Nanofluidic device for extraction of elastic bio-entities

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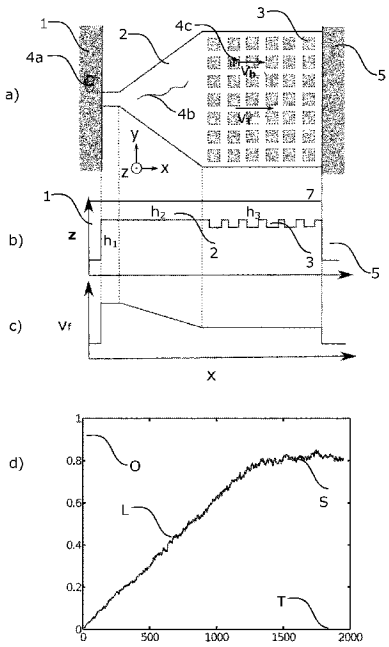
Fig. 1



(57) Abstract: The invention relates to a nanofluidic device for extraction of elastic bio-entities suspended in liquid. The device comprises a main passage and a plurality of nanoslits extending from a sidewall of the main passage. The main passage has a first height and each nanoslit has a second height so that the second height is lower than the first height. Further, each nanoslit comprises a plurality of nanopits, defining a third height which is larger than the second height, and each nanopit being at the bottom of a nanoslit. Each nanoslit has a width which increases from the sidewall of the main passage towards the nanopits.

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NANOFLUIDIC DEVICE FOR EXTRACTION OF ELASTIC BIO-ENTITIES

FIELD OF THE INVENTION

The present invention relates to a nanofluidic device for extraction of elastic bio-entities, such as DNA, enzymes, liposomes, etc., suspended in liquid.

BACKGROUND OF THE INVENTION

Detection and analysis of DNA and similar entities is more and more important for a number of interrelated bio- and nano- technology disciplines. It is a challenge to have a micro- or nano-scale device that is efficient in extraction and analysis of cell genomes and other bio-entities. With scaling down devices for DNA analysis, efficient confinement of DNAs may become a problem.

Previous design of a nanofluidic device described in a scientific paper "Directed self-organization of single DNA molecules in a nanoslit via embedded nanopit arrays", PNAS, 106.1 (2009), p 79-84, Reisner et al., discloses an array of so-called nanopit structures etched in a nanoslit. The array of nanopit structures controls the positioning and conformation of single DNA molecules in the nanofluidic device. By the design described therein, the energy provided to the DNA to enter the array of nanopit structures also moves the DNA outside of the array of nanopit structures making the device not so efficient in terms of accumulating the DNA in the device.

OBJECT OF THE INVENTION

It is an object of embodiments of the invention to provide an efficient way of capturing of different bio-entities, such as DNA, lipid vesicles, etc.

It is a further object of embodiments of the invention to provide an entropic DNA trap with a special geometry, which enables efficient trapping of DNA and the like.

DESCRIPTION OF THE INVENTION

The invention relates to an improved design of a nanofluidic device for extracting elastic bio-entities such as DNA, enzymes, liposomes or similar.

In a first aspect, the present invention relates to a nanofluidic device for extraction of elastic bio-entities suspended in liquid, the device comprising a main passage and a plurality of nanoslits extending from a sidewall of the main passage;

- the main passage having a first height,

- each nanoslit having a second height, the second height being lower than the first height,

- each nanoslit comprising a plurality of nanopits, defining a third height being larger than the second height, and

- each nanopit being at the bottom of a nanoslit;

wherein each nanoslit has a width which increases from the sidewall of the main passage towards the nanopits.

A nanofluidic device may have a structure of nanometre dimensions, configured to control fluids confined within the structure. In the present context, the term "extraction" is to be understood as separation of elastic bio-entities from liquid they are suspended in, and further capturing of the separated elastic bio-entities. The elastic bio-entities may be DNA, enzymes, liposomes or the like. The liquid in which the elastic bio-entities are suspended may be blood plasma, urine, saliva, milk or any other body liquid that may comprise one or more of aforementioned elastic bio-entities.

The nanofluidic device comprises a main passage where the bio-entities suspended in the liquid may be introduced at first. The main passage comprises two sidewalls. The main passage may ensure a continuous flow of the liquid and bio-entities suspended therein. The liquid flow through the main passage may be pressure-driven. Namely, there may be a difference in pressure along the main passage. This pressure difference may enable the liquid to move inside the nanofluidic device. The pressure may gradually decrease from a first end of the main passage, i.e. position where the liquid is injected towards a second end of the main passage where eventual excess of the liquid may exit the main passage.

A plurality of nanoslits extends outwardly from any of the sidewalls of the main passage. Nanoslits may represent side passages which also ensure a constant flow of the liquid and bio-entities suspended therein. The pressure in each nanoslit may be lower than the pressure in the main passage and the pressure in one nanoslit closer to the first end of the main

passage may be higher than the pressure in a subsequent nanoslit which is closer to the second end of the main passage.

The main passage has a first height. The main passage has a main upper and lower surface. The distance between the main upper and lower surface may define the height of the main passage.

Each nanoslit has a second height. The nanoslits also have a slit upper and lower surface. The main upper surface may coincide with the slit upper surface. The distance between the slit upper and lower surface may define the height of the nanoslit. The second height is lower than the first height.

Further, each nanoslit comprises a plurality of nanopits. The nanopits may be square-, round-, hexagonal-, rectangular-, or randomly-shaped depressions or wells. The nanopits may be formed by an etching process. The size of both nanoslits and nanopits as well as the shape of the nanopits may play an important role in trapping of the elastic bio-entities. The smaller the size of the nano-structures, the higher the confinement, resulting in more effective conformation of the bio-entities within the nanopits. The nanopits have third height which is larger than the second height. The third height may be lower than the first height. The upper surface of both the nanopits and nanoslits may coincide. Each nanopit is at the bottom of a nanoslit. The elastic bio-entities may be forced into the nanopits by the action of flow of the liquid. Confinement within the nanopits may affect conformation of the elastic bio-entities.

Each nanoslit has a width, which increases from the sidewall of the main passage towards the nanopits. This geometry of the nanoslits modulates the flow velocity of the liquid within the nanoslit. The nanofluidic device may also modulate the energy provided to the elastic bio-entities. Namely, the energy may be changed along the nanofluidic device and especially within the nanoslits. Thus, at given operation parameters, e.g., hydrodynamic flow rate, the elastic bio-entities may be provided with more energy to enter the nanoslit from the main passage than to escape the nanopits as each nanopit may provide lower confinement than each nanoslit due to the height difference.

In one embodiment of the present invention, the bottom of the main passage may be lower than the bottom of the nanopits, i.e. the bottom surface of the main passage may be below the bottom surface of the nanoslits, compared to the upper surface. The bottom of the nanopits may be lower than the bottom of the nanoslit, i.e., the bottom surface of the nanopits may be below the bottom surface of the nanoslits, compared to the upper surface.

As stated above, by having different heights along the nanofluidic device the energy of the bio-entities may be controlled and the above described trapping mechanism therefore occurs.

In one embodiment, the main passage may comprise a first inlet for introducing the liquid with the bio-entities suspended therein into the nanofluidic device, the inlet being at the first end of the main passage. The liquid may be introduced manually by using for instance a syringe or similar device. Another option for introducing the liquid into the nanofluidic device may be to have the device connected with a system of capillaries guiding the liquid towards the first inlet. The liquid introduced at the first inlet comprises the largest number of elastic bio-entities and also the highest volume, which may provide the highest pressure of the liquid at the first inlet.

In another embodiment, the main passage may comprise a first outlet for discharging the liquid from the device, the outlet being positioned at the second end of the main passage. At this position the liquid to be discharged may comprise very little or no bio-entities as these may be already trapped within the nanopits. The fluidic pressure at the second end may be significantly lower than that at the first end. As stated above this pressure difference may drive the liquid flow along the main passage and throughout the entire nanofluidic device. The first outlet may be further connected to a system of capillaries, which may further guide the excess liquid. The system of capillaries may form part of the nanofluidic device.

In yet another embodiment, each nanoslit comprises a slit outlet for discharging excess liquid from each nanoslit. As at the first outlet, also at the slit outlet, the liquid to be discharged may comprise very little or no bio-entities as these may be already all trapped within the nanopits positioned within the corresponding nanoslit. The slit outlet may be further connected to a system of capillaries, which may further guide the excess liquid. The system of capillaries may also form part of the nanofluidic device. Similarly, to the first outlet, the slit outlet at each nanoslit may create a fluidic pressure drop creating an overall difference between the pressure at the entrance of the nanoslit at the sidewall of the main passage towards the slit outlet. As above, this pressure difference may create a hydrodynamic flow of the liquid through the nanoslits. In another embodiment, each nanoslit may have two or more branches. More than two branches may be present, and each branch may in turn have two or more branches. In yet another embodiment there may be a system of capillaries between the main passage and each nanoslit.

In one embodiment, the plurality of nanopits may be arranged in a regular array/matrix. In another embodiment, the plurality of nanopits may be arranged in a triangular-shaped array, diamond-shaped array, circle-shaped array, star-shaped array, or the like. There is no limitation on a number of nanopits, it may be 1 within one nanoslit or it may be hundreds.

One nanoslit may contain 1-2000 nanopits, such as 1-1000, such as 1-500, such as 10-250, such as 50-100. The number of nanopits may determine a number of DNA molecules to be trapped by the nanopits. The array of nanopits may be etched in a nanoslit and may control the positioning and conformation of elastic bio-entities within the nanofluidic device. By organizing the array of the nanopits, namely a number of nanopits within a nanoslit, spacing between two neighbouring nanopits, spacing between the first nanopit and the sidewall of the main passage, etc. high control over the elastic bio-entities may be achieved. If the two neighbouring nanopits are separated by a distance, which is smaller than a single elastic bio-entity, then the bio-entity may extend between two adjacent nanopits. Furthermore, having the nanopits organized in a regular array/matrix may ensure better utilization of space within one nanoslit. Moreover, the position of each nanopit may be known when arranged in a regular array. Knowledge about the nanopit position and geometry of the nanopit array may be used in further measurements of DNA. However, the nanopits may not be organized in a regular array but may be positioned randomly within one nanoslit.

In one embodiment, the nanofluidic device may be formed of polymer material such as cyclic-olefin copolymer, or it may be formed of a silica wafer. Having a device made of polymer or silica provides a transparency of the device so that radiation can be introduced into the device if needed. Once the elastic bio-entities are trapped by the nanopits, further analysis may be performed. These analyses may be performed by ultra-violet (UV), infra-red (IR) or some other light wavelength range. Furthermore, fabrication processing of both polymer materials and silica is well-known and well-established, enabling fabrication of highly complex nano-devices.

In one embodiment of the invention, the first height of the main passage may be larger than 100 nm, such as larger than 500 nm, such as larger than 1 μm. The second height of the plurality of nanoslits may be below 1 μm such as below 200 nm, such as below 100 nm. The ratio between the width of the nanoslit at the sidewall of the main passage and the width of the nanoslit at its end may be larger than 10, such as larger than 20, such as larger than 30. The third height defining nanopits may be larger than 100 nm, such as larger than 200 nm. The width of nanopits may be in the range of 10 – 10000 nm. A nanopit may occupy an area such as 10x10 nm^2, such as 100x100 nm^2, such as 100x100 nm^2, such as 400x100 nm^2, such as 600x100 nm^2, such as 600x600 nm^2, such as 800x800 nm^2, such as 1000x1000 nm^2, such as 1000x500 nm^2. The edge distance between two adjacent nanopits may be in the range of 100 – 10000 nm. However, there is not a strict limit on both width of nanopits as well as the edge distance between two adjacent nanopits, i.e. an upper limit may be larger than above stated.

In a second aspect the present invention relates to a method for extraction of elastic bio-entities suspended in liquid, the method comprising:

a) providing a nanofluidic device according to the first aspect of the invention,

b) passing the liquid with the elastic bio-entities suspended therein through the main passage starting from the first inlet towards the first outlet

such that at least a portion of said elastic bio-entities suspended in liquid pass through said nanoslits,

and such that at least a portion of said elastic bio-entities suspended in the liquid are trapped in at least a portion of said nanopits and can be extracted therefrom.

As mentioned above, the main passage comprises a first inlet for introducing the liquid with the bio-entities suspended therein into the nanofluidic device, the inlet being the first end of the main passage. After the liquid is introduced through the first inlet of the main passage, it will continue to flow through the main passage and towards the first outlet. The first outlet is positioned at the second end of the main passage and may serve for discharging the liquid from the nanofluidic device. The fluidic pressure at the first outlet may be significantly lower than that at the first inlet. This pressure difference may drive the liquid flow along the main passage and through the entire nanofluidic device.

While passing through the main passage, at least a portion of the liquid together with suspended bio-entities will pass through the nanoslits extending outwardly from the sidewalls of the main passage. The pressure in each nanoslit may be lower than a pressure in the main passage and this pressure difference may drive the flow of liquid through the nanoslits.

While passing through the nanoslits, at least a portion of the elastic bio-entities suspended in the liquid will be trapped in at least a portion of nanopits within each nanoslit. This may enable further on-chip use and analysis of elastic bio-entities without the need for external actuation. The elastic bio-entities can also be extracted from the nanopits. If there is a need for the elastic bio-entities to be released from the nanopits the flow velocity may be increased so that bio-entities move quickly through the array of the nanopits.

In one embodiment of the invention, each nanopit may form an entropic trap which traps the elastic bio-entities. Each nanopit being a depression within a nanoslit, may trap an elastic bio-entity as the nanopit may be the place where the bio-entity may have the lowest entropy. For practical use and with a current technology 10 – 1000 nanopits per nanoslit may be

achieved. However, as stated above, there is no limitation on a number of nanopits per nanoslit. A number of nanopits may only determine a number of elastic bio-entities to be trapped therein. This number may not influence the trapping efficiency.

In one embodiment of the invention, the elastic bio-entities may self-assemble in the plurality of nanopits. The reason for this self-assembling is that the device modulates the energy provided to the elastic bio-entities over the entropic trap array. Thus, at given hydrodynamic flow rate, the bio-entities have more energy for entering the nanoslit from the main channel than from the nanopits, i.e. for escaping the entropic traps. This energy modulation is done by varying the width of the nanoslits so that the flow velocity varies. Varying the width of the nanoslits is to be understood as a continuous increase of the nanoslit width starting from the sidewall of the main passage towards the nanopit array. Thanks to this self-assembling the device may be used to purify for instance genomic DNA and an enzymatic reaction may be performed on the DNA while sitting in the entropic traps, i.e., nanopits.

In one embodiment, there may be an entropic energy cost of introducing the bio-entities into the nanopits. The elastic bio-entities are then physically trapped inside the nanopits and the entropic cost may further be used to keep them there. A force which may provide the entropic energy cost and which may force the elastic bio-entities into the nanopits may be the action of hydrodynamic flow of the liquid in which the elastic bio-entities are suspended. In another embodiment, the elastic bio-entities may be forced into the nanopits by an electrical force (electrophoresis), by an optical force, thermophoresis force or any other force suitable for this nanofluidic device. The remaining liquid and other possible cellular content may be washed away under the hydrodynamic flow.

In one embodiment of the invention, a second liquid may be applied to a nanofluidic device and through the plurality of nanopits without moving the elastic bio-entities once these have been settled in the nanopits. The elastic bio-entities would not be displaced from the nanopits as they have the highest entropy when sitting inside the nanopits. The second liquid may comprise chemical reagents such as enzymes to perform many reactions on bio-entities. By applying the second liquid as an enzymatic composition to a nanofluidic device, a reaction may be performed. In case the bio-entities are DNA molecules the reactions may be amplification, labelling, cutting with restriction enzymes, ligation, or the like. The second liquid may be any composition including but not limited to a composition that comprise enzymes, antibodies and/or specific ligands for reactions for labelling, detection, etc.

In yet another embodiment of the present invention, the hydrodynamic flow may be controlled by a pressure difference which may exist between the first inlet and the first outlet

of the main passage. This pressure difference may be formed by difference in the width of the main channel, namely the main passage may form a V-shape, being widest at the first inlet and narrower at the first outlet. Furthermore, a pressure difference may also exist between the main passage and the nanoslits extending from the sidewall of the main passage. This pressure difference may be created by difference in height which may exist between the main passage, nanoslits, and the slit outlets. It also may be created by difference in width which may exist along the main passage and/or nanoslits. At parts of the nanofluidic device with the highest pressure, the flow velocity of the liquid may be highest as well. Typically, at the places having the smallest width, the pressure may be the highest and therefore the flow velocity may also be highest.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates top view of a part of a nanofluidic device (a), cross-sectional view of the nanofluidic device (b), profile of the flow velocity inside the nanofluidic device (c), occupancy of the nanopits by the elastic bio-entities over time (d).

Fig. 2 illustrates an optical image of a nanofluidic device (a), enlarged view of a part of the illustration in Fig. 2a (b).

Fig. 3 is described in the experimental section and examples and illustrates occupancy at steady-state as a function of the pressure drop P (a), waiting time of 48 kb dsDNA molecules in single nanopits as a function of the pressure drop and nanopit size (b), waiting time for one (down triangle) and two (up triangle) molecules per nanopit events in 1000 nm pits (c). Error bars are the standard error on the mean. Waiting time is fitted according to transition state theory (dotted line).

DETAILED DESCRIPTION OF THE INVENTION

Fig. 1a illustrates top view of a part of a nanofluidic device. The nanofluidic device comprises a main passage 1. Only a part of the main passage is shown, namely only a part of the width along the axis x. When the liquid with the elastic bio-entities is injected into the device, a plurality of the bio-entities will be present within the main passage. For the purpose of illustration, only one bio-entity 4a is shown in Fig. 1a. A nanoslit 2 extends from a sidewall of the main passage 1. The nanoslit 2 has at first a small width, which then increases from the sidewall of the main passage 1 towards the matrix of nanopits 3. The flow velocity V_f therefore varies between the entrance from the main passage 1 and towards an array of nanopits. Namely, as the width increases the cross section increases and therefore the flow

velocity decreases. The nanoslit 2 comprises a plurality of nanopits 3 arranged in a 6x7 regular matrix. The hydrodynamic flow of the liquid drags the elastic bio-entity from the position 4a at first into the nanoslit 2, as indicated by position 4b. At this stage the bio-entity may expand under hydrodynamic force, or electrophoretic force if electrophoresis is used to move the bio-entities. Usually, one bio-entity may be trapped by one nanopit. In another embodiment there may be more than one bio-entity per nanopit. In yet another embodiment one bio-entity may be extended over several nanopits. Further, under the flow of the liquid the bio-entity 4b will be trapped by one of the plurality of nanopits 3 as indicated by a position 4c. Depending on the speed V_b of the bio-entity at the position 4c, the bio-entity may be permanently trapped or may continue to travel and finally be trapped by other nanopits. The nanoslit ends with the slit outlet 5.

In Fig. 1b it can be seen that the main passage 1 has a first height h_1 defined along the z axis from the top 7 of the nanofluidic device. Next section shows a nanoslit 2 having a second height h_2. The second height h_2 is smaller than the first height h_1. The third section of Fig. 1b shows that the nanoslit 2 comprises a plurality of nanopits 3, defining a third height h_3 being larger than the second height h_2. From the cross-sectional view of the nanofluidic device it can be seen that each nanopit 3 is placed at the bottom of the nanoslit 2. The slit outlet 5 has a height which is larger than the first height h_1 of the main passage.

A profile of the flow velocity V_f inside the nanofluidic device is illustrated in Fig. 1c. It can be seen that the velocity is the lowest in the first region, within the main passage, then it reaches its maximum at the part of the nanoslit having the smallest width. Further, the velocity decreases within the nanoslit. The decrease in the velocity is proportional to the nanoslit width change. When reaching the area with the nanopits the velocity remains constant, until it reaches its minimum at the slit outlet.

The graph shown in Fig. 1d illustrates occupancy of the nanopits by the elastic bio-entities versus time. Occupancy is represented on the axis O, and 1 corresponds to 360 nanopits occupied by the elastic bio-entities for a particular design of the nanofluidic device. The time is represented on the axis T with second time scale. The nanopits fill up linearly at the flow rate of the incoming bio-entities as represented by a region L. After this region there is a region S representing time when the flow reaches a steady-state where on average, the number of bio-entities coming into the nanopits balances out the bio-entities leaving the nanopits.

Fig. 2 illustrates an optical image of a nanofluidic device with a plurality of nanoslits. Fig. 2a illustrates the main channel 1 with two nanoslits 2 extending therefrom. The nanoslit 2 has two branches, i.e. nanoslits 2a and 2b. More branches may be present, and each branch may

in turn have two or more branches. In Figure 2a, each first-level branch is shown to split into two second-level branches. Nanoslits 2a and 2b comprise array of nanopits 3a and 3b. Fig. 2b illustrates zoom-in of the next stage of the nanoslit 2b illustrated in Fig. 2a. Arrays of the nanopits can be clearly identified in both nanoslits.

EXPERIMENTS AND EXAMPLES

An enzymatic labelling of genomic DNA arranged in a dense array of single molecules without surface attachment to the enzyme or the DNA is performed. To realise the dense array of single DNA molecules, genomic DNA is concentrated to 100 µg/mL in an array of nanopits being entropic traps. Genomic DNA placed in a nanoslit settle inside the nanopits to minimise its entropic energy. Genomic DNA are arranged therein before the enzymatic reaction takes place. As mentioned above, DNA can be moved from the liquid where they are suspended to the confinement region using hydrodynamic force, electrophoresis, or the like. The nanopits may be loaded to more than 90% of its total capacity at a flow velocity of 0.01 nL/min. By adding reagents to the genomic DNA, without displacing them from the nanopits, labelling by synthesis using polymerase can be performed.

The nanofluidic device used for this experiment is fabricated by injection moulding of a cyclic-olefin copolymer with a Ni master. The array of nanopits is defined at a 200 nm depth by E-beam lithography and reactive ion etching in a silicon substrate. The nanoslits are defined by UV-lithography and etched at a 100 nm depth. The main passage is defined by UV-lithography and etched a 5 µm depth. The square nanopits are arranged in square array with a 2 µm pitch and the nanopit area is 600×600, 800×800 or 1000×1000 nm^2. The nanoslit is 5 µm wide at the entry and branches out in eight parallel 20 µm wide nanoslits over the nanopit array thus providing a 32 fold reduction of the flow velocity that enables to accumulate DNA in the nanopit array (see Fig. 2a). The Ni master is replicated in COC (TOPAS 5013) by injection moulding. The injection moulded parts are sealed with a 150 µm thick COC foil. λ-phage DNA is stained with YOYO-1 (Invitrogen) at a 5:1 staining ratio raising the persistence length to 64 nm and the contour length of λ-phage DNA to 21 µm. DNA is diluted to 1 µg/mL in a buffer consisting of 0.5TBE + 1% BME + 0.5% Triton-X 100 to suppress photo nicking and stiction to the polymer surface.

For the enzymatic reaction, the device was coated using a 1mM solution POPC:POPG 3:1 in 5% ethanol (Avanti Lipids). DNA is diluted to 1 µg/mL in a buffer consisting of 0.5×TBE + 1% BME and loaded into the array. The reaction was performed under a mild flow of a reagent mix (Polymerase Phi 29 1 unit, 20 µM of dATP, dGTP, dTTP, 10 µM of dCTP and 25 µM of Cy3-dCTP in 1× reaction buffer) for 2 hours at 30°C. The DNA was washed in

0.5×TBE + 1% BME before imaging. For waiting time measurements, λ-phage DNA molecules were introduced to the array of nanopits so they become isolated in single nanopits. The waiting time was exponentially distributed for a given pressure drop and nanopit size (P-value check). The mean value was plotted against the pressure drop with the standard error on the mean as error bars. The waiting time was fitted to the transition state theory model. Experimental values for the flow rate of incoming molecules, r_{in}, were obtained from the linear part of the filling curves (Fig. 1d) for each pressure drop and nanopit size. The r_{in} values used for simulation were extrapolated from an origin-intersecting fit. The waiting time values were extrapolated from the fit in Fig. 3b and used in the simulation of the nanopit array filling used to plot the fractional occupancy at steady state in Fig. 3a.

As a model system, DNA is labelled with λ-DNA non-specifically by incorporating labelled nucleotides directly into the double-stranded DNA using polymerase phi29. Phi29 has the ability to synthesise the complementary DNA strand at a single strand nick. Consequently, demonstrating DNA synthesis by phi29 can be considered a model reaction for sequencing-by-synthesis or sequence barcoding. The densely packed array of single DNA molecules was realised by the particular nanoslit geometry of the nanofluidic device described above. By designing the nanopit array with a 2 µm pitch larger than the diffraction limit, it is ensured that single DNA molecules labelled with YOYO-1 can be addressed individually by epifluorescence microscopy. The experiment is designed so that DNA has a uniform and only one length (48 kb) so it is possible to distinguish cases where single molecules occupy n = 1, 2,… nanopits. The focus is on the mobility of DNA through such an array of nanopits and on conditions to maximise the nanopit occupancy under a constant flow. The largest entropic barrier to be overcome by the DNA is to transit from the main passage to the nanoslits (see Fig. 1a). Once DNA is in the nanoslit, it settles into the nanopits to minimise its entropic energy. The entropic barrier to be overcome for escaping the nanopits is smaller. However, the probability for the molecules to escape confinement of the nanopits depends on the entropic barrier and the Stokes drag, i.e. on the fluid velocity. As described above, in order to accumulate the DNA molecules in the nanopit array, the fluid velocity is modulated by varying the nanoslit width so that the molecules experience a lower Stokes drag (more than an order of magnitude) when settled in the nanopits than when entering the nanoslit. As a result, the DNA accumulates in the entropic trap array under constant flow as it is shown in Fig. 1c

As mentioned above, an injection moulded polymer device made of cyclic-olefin copolymer is used in the experiment. Before introducing the DNA, the entropic trap array is coated by phospholipids forming a supported monolayer on the hydrophobic polymer surface and thus preventing nonspecific interaction of the polymer surface with the DNA and the enzyme. The reaction is performed by introducing a reaction buffer including phi29 polymerase,

magnesium, ATP and the four nucleotides one of which is labelled with the fluorophore Cy3. After the reaction, excess nucleotides can effectively be washed away and the fluorescence signal of the incorporated nucleotides is observed on the genomic DNA remaining in the individual nanopits. The signal of the Cy3-labelled nucleotides incorporated to the DNA is subjected to Brownian motion in the nanopits and is thus easily distinguishable from surface defects or defects in the lipid coating. The molecules that are labelled are distributed randomly in the array which indicates the polymerase and the reagents have been introduced to all molecules in the array. There is no indication that Cy3 signal and YOYO-1 signal are correlated and there is no significant background Cy3 signal in the wells that are not occupied by DNA. Moreover, in nanopits where both YOYO-1 and Cy3 are detected, it is observed that the Cy3 signal moves around the nanopit while the DNA signal covers the whole area of the nanopit. This is consistent with the polymerase incorporating labelled nucleotides locally along the DNA molecule.

In this experiment single strand nicks is detected on genomic DNA by fluorescence labelling. Relatively few molecules carry such a nick as expected since nicks occur accidentally by mechanical shearing during the handling or by photo nicking during imaging. Instead of labelling the nicks by a polymerase reaction, one could repair nicks using a ligase as done in library preparation protocols for sequencing. In addition, single strand nicks can be created by a sequence specific enzyme thus our experiment is a step toward on-chip labelling for optical mapping.

The flow conditions for loading a nanopit array with single molecules as densely as possible is dependent on the geometry of the nanoslit and nanopits as well as the length of the DNA molecules. The experiment is performed for three different devices where the nanopit area is varied (600×600, 800×800 or 1000×1000 nm^2) so that the hopping rate of DNA molecules between the nanopits is modified. Fig. 3a illustrates steady-state occupancy (axis O) and Fig. 3b illustrates waiting time (axis T in seconds) of single molecules in single nanopits as a function of increased pressure drop (axis P in bar). As expected, the smaller the nanopit size is, the longer the waiting time is and so the lower the fractional occupancy at steady-state is.

The waiting time depends exponentially on the energy barrier to escape the trap according to transition state theory. In the case of 800 nm and 1000 nm nanopits, the fractional occupancy at steady-state can be simulated using the r_{in} and the hopping rates. Using the values of r_{in} extracted from curves as in figure 1d and the hopping rate of the first molecule (Fig. 3b) and the second molecule (Fig. 3c) in 1000 nm and 800 nm nanopits, it is possible to simulate the fractional occupancy versus pressure curves (Fig. 3a). A flow rate within the device is 8 pl/min (i.e. the flow velocity is 9 µm/s at 0.25 bar) allows to load an array of 800 nm and 1000 nm pits to 90% of the capacity with 48 kb-long molecules. Decreasing the

flow rate further does not increase the loading significantly. In Fig. 3a the loading of the array is dramatically reduced for nanopit size of 600 nm compared to a nanopit size of 800 nm and 1000 nm. In a 600 nm nanopit array, 48 kb-long DNA molecules can extend over two nanopits at rest while it fits in a single nanopit for the larger nanopits. When a molecule occupies more than one nanopit, it experiences a higher drag and moves faster through the array before it collapses in a single nanopit or escapes the array. The fraction of the incoming molecules that initially settle in several nanopits is thus much higher in the 600 nm nanopit array and thus explains the dramatic reduction of loading observed (Fig. 3a) beyond the mere reduction in waiting time for molecules that are settled in a single 600 nm nanopit.

Each nanopit size thus represents a different case that help understand what would happen if a sample of DNA molecules with heterogeneous sizes are accumulated in an entropic trap array. At 800 nm, a single 48 kb molecule can fit in one nanopit while at 1000 nm two molecules can fit in and at 600 nm, a single molecule can extend in two neighbouring nanopits. This is observed in the experiment at $\Delta P=0$ and confirms the entropic energy calculations using the molecule contour length and the nanopit array geometry. It shows that in order to accumulate molecules of different sizes at a constant rate, the nanopit size should be varied through the array according to the size distribution of the DNA sample.

The DNA concentration in the array at complete loading is 100 µg/mL corresponding to a 100 fold increase. Higher loading can be achieved by increasing the waiting time i.e. the entropic barrier. Since reducing the slit height results in a much lower flow rate, higher loading should be achieved by increasing the nanopit depth. Increasing the total length of the array also increases the fraction of the DNA retained in the nanopit array however, this also yield to a lower local density of DNA (i.e. fractional loading). The 1000 nm nanopit array contains 14.4 Mbp of DNA. It can be estimated that in the experiment, a 0.6 mm^2 array could accommodate the whole genome of a human cell (2×3.4 Gb), namely one cell in one mm^2.

LIST OF REFERENCES

1 – main passage
2 – nanoslit
3 – nanopits
4 – elastic bio-entities
5 – slit outlet
7 – top surface of the nanofluidic device
V_b – velocity of the elastic bio-entity

V_f – flow velocity

CLAIMS

1. A nanofluidic device for extraction of elastic bio-entities suspended in liquid, the device comprising a main passage and a plurality of nanoslits extending from a sidewall of the main passage;

   - the main passage having a first height,
   - each nanoslit having a second height, the second height being lower than the first height,
   - each nanoslit comprising a plurality of nanopits, defining a third height being larger than the second height, and
   - each nanopit being at the bottom of a nanoslit;

   wherein each nanoslit has a width which increases from the sidewall of the main passage towards the nanopits.

2. The nanofluidic device according to claim 1, wherein the bottom of the main passage is lower than the bottom of the nanopits and the bottom of the nanopits is lower than the bottom of the nanoslit.

3. The nanofluidic device according to claims 1 or 2, wherein the main passage comprises a first inlet for introducing the liquid with the bio-entities suspended therein into the nanofluidic device, the inlet being positioned at one end of the main passage.

4. The nanofluidic device according to any of the preceding claims, wherein the main passage comprises a first outlet for discharging the liquid from the device, the outlet being positioned at the end of the main passage from the first inlet.

5. The nanofluidic device according to any of the preceding claims, wherein each nanoslit comprises a slit outlet for discharging excess liquid from each nanoslit.

6. The nanofluidic device according to any of the preceding claims, wherein a nanoslit has two or more branches, wherein each branch comprises a plurality of nanopits.

7. The nanofluidic device according to any of the preceding claims, wherein the plurality of nanopits are arranged in a regular array/matrix.

8. The nanofluidic device according to any of the preceding claims, wherein the device is formed of polymer material or a silica wafer.

9. The nanofluidic device according to any of the preceding claims, wherein the first height being larger than 100 nm, such as larger than 500 nm, such as larger than 1 µm, the second height being below 1 µm such as below 200 nm, such as below 100 nm, the third height being larger than 100 nm, such as larger then 200 nm, and wherein the ratio between the width of the nanoslit at the sidewall of the main passage and the width of the nanoslit at the end is larger than 10, such as larger than 20, such as larger than 30.

10. A method for extraction of elastic bio-entities suspended in liquid from said liquid, the method comprising:

a) providing a nanofluidic device according to claims 1-9,

b) passing the liquid with the elastic bio-entities suspended therein through the main passage starting from the first inlet towards the first outlet

such that at least a portion of said elastic bio-entities suspended in liquid pass through said nanoslits,

and such that at least a portion of said elastic bio-entities suspended in the liquid are trapped in at least a portion of said nanopits and can be extracted therefrom.

11. The method according to claim 10, wherein each nanopit forms an entropic trap trapping the elastic bio-entities.

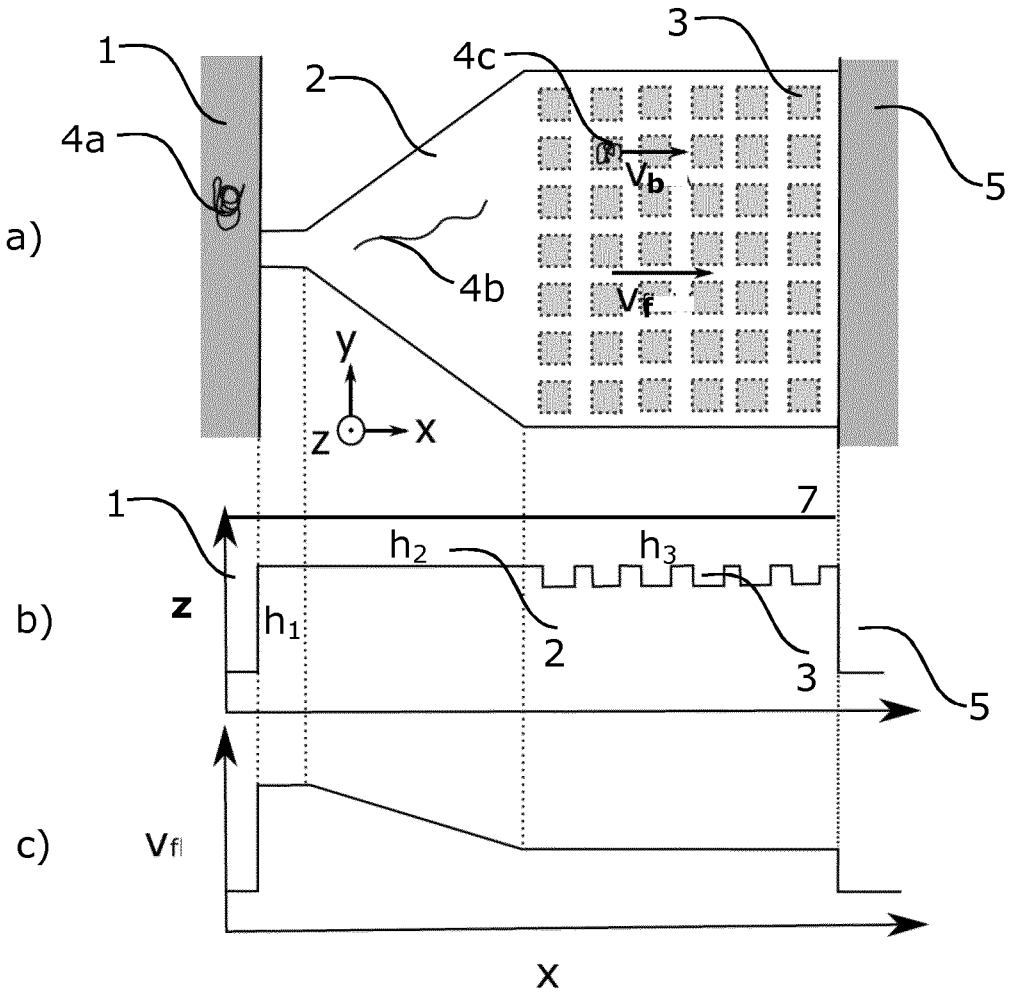
12. The method according to claims 10 or 11, wherein the elastic bio-entities self assemble in the plurality of nanopits.

13. The method according to any of claims 10-12, wherein the elastic bio-entities are forced into the nanopits by the action of flow of the liquid in which the elastic bio-entities are suspended.

14. The method according to any of claims 10-13, wherein a second liquid is applied through the plurality of nanopits without moving the elastic bio-entities from the nanopits.

15. The method according to any of claims 10-14, wherein the liquid flow is controlled by a pressure difference which exists between the first inlet and the first outlet of the main passage.

Fig. 1 1/4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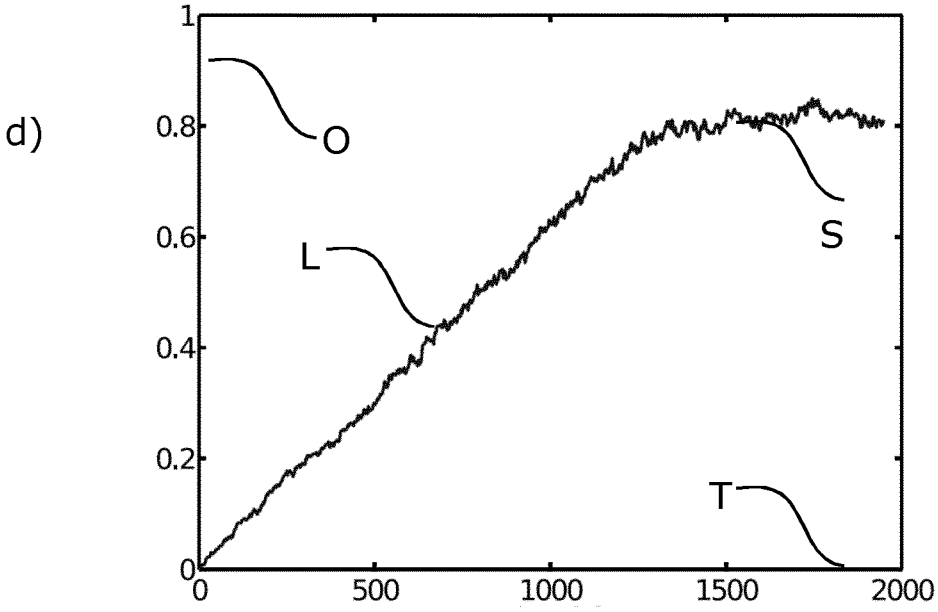
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Fig. 2

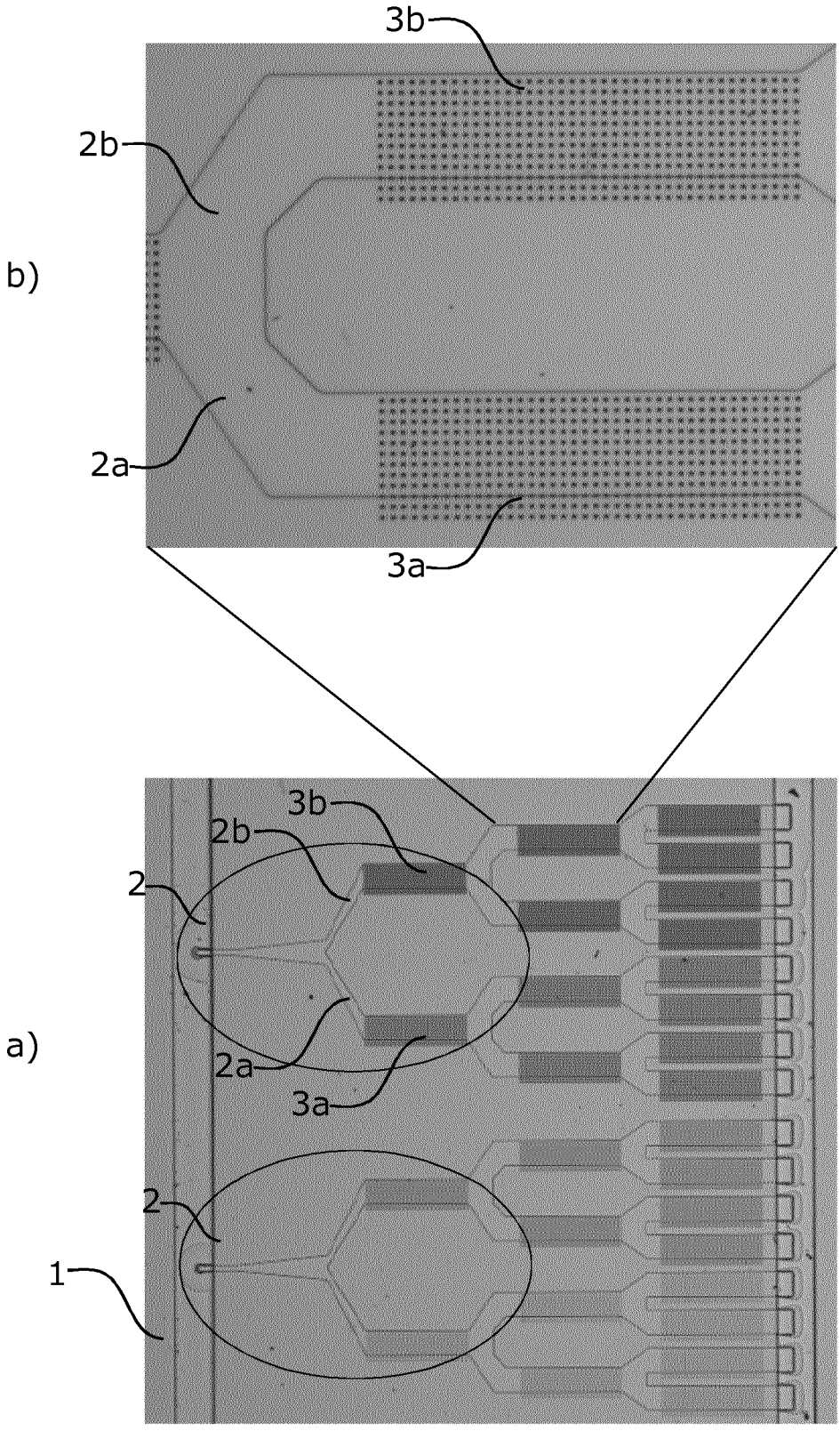
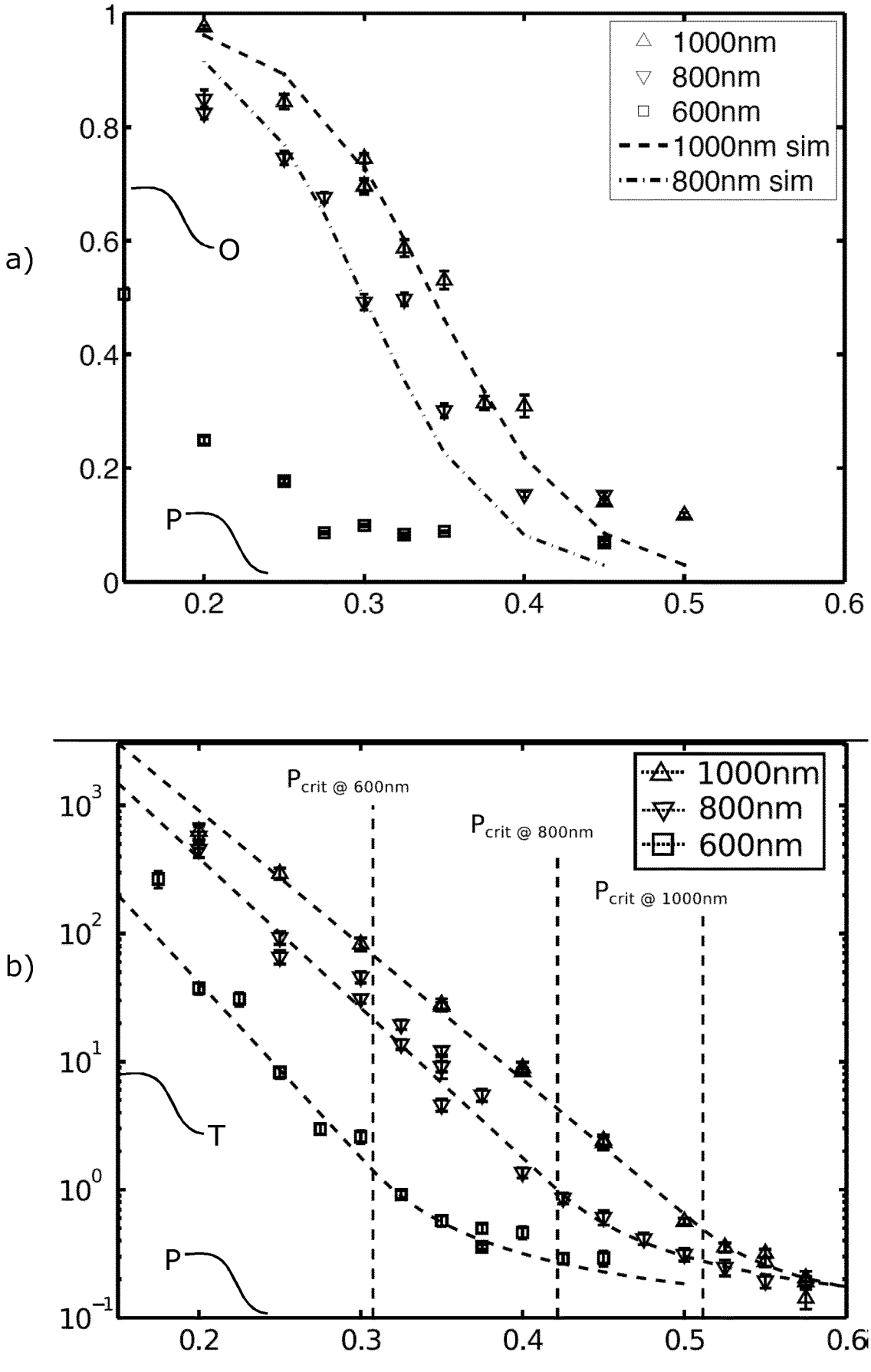


Fig. 3


a)
1000nm
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600nm
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P
b)
$P_{crit\ @\ 600nm}$
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$P_{crit\ @\ 1000nm}$
1000nm
800nm
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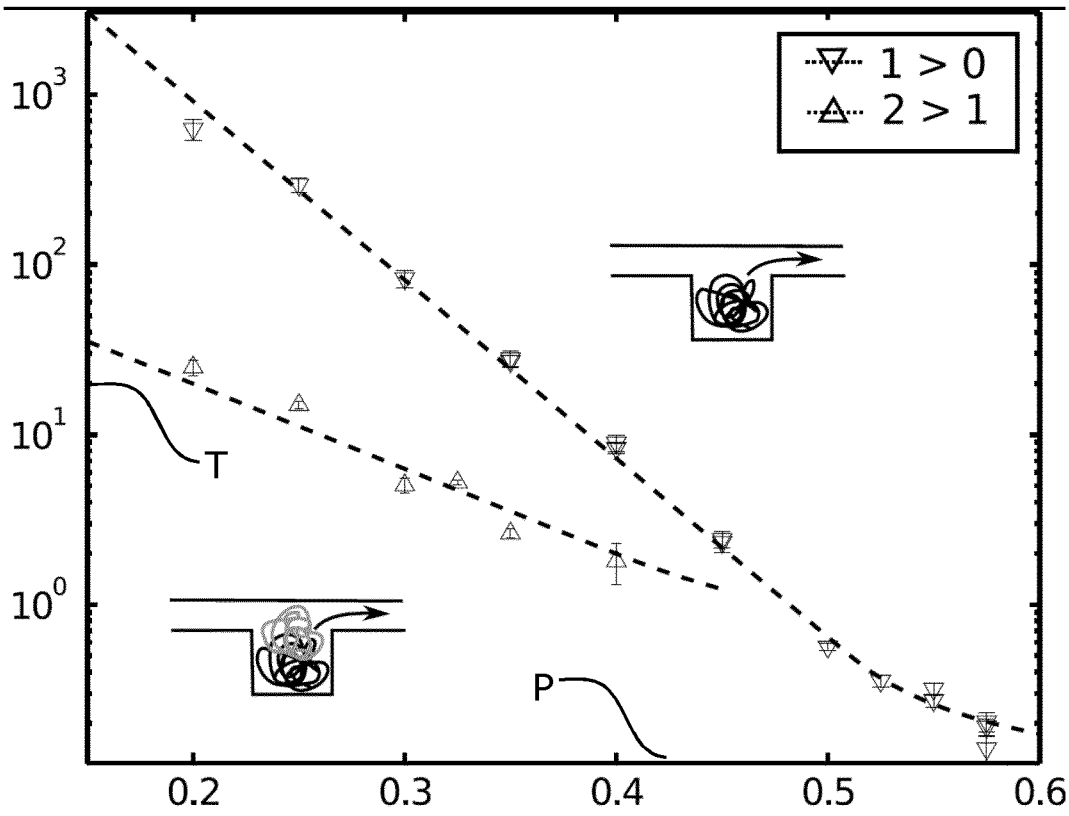
P

Fig. 3

c)



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/066894

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L3/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01L

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2015/323499 A1 (YOBAS LEVENT [CN] ET AL) 12 November 2015 (2015-11-12) paragraphs [0002], [0058]; figures 1a, 1c	1-15
A	US 2010/307617 A1 (MIURA TORU [JP] ET AL) 9 December 2010 (2010-12-09) paragraph [0281]; figure 30a	1-15
A	WO 2007/088517 A2 (ECOLE POLYTECH [CH]; RENAUD PHILIPPE [CH]; LINDERHOLM PONTUS [CH]; BRA) 9 August 2007 (2007-08-09) figure 0040	1-15

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

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2017	07/09/2017

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	W. REISNER ET AL: "Directed self-organization of single DNA molecules in a nanoslit via embedded nanopit arrays", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 106, no. 1, 2 January 2009 (2009-01-02), pages 79-84, XP055328276, US ISSN: 0027-8424, DOI: 10.1073/pnas.0811468106 the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/066894

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