said scanning device comprises displacement of the process chamber on an X-Y stage as a means to change the position of the laser beam on the substrate.

12. The apparatus according to any of the preceding claims, wherein said scanning device comprises displacement of the process chamber in a rotational movement as a means to change the position of the laser beam on the substrate.

13. The apparatus according to any of the preceding claims, wherein said scanning device comprises displacement of the process chamber in one direction and actively positioning the laser beam in a direction perpendicular to the first direction as a means to change the position of the laser beam on the substrate in the X-Y plane.

14. The apparatus according to any of the preceding claims, wherein said scanning device comprises displacement of the process chamber as a means to change the position of the laser beam on the substrate
15. The apparatus according to any of the preceding claims, wherein said process chamber is a flow cell.

16. The apparatus according to any of the preceding claims, further comprising a synthesizer.

17. The apparatus according to any of the preceding claims, further comprising an interrupter system.

18. The apparatus according to any of the preceding claims, wherein the light from the light source is a laser beam, preferably a focused laser beam.

19. The apparatus according to any of the preceding claims, wherein said substrate comprises channels.

20. The apparatus according to claim 19, wherein said channels comprise said active surface.

21. The apparatus according to claim 19, wherein said active surface is present in said channels.

22. A method of synthesizing arrays of oligonucleotides comprising the steps of:
   (a) providing an apparatus according to any of claims 1 to 21, wherein said substrate comprises active sites for DNA synthesis;
   (b) directing the light from the light source to a selected position on the substrate by the scanning device to illuminate those positions in the array on the substrate which are to be activated to deprotect OH groups thereon to make them available for binding to chemically activated nucleosides or nucleotides;
   (c) providing a fluid containing an appropriate nucleosides or nucleotides to the substrate and binding the selected base to the illuminated positions;
   (d) providing new position coordinates to the scanning device to select new selected positions on the substrate to illuminate those positions in the array on the substrate which are to be activated to deprotect OH groups thereon to make them available for binding to chemically activated nucleosides or nucleotides;
   (e) repeating steps (c) through (d); and.
(f) providing position coordinates to the scanning device to direct light to the at least one sensor unit.

23. The method of according to claim 22, wherein steps (c) through (d) are repeated a selected number of times to build up a selected number of levels of nucleosides or nucleotides in an oligonucleotide array on the substrate.

24. A method of synthesizing arrays of peptides and proteins comprising the steps of:
(a) providing an apparatus according to any of claims 1 to 21, wherein said substrate comprises active sites for peptide or protein synthesis;
(b) directing the light from the light source to a selected position on the substrate by the scanning device to illuminate those positions in the array on the substrate which are to be activated to deprotect reactive groups thereon to make them available for binding to chemically activated amino acids;
(c) providing a fluid containing an appropriate amino acid to the substrate and binding the selected amino acid to the illuminated positions;
(d) providing new position coordinates to the scanning device to select new selected positions on the substrate to illuminate those positions in the array on the substrate which are to be activated to deprotect reactive groups thereon to make them available for binding to chemically activated amino acids;
(e) repeating steps (c) through (d); and
(f) providing position coordinates to the scanning device to direct light to the at least one sensor unit.

25. The method of according to claim 24, wherein steps (c) through (d) are repeated a selected number of times to build up a selected number of levels of amino acids in an peptide or protein array on the substrate.

26. The method according to claim 22 or 23, wherein a selected nucleotide base is flowed over the substrate in step (c) to bind to selected positions utilizing phosphoramidate nucleotide synthesis.

27. The method according to claim 24 or 25, wherein a selected amino acid is flowed over the substrate in step (c) to bind to selected positions according to SPPS (Solid Phase Peptide Synthesis) protocols.
28. The method according to any of claims 22 to 27, wherein said step (f) is repeated after each cycle of steps (c) through (d).

29. The method according to any of claims 22 to 28, wherein the light, preferably a laser beam, directed to the at least one sensor unit is correlated with the position coordinates of the light.

30. The method according claim 29, further comprising the steps of:
   (i) directing the light to selected positions at the substrate and/or at or alongside the process chamber;
   (ii) detecting the light received at a sensor unit, wherein a location of the detected light produces light position coordinates;
   (iii) forming scanning device position data versus light position data by creating associations between light position coordinates and scanning device position coordinates corresponding to the position of the scanning device when the light is detected;
   (iv) determining light position coordinates for a selected position of the light; and
   (v) calculating selected scanning device position coordinates that correspond with the selected position based on the scanning device position versus light position data.

31. The method according to claim 30, further including the step of using the selected scanning device position coordinates to position the light at the selected position.

32. The method according to claim 31, wherein the step of calculating the selected scanning device position coordinates comprises the steps of:
   - determining if coordinates of the selected position match light position coordinates in the scanning device position versus light position data; and
   - if the coordinates of the selected position match light position coordinates, using scanning device position coordinates that correlate with matching light position coordinates for the selected scanning device position coordinates.

33. The method according to claim 31, wherein the step of calculating the desired scanning device position coordinates comprises the steps of:
- determining if coordinates of the selected position match light position coordinates in the scanning device position versus beam position data; and
- if the coordinates of the selected position do not match light position coordinates, using interpolation techniques to determine the selected scanning device position coordinates based on light position coordinates in close proximity to the selected position based on the scanning device position versus light position data.

34. The method according to any of claims 22 to 33, wherein the sensor unit for detecting the light is an array of photodetectors, each photodetector having a plurality of pixels, and the method further includes the steps of:
- calculating an intensity weighted centroid of pixels in the photodetectors illuminated by the light; and
- using the intensity weighted centroid as the light position coordinates when forming the scanning device position versus light position data.

35. The method according to one of claims 22 to 34, wherein the step of directing the light includes the step of using pre-selected scanning device position coordinates to direct and detect the light.

36. The method according to one of claims 22 to 35, wherein the light is detected by the sensor units at predetermined intervals.

37. The method according to claim 36, wherein the predetermined intervals are intervals of time.

38. The method according to claim 36, wherein the predetermined intervals are intervals of space.

39. The method according to any of claims 22, 23 or 26, wherein at least part of the synthesized oligonucleotides bound to said selected positions form a pattern.

40. The method according to any of claims 24, 25 or 27, wherein at least part of the synthesized peptides or proteins bound to said selected positions form a pattern.
41. The method according to claim 39 or 40, wherein said patterns comprise non-square shapes, preferably selected from the group consisting of circles, lines, symbols, such as characters, fonts and icons, graphic images, such as logos, and patterns of irregular shapes.

42. The method according to claim 39 or 40, wherein said patterns comprise patterns of shapes on non-rectangular grids, preferably selected from the group of circles in hexagonal dense packing, regular patterns of non-square shapes, and patterns made up of subgroups of shapes where the pattern of the shapes or the pattern of the subgroups is superimposed on a flat or non-flat pattern on the substrate, such as grooves or wells.

43. The method according to any of claims 22 to 42, wherein at least one selected position is located in a channel.

44. The method according to claim 43, wherein said selected position extends to the sides of said channel.

45. The method according to claim 43 or 44, wherein said selected position on the substrate overlaps a channel.

46. The method according to any one of claims 43 to 45, wherein said selected position extends over the sides of said channel.

47. The method according to any one of claims 43 to 46, wherein said selected position is larger than the cross section of said channel.

48. An array comprising oligonucleotide bound to selected positions, wherein said oligonucleotides are synthesized by the method according to any of the claims 22, 23 and 26.

49. The array according to claim 48, wherein said oligonucleotides form patterns.

50. The array according to claim 49, wherein said pattern is used for determining, e.g. detecting quality of, binding between receptor and ligand.
51. The array according to claim 49, wherein said pattern is used for identifying individual arrays.

52. An array comprising peptides or proteins bound to selected positions, wherein said peptides or proteins are synthesized by the method according to any of the claims 24, 25 and 27.

53. The array according to claim 52, wherein said peptides or proteins form patterns.

54. The array according to claim 53, wherein said pattern is used for determining, e.g. detecting quality of, binding between receptor and ligand.

55. The array according to claim 53, wherein said pattern is used for identifying individual arrays.

56. A method for determining binding between receptor and ligand on a substrate, comprising providing an array comprising oligonucleotides according to claim 49 or peptides or proteins according to claim 53, contacting said oligonucleotides or peptides or proteins with a ligand under conditions allowing binding between said oligonucleotides or peptides or proteins with a ligand and identifying a pattern, wherein said pattern determines binding between said oligonucleotides or peptides or proteins and said ligand.

57. A substrate comprising at least one channel and at least one biopolymer at a spatially selected region, characterized in that said spatially selected region overlaps a channel.

58. A substrate comprising at least one channel and at least one biopolymer at a selected position, wherein said at least one selected position is located in a channel, characterized in that the diameter of said at least selected position is larger than the cross section of said channel.

59. The substrate according to claim 58, wherein said selected position extends to the sides of said channel.
60. The substrate according to claim 58 or 59, wherein said selected position overlaps said channel.

61. The substrate according to any one of claims 57 to 60, wherein said selected position extends over the sides of said channel.
FIGURE 1
A. CLASSIFICATION OF SUBJECT MATTER

INV. B01J19/00  C40B50/14  C40B60/14

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)

BOIJ  C40B

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

See patent family annex.

Special categories of cited documents:

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

"E" earlier document but published on or after the international filing date

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

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

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

Date of the actual completion of the international search

19 March 2008

Date of mailing of the international search report

02/04/2008

Name and mailing address of the ISA/

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Fax: (+31-70) 340-3016

Authorized officer

Nazario, Luis
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### Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. □ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

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

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

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

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

see additional sheet

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

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

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

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

**Remark on Protest**

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

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

- □ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-56

Independent claims 1, 22, 24, 48, 52 and 56 (and dependent claims 2-21, 23, 25-47, 49-51, 53-55) relate to an apparatus for synthesising an array of biopolymers, methods for synthesising oligonucleotides, peptides or proteins, to the arrays therein produced and their use in binding reactions between a receptor and a ligand. The device and the method include the provision of sensor units to correlate the position coordinates of the light.

2. claims: 57-61

Independent claims 57 and 58 (and dependent claims 59-61) relate to substrates comprising at least one channel and a biopolymer at a spatially selected region that either overlaps the channel or its diameter is larger than the channel's cross-section.
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