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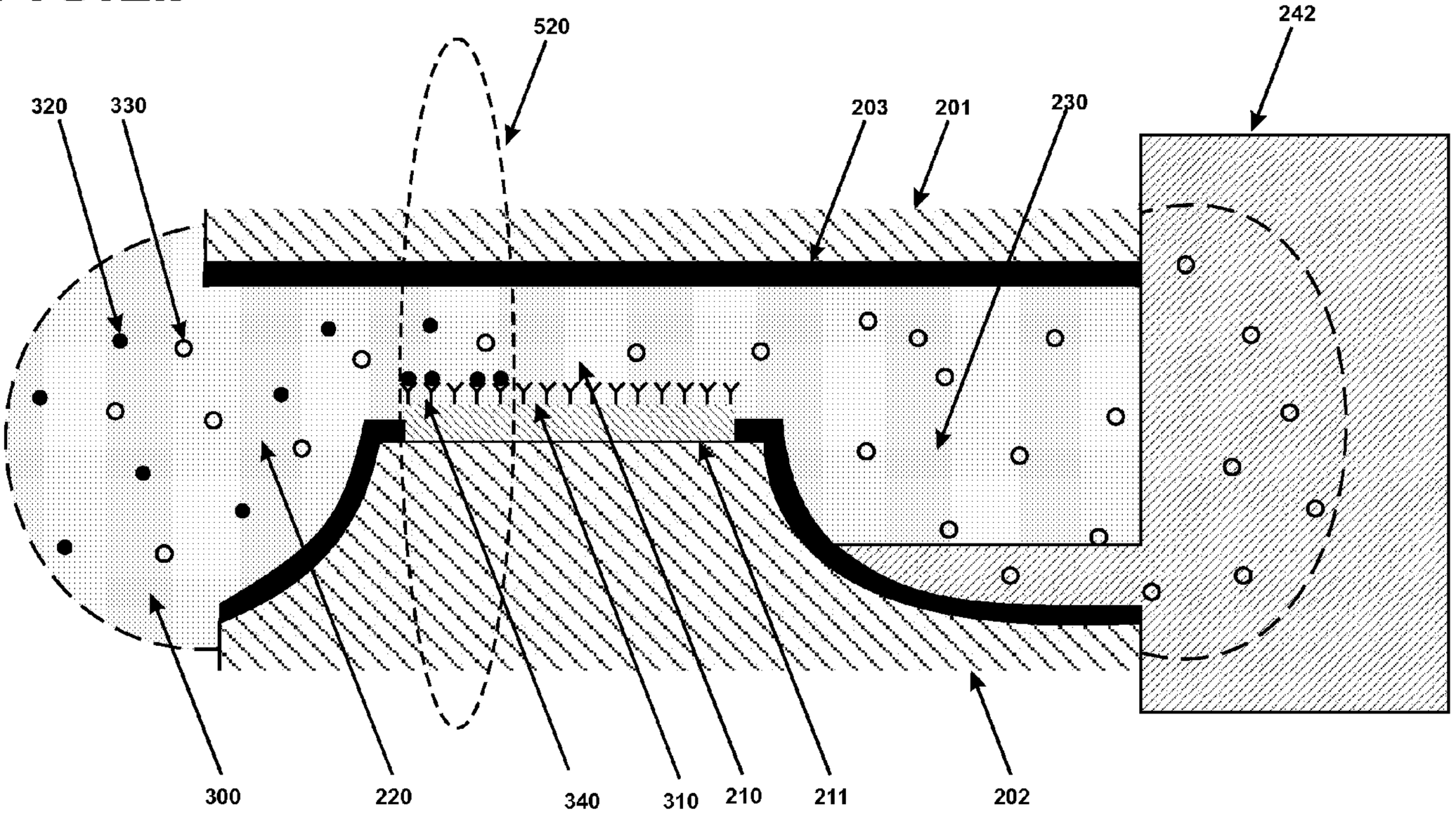
(54) Titre : QUANTIFICATION RAPIDE DE BIOMOLECULES DANS UN BIOCAPTEUR NANOFLUIDIQUE SELECTIVEMENT FONCTIONNALISE ET PROCEDE ASSOCIE

(54) Title: RAPID QUANTIFICATION OF BIOMOLECULES IN A SELECTIVELY FUNCTIONALIZED NANOFLUIDIC BIOSENSOR AND METHOD THEREOF

FIG.2b



(57) Abrégé/Abstract:
A method and device for the rapid quantification of biomolecules (320) present in a nanochannel (210) is claimed. In particular, the present invention relates to a novel concept of liquid actuation and selectively functionalized surfaces in a nanochannel that create a concentration gradient of transitory immobilized biomolecules (340) across the nanochannel. The present concept enables the quantification of biomolecular interactions of interest (320).

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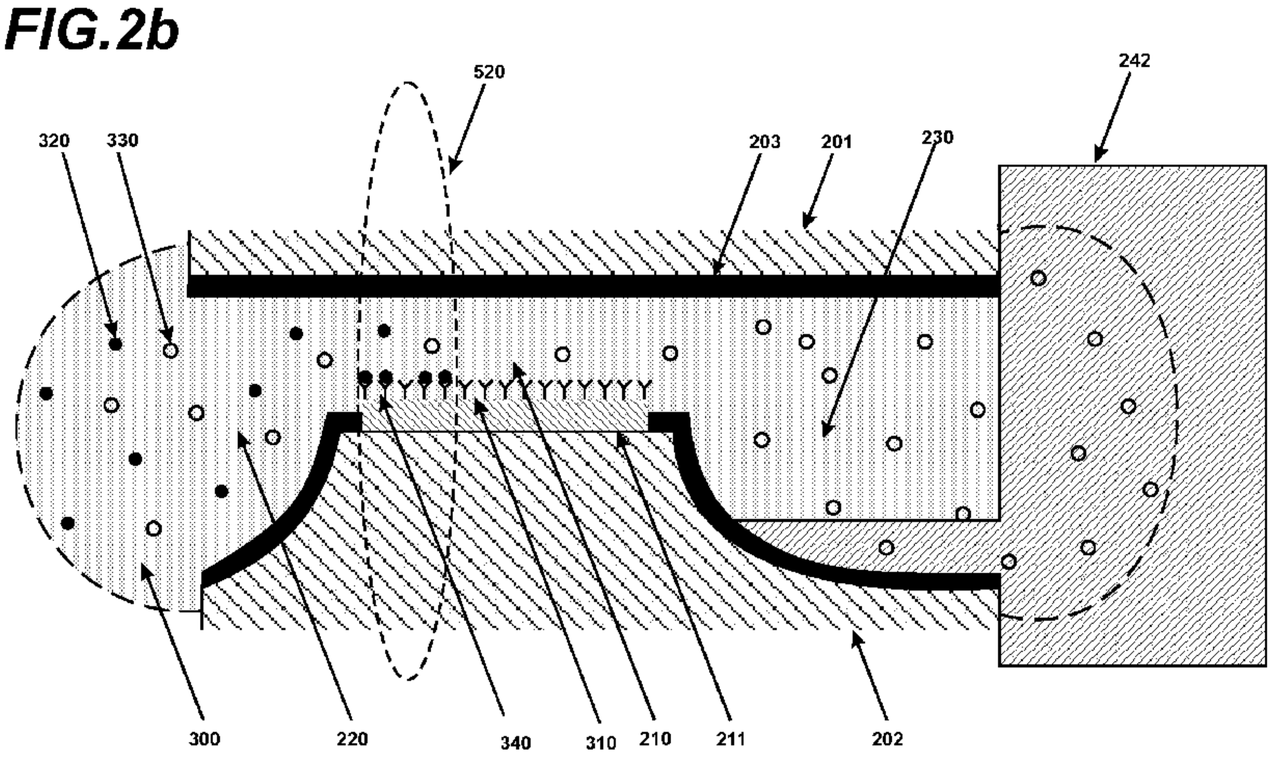
(54) Title: RAPID QUANTIFICATION OF BIOMOLECULES IN A SELECTIVELY FUNCTIONALIZED NANOFLUIDIC BIO-SENSOR AND METHOD THEREOF

FIG.2b



(57) Abstract: A method and device for the rapid quantification of biomolecules (320) present in a nanochannel (210) is claimed. In particular, the present invention relates to a novel concept of liquid actuation and selectively functionalized surfaces in a nanochan - nel that create a concentration gradient of transitory immobilized biomolecules (340) across the nanochannel. The present concept enables the quantification of biomolecular interactions of interest (320).

WO 2012/120387 A1

RAPID QUANTIFICATION OF BIOMOLECULES IN A SELECTIVELY FUNCTIONALIZED NANOFLUIDIC BIOSENSOR AND METHOD THEREOF

Field of invention

[0001] The present invention relates to methods and devices for the detection of fluorescently labeled biomolecules in selectively functionalized nanofluidic biosensors, using an optical system. The present invention may advantageously be used for rapid quantification of biomedical and biological samples.

Background of the invention

[0002] Nanofluidic biosensors are defined as fluidic systems with nanometer-sized confinements and/or lateral apertures, which are used to quantify the presence of biomolecules in a solution. A majority of the current nanofluidic biosensor developments are intended for bioengineering and biotechnology applications. In the scope of this invention, biosensors are used to quantify the presence of biomolecules in solution for in vitro diagnostic applications.

[0003] Swiss patent application CH 01824/09 discloses biosensors with lateral apertures for the detection of biomolecular interactions and PCT application IB2010/050867 discloses their use with simple optical systems. The diffusion of biomolecules in these configurations are slow and require either long waiting times to attain stable measurement conditions or highly concentrated solutions for the observation of the biomolecular interactions.

[0004] Biomarkers, also called biological markers, are substances used as specific indicators for detecting the presence of biomolecules. It is a characteristic that is objectively measured and evaluated as an indicator of biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

[0005] Current practices for the detection of specific biomolecules can be divided in two categories: (a) the labeled techniques and (b) the label-free techniques.

[0006] Among the labeled techniques, the widely used are fluorescence, colorimetry, radioactivity, phosphorescence, bioluminescence and chemiluminescence. Functionalized magnetic beads can also be considered as labeling techniques. Labeled techniques advantages are the sensitivity in comparison to label-free methods and the molecular recognition due to specific labeling.

[0007] Among the label-free techniques, the widely used are electrochemical biosensors, referring to amperometric, capacitive, conductometric or impedimetric sensors, which have the advantage of being rapid and inexpensive. They measure the change in electrical properties of electrode structures as biomolecules become entrapped or immobilized onto or near the electrode, but all these concepts lack molecular specific contrast, sensitivity and reliability.

[0008] Enzyme linked immunosorbent assay (ELISA) is an important biochemical technique mainly used to detect the presence of soluble biomolecules in serum, and thus is widely used as diagnostic tool in medicine and quality control check in various industries. ELISA analysis are however expensive, require large amounts of solution and is time consuming.

[0009] The other important technologies for biomolecular diagnostics are Western and Northern blots, protein electrophoresis and polymerase chain reaction (PCR). However, these methods require highly concentrated analytes and do not allow high throughput samples testing.

Objectives

[0010] It is an object of this invention to provide inexpensive and rapid nanofluidic biosensors, which do not require complex manipulations.

[0011] Still another object of the invention is to geometrically confine the optical measurement volume using nanofluidics, and to selectively functionalize nanochannel surfaces in order to obtain a high sensitivity of the biosensor.

[0012] Still another object of the invention is to enhance the sensitivity of the detection by forcing a convective flow across a nanometer-sized confinement

(nanochannel) in order to increase the probability for the biomolecules to interact with immobilized biomarkers.

[0013] These and other objects of the present invention will become increasingly apparent with reference to the following drawings and preferred embodiments.

Summary of the invention

[0014] This invention is based on the discovery that forcing biomolecules to enter into a nanometer sized confinement that has selectively functionalized surfaces strongly increase the probability for the biomolecules to interact with immobilized biomarkers. This allows quantifying the presence of fluorescently-labeled biomolecules at ultra-low concentration.

[0015] This invention is also based on the discovery that monitoring the photobleaching of the fluophores attached to the biomolecules can be used to differentiate between biomolecules that have interacted with biomarkers and are immobilized in the nanochannel, and those that are simply diffusing through the detection volume.

[0016] Furthermore, this invention highlights the possibility to use a driving component to force the convective flow of the solution to analyze through the nanochannel.

[0017] In the present text the term “driving component” has to be understood as any element, for instance an absorbing element, which can be used for facilitating the solution flow through the nanochannel.

[0018]

[0019] In the scope of this invention, nanofluidics is used because of its high surface-to-volume ratio, meaning that the surfaces included in the detection volume, maximize the probability of the interactions between biomolecules and immobilized biomarkers on surfaces. It also strongly reduces the background signal of the solution due to the small portion of substrate that is within the detection volume.

[0020] The invention therefore relates to a biosensor as defined in the claims.

[0021] It also relates to an assembly and a method using said biosensor.

Brief description of the drawings

[0022] FIGURE 1a is a perspective view of a capsule system **101** containing an array of nanofluidic biosensors **200**. A solution **300** containing fluorescently-labeled biomolecules is deposited inside the capsule **101** by a pipet system **400**. An optical system **500** based on a laser beam **510** is used for the measurement.

[0023] FIGURE 1b is a perspective view of a surface **102** containing an array of nanofluidic biosensors **200**. A solution **300** containing fluorescently-labeled biomolecules is deposited on the surface **102** by a pipet system **400**. An optical system **500** based on a laser beam **510** is used for the measurement.

[0024] FIGURE 2a shows a cross section of the nanofluidic biosensor defined by two substrates **201** and **202** that are locally structured by areas **211** that are functionalized by biomarkers **310** and other areas **203** that prevent that functionalization. Reagent solution **300** containing biomolecules enter the nanochannel **210** and is actuated by the external driving component **241**. The laser beam **510** monitors the photobleaching of the immobilized biomolecules **340** in the detection volume **520**.

[0025] FIGURE 2b shows a cross section of the nanofluidic biosensor defined by two substrates **201** and **202**. Only one of the substrates is locally structured by area **211** that is functionalized by biomarkers **310** and other areas **203** that prevent that functionalization. Reagent solution **300** containing biomolecules enter the nanochannel **210** and is actuated by the internal driving component **242**. The laser beam **510** monitors the photobleaching of the immobilized biomolecules **340** in the detection volume **520**.

[0026] FIGURE 3 illustrates the concentration evolution with time of specific biomolecules over the nanochannel length.

[0027] FIGURE 4 shows the concentration profile of specific biomolecules over the nanochannel length for a given time t_1. The marked area represents the detected portion of specific biomolecules.

[0028] FIGURE 5 illustrates the concentration evolution with time of non-specific biomolecules (background) over the nanochannel length.

[0029] FIGURE 6 shows the concentration profile of non-specific biomolecules over the nanochannel length for a given time t_1. The marked area represents the detected portion of specific biomolecules, corresponding to the background noise.

[0030] FIGURE 7 illustrates a standard photobleaching curve of fluorophores attached to immobilized specific biomolecules.

[0031] FIGURE 8 illustrates the fluorescence intensity curve in function of time for non-specific biomolecules inside the nanochannel, showing that only background noise is detected.

[0032] FIGURE 9 shows the solution flow velocity inside the nanochannel in function of time.

Detailed description of the invention

[0033] As used herein, the term "biomolecules" is intended to be a generic term, which includes for example (but not limited to) proteins such as antibodies or cytokines, peptides, nucleic acids, lipid molecules, polysaccharides and virus.

[0034] As used herein, the term "nanochannel" is intended to be a generic term, which means well-defined microfabricated structure with at least one nanometer-sized dimension. The nanometer-sized dimension of the nanochannel is defined to be higher than 2 nm because of the size of the smallest biomolecules to be detected that have to enter into the slit and that are in the same order of magnitude. The present invention is limited to nanochannels with a height lower than one micron, because of the range of the detection volume of the optical system that are typically in the same order of magnitude.

[0035] The present invention aims to enhance the detection of biomolecules by increasing the probability of interactions with specific biomarkers due to the confinement of functionalized surfaces. As shown in **FIGURE 1a** and **FIGURE 1b**, an array of nanofluidic biosensors **200** is immobilized in a capsule system **101** or on a surface **102**. A mix solution **300** containing the fluorescently-labeled biomolecules of interest is disposed inside the capsule **101** or on the surface **102** by a pipet system **400**. The capsule **101** may be hermetically closed in order to avoid contamination.

Finally, an optical unit **500** is used to measure the biomolecular interactions inside the biosensors **200** by focusing the laser beam **510** inside the biosensors nanochannel.

[0036] FIGURE 2a and **FIGURE 2b** illustrate the principle of detection and the cross-section of a biosensor according to the invention. The system is composed of a nanochannel **210** linking a lateral input aperture **220** with a lateral output aperture **230**. A driving component that can be external (**241**) or internal (**242**) is located next to the lateral output aperture **230**. First, biomarkers **310** are immobilized on selectively functionalized nanochannel surfaces of one or both substrates **201** and **202**. The other nanochannel surfaces and the lateral aperture surfaces may be protected by the deposition of a non-functionalized layer **203**. The detection volume **520** has to be focused inside the nanochannel **210** such as the intersection volume defined by the volume of the nanochannel **210** and the detection volume **520** is maximal, and directly next to the lateral input aperture **220**. Next, the solution **300** containing the fluorescently labeled specific biomolecules **320** and non-specific biomolecules **330** is filled into the system from the lateral input aperture **220** by capillarity. When reaching the driving component **241** or **242**, the solution **300** fills the driving component by absorption for example, leading to a forced convective flow across the biosensor. When the driving component **241** or **242** achieves its maximum filling capacity, the convective flow stops and the system reaches equilibrium. During the convective flow and thanks to Brownian motion, biomolecules **320** interact with the biomarkers **310** immobilized inside the nanochannel **210** and may create molecular complexes **340**. A concentration gradient is obtained across the nanochannel **210**. The non-specific biomolecules **330** will diffuse in the nanochannel **210** but will not form molecular complexes with the immobilized biomarkers **310**. Non-specific biomolecules **331** will be present in the lateral output aperture **230**, and some **332** may also be present inside the driving component **241** or **242**. When excited by the laser beam **510**, the immobilized fluorescently emitting complexes **340** and the diffusing fluorescently emitting biomolecules **330** diffusing across the optical detection volume are both detected by the optical system.

[0037] The present invention is distinguishable from techniques currently being used to detect molecular interactions. The unique method of measuring the concentration of immobilized complexes across the selectively functionalized nanochannel being linked to lateral apertures is different from current techniques based on measuring

interactions on a single surface or reservoir. These solutions do not benefit from the increased probability of interaction events that occur in the unique design presented in this patent.

[0038] FIGURE 3 shows the evolution of concentration with time across the biosensor when the solution contains specific biomolecules. Directly after the capillary filling, at time t_0, there is a background concentration c_0 of fluorescently labeled molecules inside the lateral input aperture. Specific biomolecules that enter into the nanochannel interact quickly with the nanochannel functionalized surfaces, leading to an increase of concentration (dashed curve). The maximum concentration c_{sat} corresponds to the case where, for a given x position, all biomarkers have interacted with specific biomolecules. In function of time, the concentration gradient will tend to the t_{inf} dotted curve, corresponding to the total saturation of the nanochannel biomarkers (dotted curve).

[0039] FIGURE 4 illustrates the concentration gradient across the biosensor at a time t_1, corresponding to the case when the solution has already filled the biosensor as well as the absorbing component. Thanks to Brownian motion, the biomolecules continue to enter the nanochannel and continue to interact with the biomarkers, but depending on the background concentration c_0, the transition to saturation t_{inf} may be very long. This allows a stable measurement of the concentration profile across the nanochannel. The measurement volume (hatched area) corresponds to the intersection of the laser beam with a width b and the nanochannel.

[0040] FIGURE 5 shows the concentration evolution with time across the biosensor when the solution contains only non-specific biomolecules. Directly after the cap$_i$llary filling, at time t_0, a background concentration c_0 of fluorescently labeled molecules is present inside the lateral input aperture and the nanochannel. No further concentration increase is expected as there is no interaction with the functionalized surfaces. In this case, the concentration c_0 remains constant for all x positions and with time.

[0041] FIGURE 6 illustrates the concentration gradient across the biosensor at a time t_1, corresponding to the case when the solution contains no specific biomolecules and has already filled the biosensor as well as the absorbing component. The measurement volume (hatched area) corresponds to the intersection of the laser beam with a width b and the nanochannel.

[0042] FIGURE 7 shows the fluorescence intensity evolution with time during measurement, for a given position inside the nanochannel, when the solution contains specific biomolecules. The measurement starts when the shutter of the optical system opens. A standard photobleaching curve is obtained containing quantitative information on the number of immobilized and fluorescently-labeled molecules present within the measurement volume.

[0043] FIGURE 8 shows the fluorescence intensity evolution with time during a measurement, for a given position inside the nanochannel, when the solution does not contain any specific biomolecules. The measurement starts when the shutter of the optical system opens. No photobleaching curve is obtained, since there are only diffusing fluorescently-labeled biomolecules inside the measurement volume leading to a constant background signal.

[0044] FIGURE 9 shows the evolution of the convective flow of the solution inside the nanochannel in function of time. First, the nanochannel is filled by capillarity during a time t_{cap}, which results in an increase of the flow velocity. When reaching the absorbing component, the solution has completely filled the nanochannel and the flow is no more driven by capillarity but rather by absorption. This results in a change of flow velocity during a time t_{act}. Finally, the solution flow inside the nanochannel tends to 0, and biomolecule movements are only due to Brownian motion. Measuring time t_m should occur after the convective flow stopped.

[0045] According to the present invention, the device offers great improvements for the detection, enumeration, identification and characterization of biomolecules interacting or not with other immobilized biomolecules. Applications of the present invention can cover biomedical, biological or food analysis as well as fundamental studies in analytical and bioanalytical chemistry.

Claims

1. A biosensor (200) for detecting and quantifying fluorescently-labeled biomolecules (320); said biosensor (200) comprising a nanochannel (210) defined between two substrates (201, 202) and containing one or several selectively functionalized areas (211) on which are immobilized biomarkers (310), said nanochannel furthermore being defined by a lateral input aperture (220) and a lateral output aperture (230), said input aperture (220) being adapted to let a solution containing biomolecules (320) to enter said nanochannel (210) and said output aperture (230) adapted to drive said solution through said nanochannel (210) by capillarity.

2. Biosensor (200) according to claim 1 wherein said biomarkers (310) are adapted to biologically or chemically interact with specific biomolecules (320) and/or not interact with non-specific biomolecules (330) contained in said solution (300).

3. Biosensor (200) according to claim 1 or 2 wherein the substrates (201, 202) are made of a material selected from the group constituted of silicon, glass, plastic and oxide compounds.

4. Biosensor (200) according to any of the previous claims wherein the output aperture (230) is containing or is in contact with a driving component (241 or 242) adapted to drive said solution through said nanochannel (210).

5. Biosensor (200) according to any of the previous claims wherein non-functionalized surfaces inside the nanochannel (210) and the lateral apertures (220, 230) contain a thin layer of material selected from the group constituted of metallic, plastic and oxide compounds, having a thickness between 1 nm and 1000 nm.

6. Biosensor (200) according to any of the previous claims wherein the lateral apertures (220, 230) have an area from 100 nm^2 to 20 mm^2 and the nanochannel (210) a height between 2 nm and 1000 nm, a width between 2 nm and 20 mm, and a length between 2 nm and 20 mm.

7. An array comprising several biosensors (200) as defined in any of the previous claims, said biosensors (200) being fixed inside a capsule system (101) or on a surface (102).

8. Assembly consisting of one or several biosensors (200) as defined in any of the previous claims and comprising optical means (500) for fluorescence excitation and detection.

9. Assembly according to claim 8 wherein said optical means (500) is a fluorescence measurement unit comprising a detector which is a single-photon detector, such as a detector array (CMOS or CCD), an avalanche photodiode (APD) or a photomultiplier tube (PMT).

10. A method for detecting and quantifying the presence of fluorescently-labeled biomolecules (320) in a solution (300) that comprises:

a) at least one biosensor (200) as defined in any of claims 1 to 6;

b) a filling mechanism of said biosensor(s) (200) from lateral input apertures (220) to the lateral output aperture (230), crossing the nanochannel (210), by depositing an aqueous solution (300) containing fluorescently-labeled biomolecules (320 and or 330) that can be specific to biomarkers (310) immobilized in the nanochannel and not into the capsule system (101) or the surface (102).

c) an optical system (500);

d) the detection of specific biomolecules (320) immobilized on biomarkers (310) inside said nanochannel (210) by means of photobleaching of fluophores attached to the biomolecules (320) as well as the determination of the concentration gradient across the length of the nanochannel (210).

11. Method according to claim 10 wherein said biomolecules (320) are proteins, DNA, RNA, antibodies, amino acids, nucleic acids, enzymes, lipid molecules, peptides, polysaccharides or virus.

FIG.1a

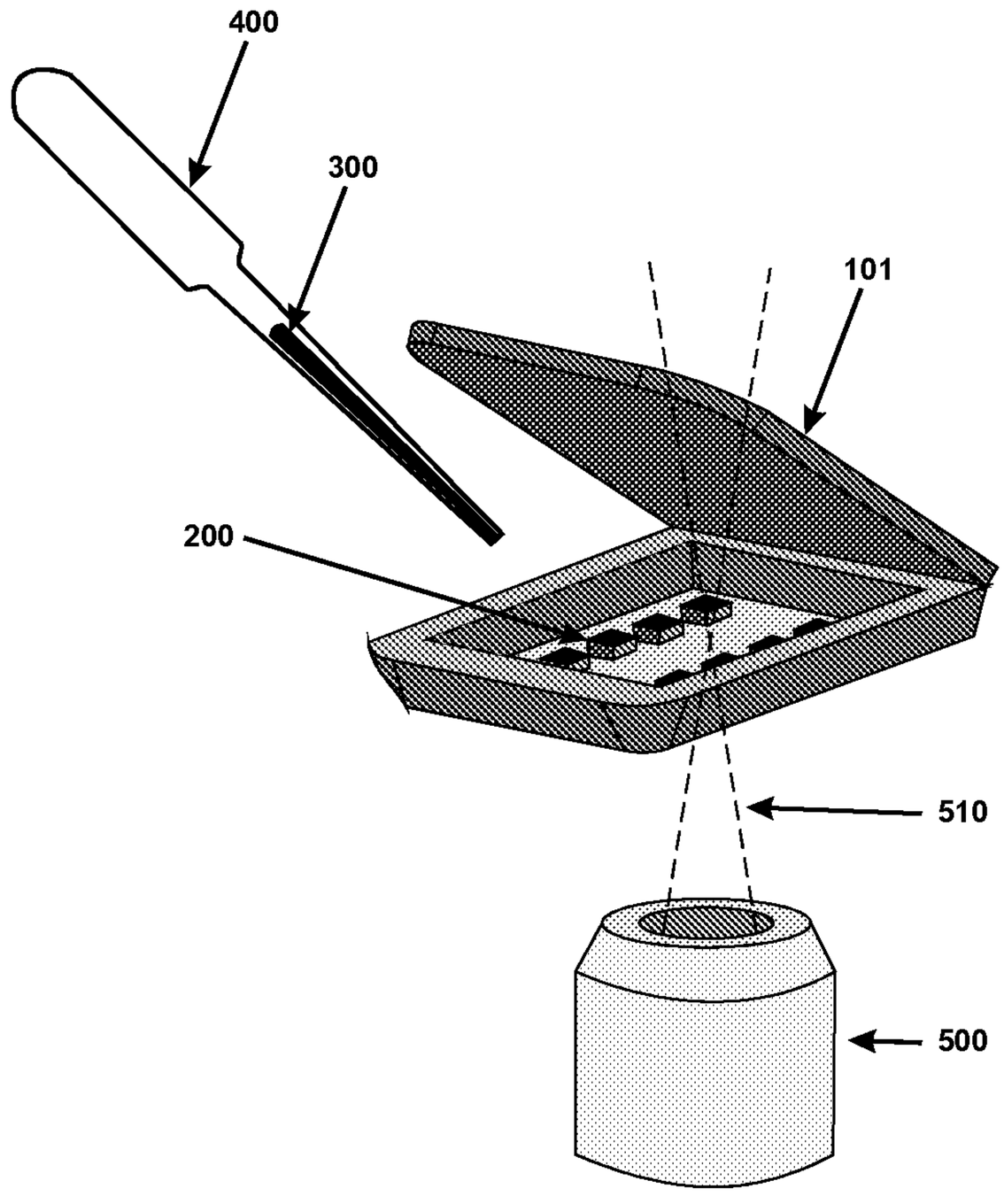


FIG.1b

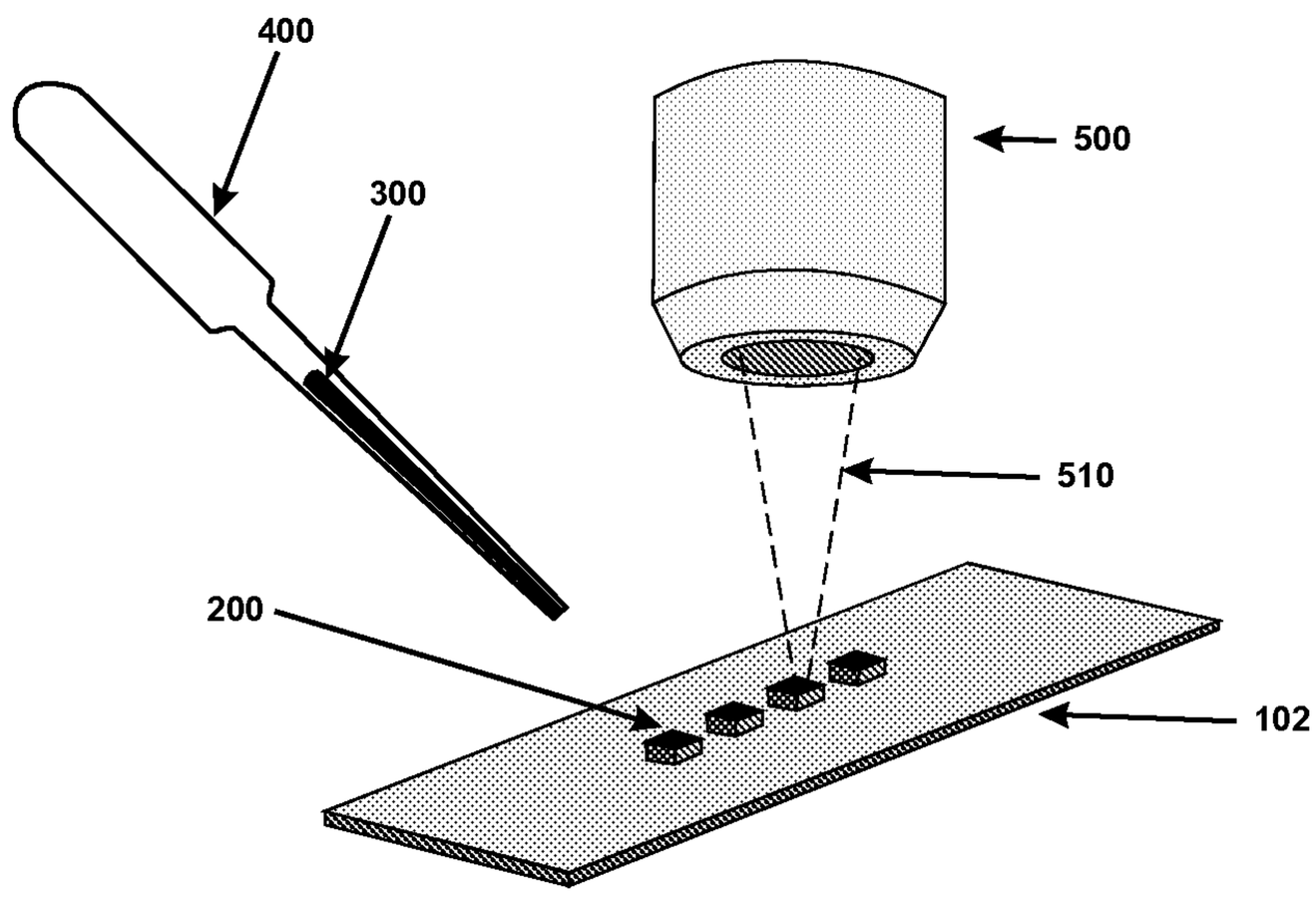


FIG.2a

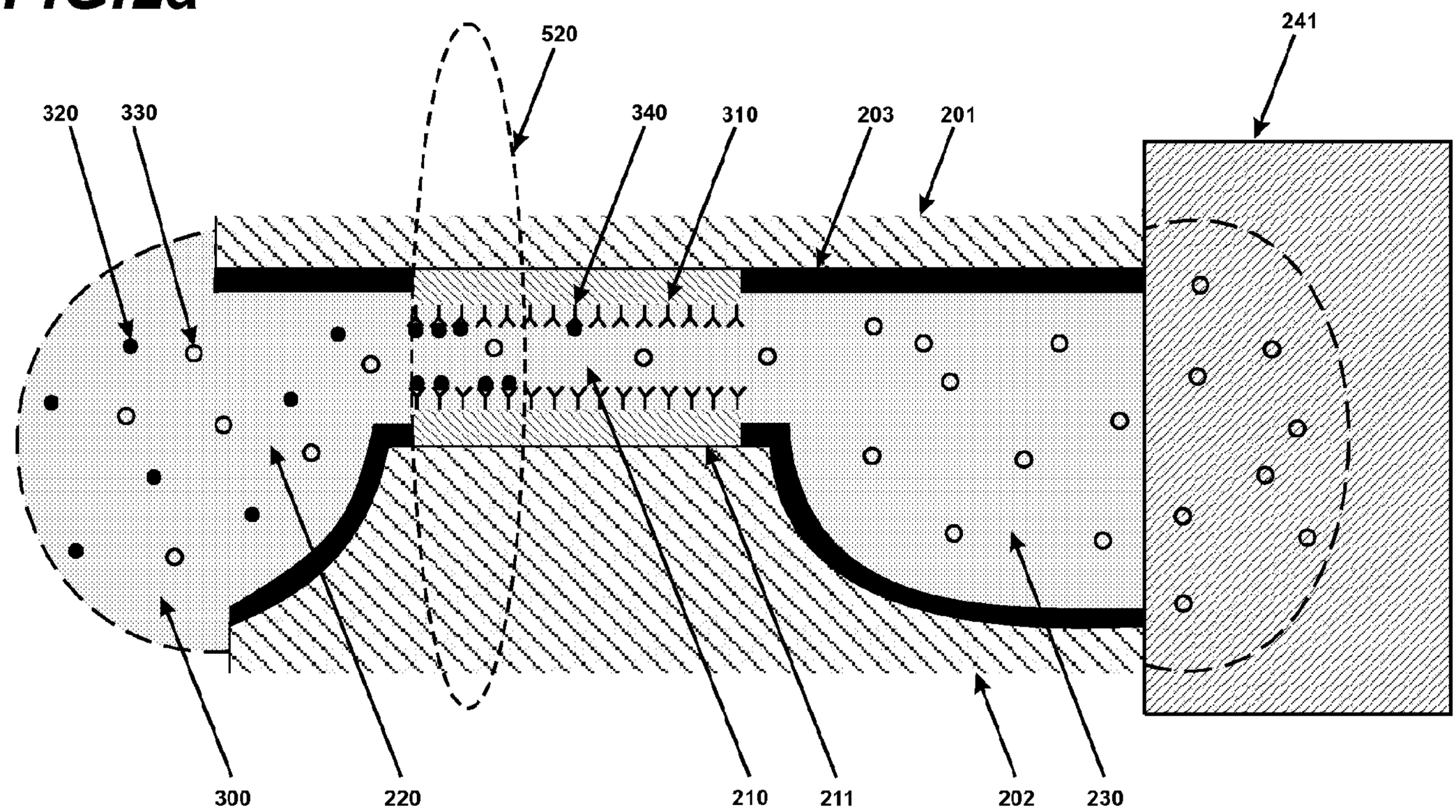

520
241
320
330
340
310
203
201
300
220
210
211
202
230

FIG.2b

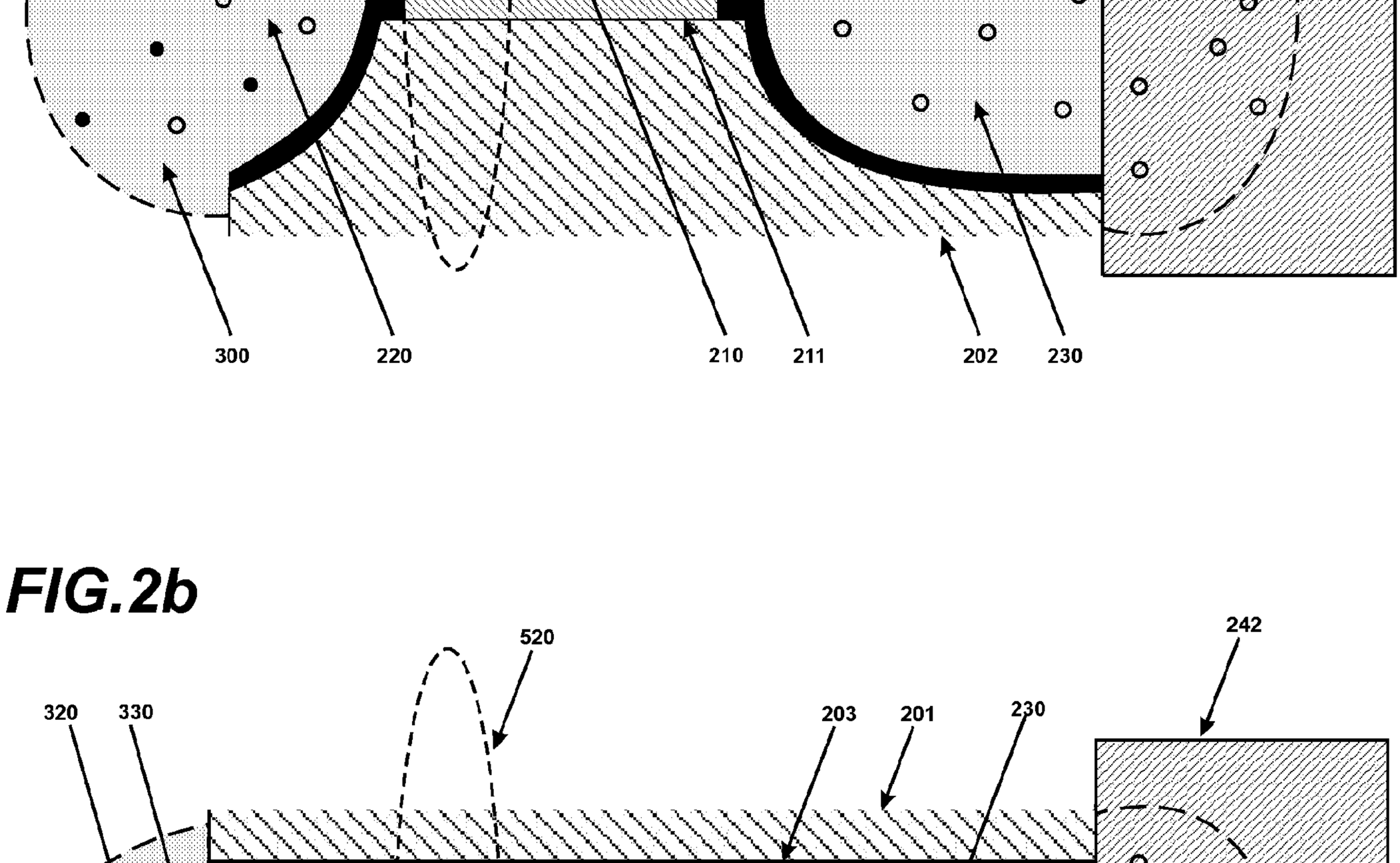

520
242
320
330
203
201
230
300
220
340
310
210
211
202

FIG.3

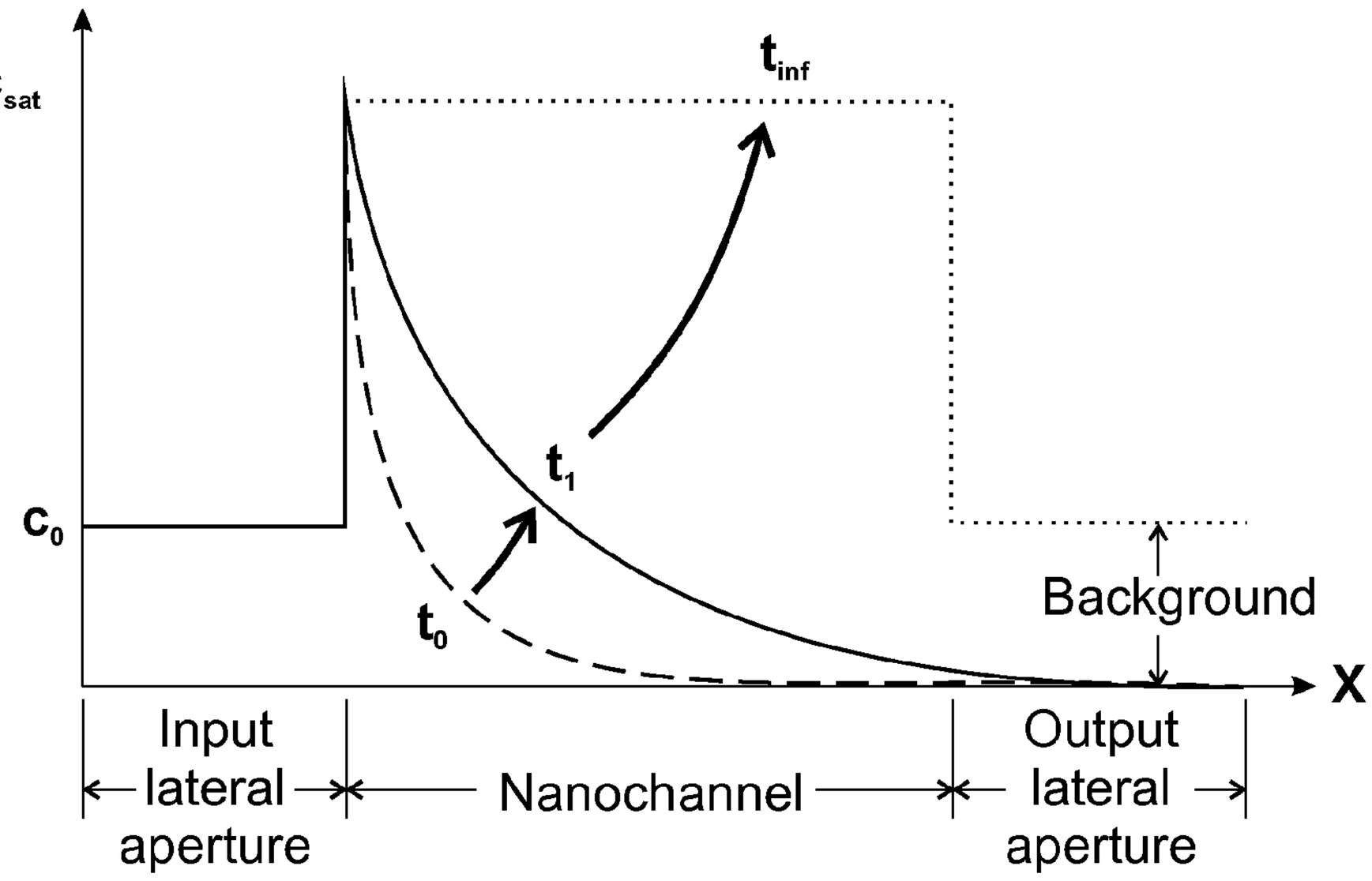

Concentration c
c_{sat}
t_{inf}
t_1
c_0
t_0
Background
X
Input lateral aperture
Nanochannel
Output lateral aperture

FIG.4

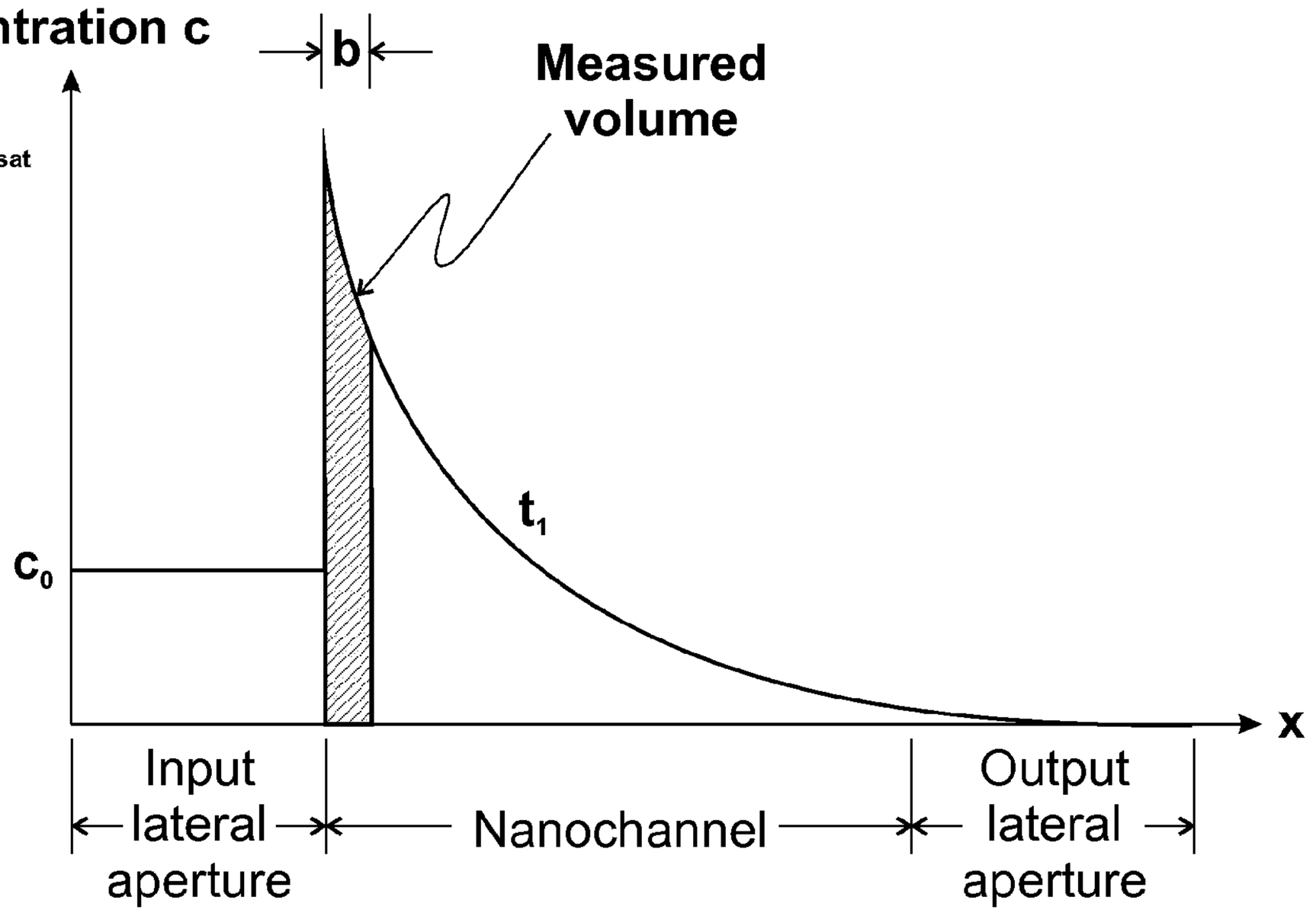

Concentration c
b
Measured volume
c_{sat}
t_1
c_0
x
Input lateral aperture
Nanochannel
Output lateral aperture

FIG.5

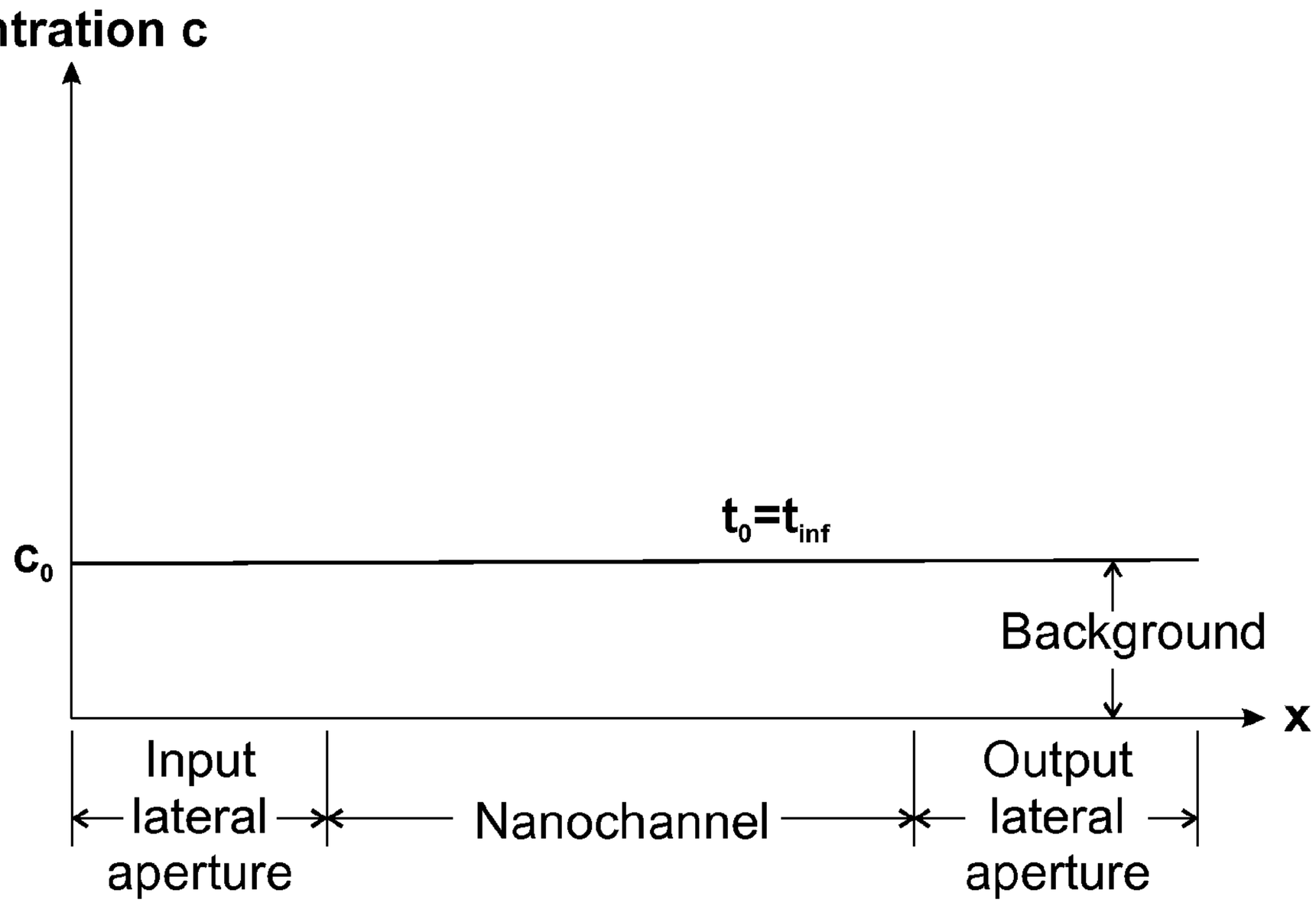

Concentration c
$t_0=t_{inf}$
c_0
Background
x
Input lateral aperture
Nanochannel
Output lateral aperture

FIG.6

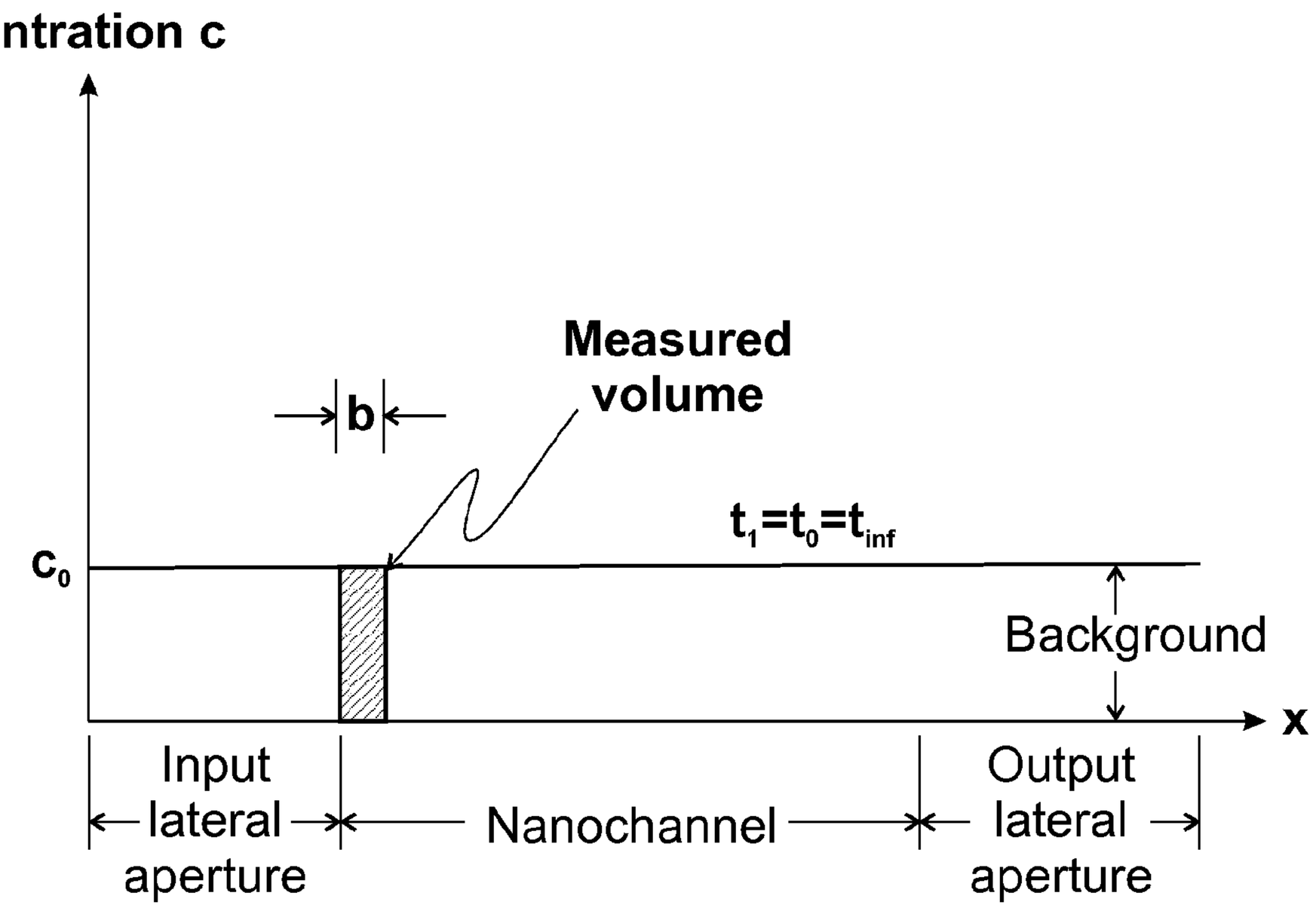

Concentration c
b
Measured volume
$t_1=t_0=t_{inf}$
c_0
Background
x
Input lateral aperture
Nanochannel
Output lateral aperture

FIG.7

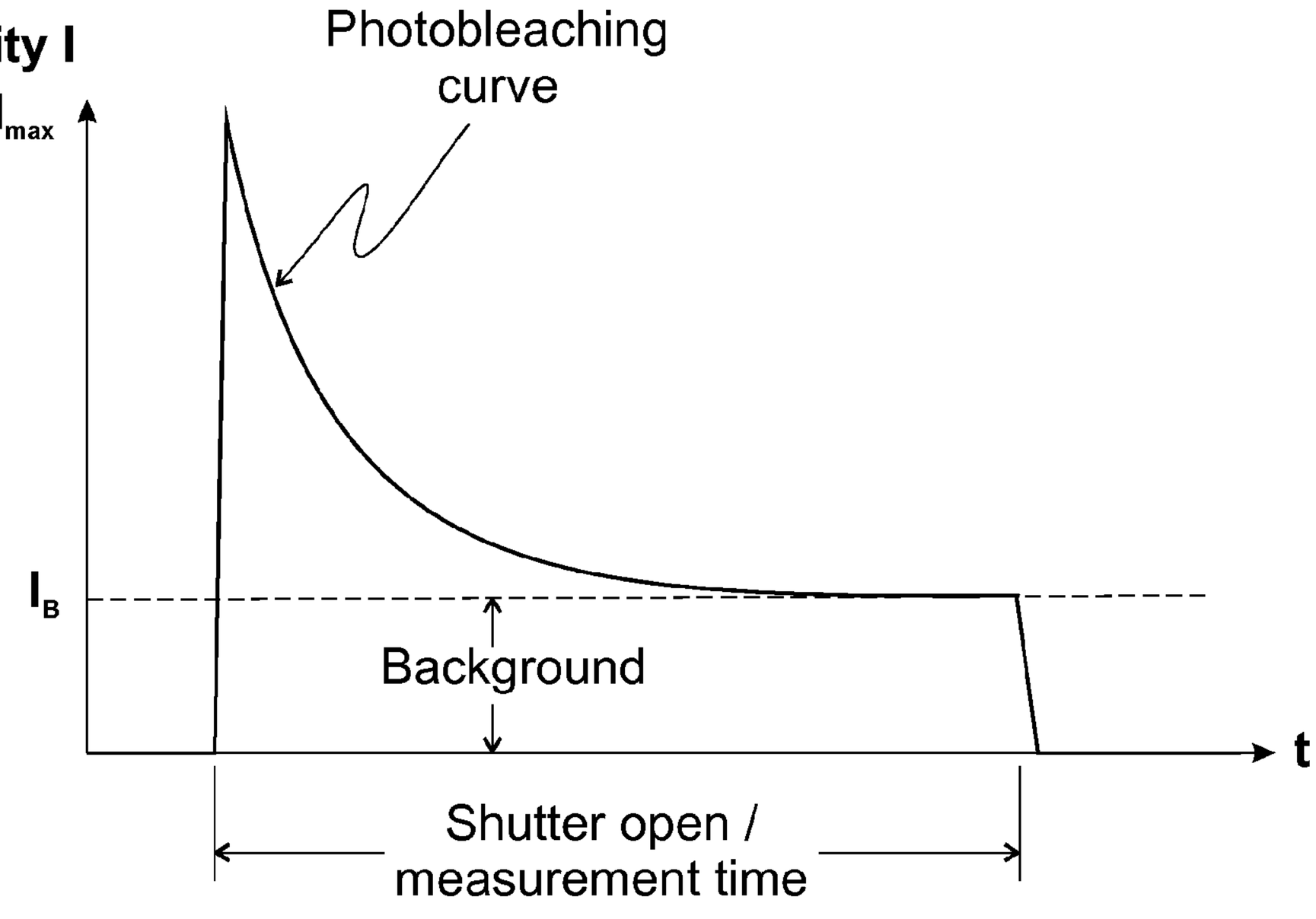

Intensity I
I_{max}
Photobleaching curve
I_B
Background
t
Shutter open / measurement time

FIG.8

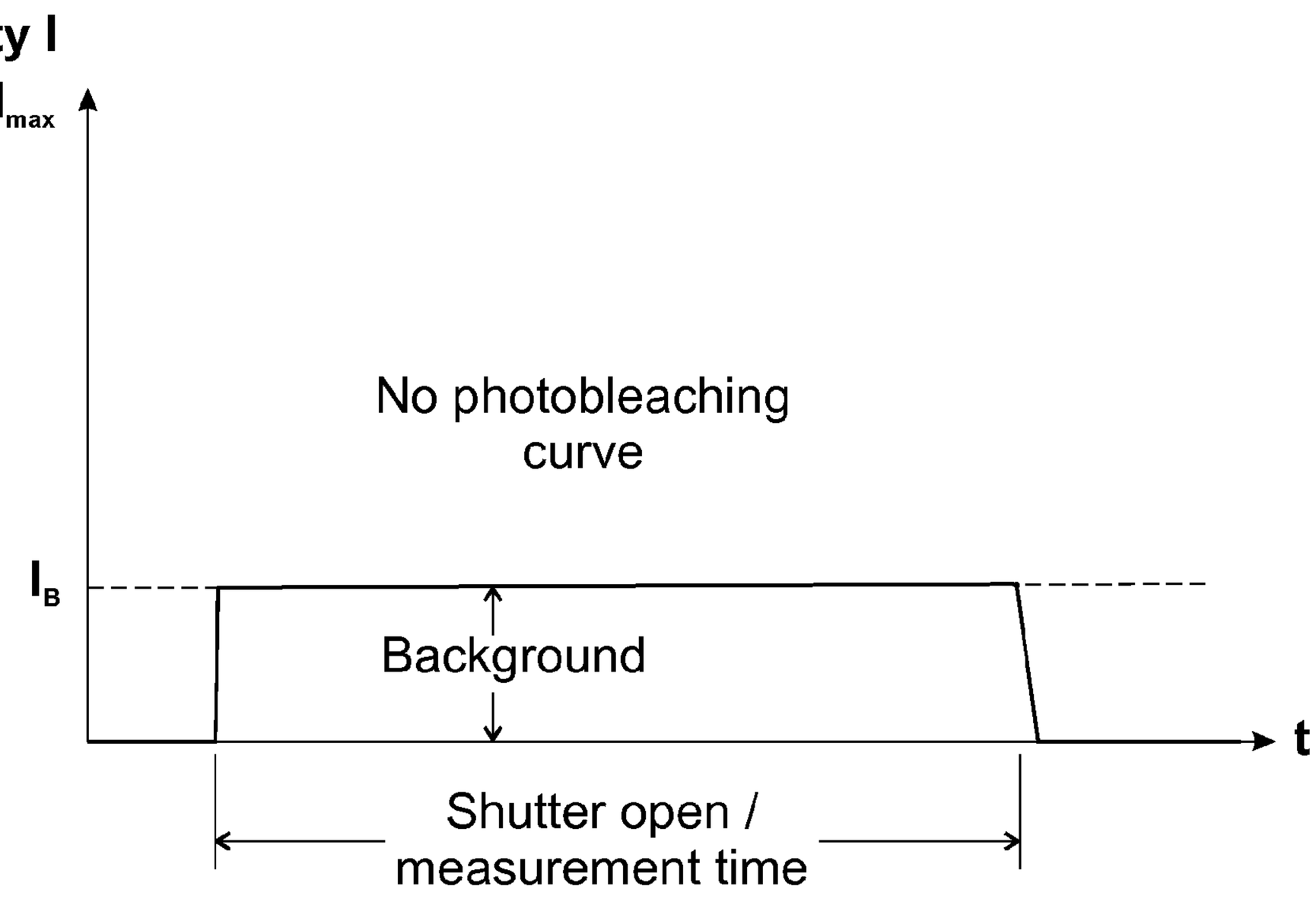

Intensity I
I_{max}
No photobleaching curve
I_B
Background
t
Shutter open / measurement time

FIG.9

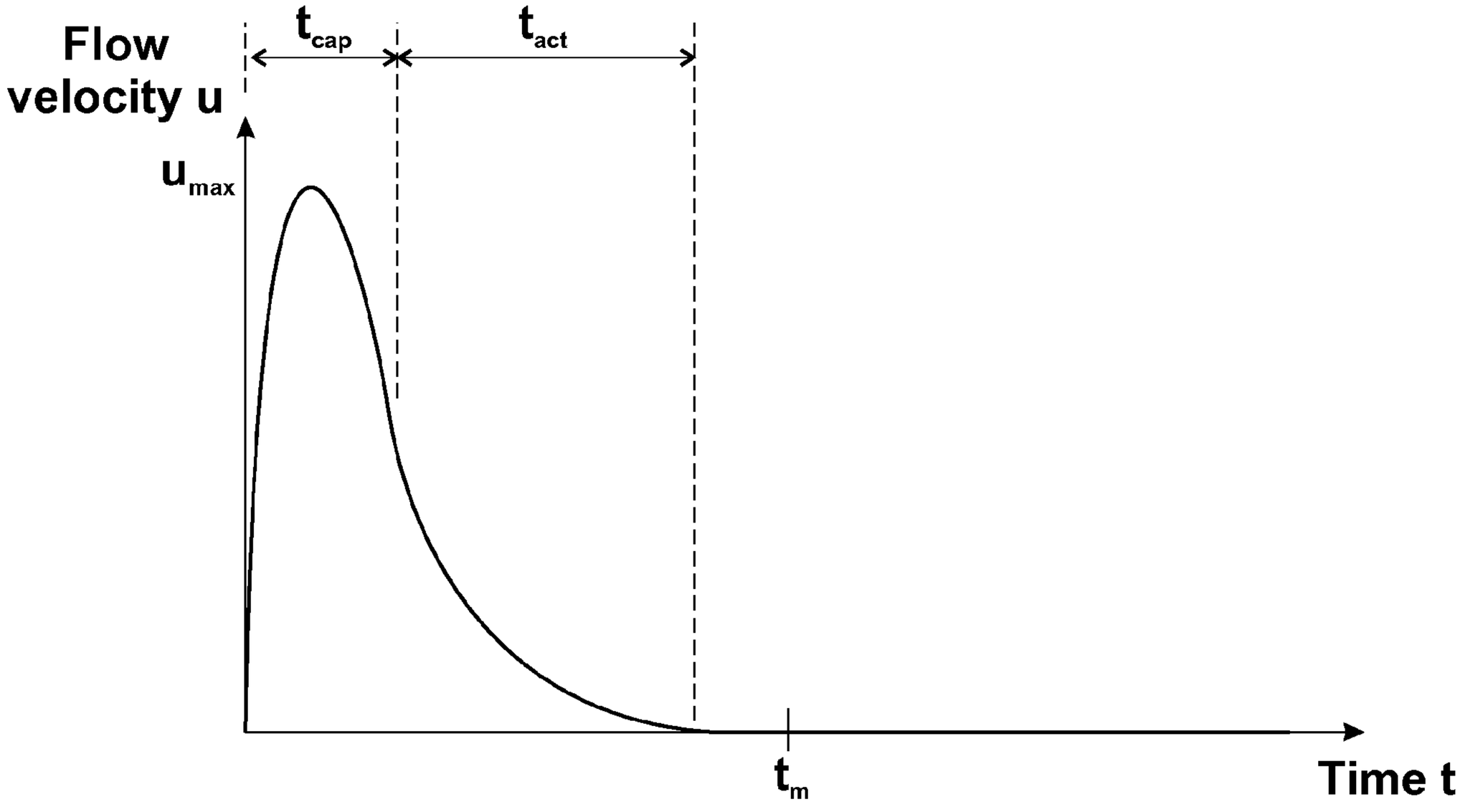


FIG.2b