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(54) Title: SYSTEM FOR OPTICAL DETECTION AND IMAGING OF SUB-DIFFRACTION-LIMITED NANO-OBJECTS

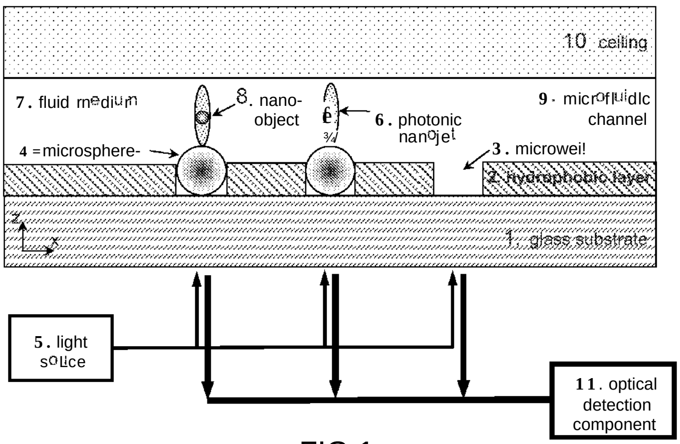

FIG.1

(57) Abstract: Nano-objects **(8)** with feature size smaller than the classical Abbe's diffraction limit can be optically detected via a microlens (4) array configured in a miniaturized microfluidic device. A hydrophilic microwell (3) array in a hydrophobic layer (2) is firstly fabricated by standard microfabrication processes. Dielectric microspheres (4) with high refractive index are used as microlenses and patterned inside the hydrophilic microwells (3) resulting in a microlens array format with high uniformity over a large surface area. These microlenses (4) focus the illuminating light into a region that is referred to as a photonic nanojet (6), which is characterized by a high optical intensity and sub-diffraction transverse dimension. The microlens array is integrated with a micro-fluidic channel **(9),** where a fluid medium **(7)** with nano-objects **(8)** is present. When the nano-objects **(8)** pass through the photonic nanojet (6), the retroreflected light from the microsphere-nano-object ensemble is highly enhanced and measured by an optical signal detection component (11) (conventional optical microscope, etc.), and the enhancement of the retroreflected light is very sensitive to the size of the nano-objects **(8).** Therefore, not only the existence of the nano-objects **(8)** can be qualitatively monitored, but also the size of these nano-objects can be quantitatively determined by the proposed technique.

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System for optical detection and imaging of sub-diffraction-limited nano-objects

Technical Field

[0001] This invention generally relates to microfluidics and optical detection methods, more particularly for examining the presence and the size of nanometric objects in fluidic media.

[0002] The present patent application claims priority from international application PCT/IB2014/063747 filed on August 6, 2014, the content of which is incorporated herein by reference.

Background Art

[0003] The unique physical, chemical, mechanical, and optical properties that materials display at the nanoscale are at the bases both of the success of the field of nanotechnology and of the increased need for environmental nanoparticles monitoring. Hence, there is a strong interest in having high-resolution, inexpensive and miniaturized devices capable of real-time in situ detection and characterization of individual nanoscale objects for applications that range from material science to biomedical research, health-care, diagnostics and environmental monitoring.

[0004] Optical microscopy has been extensively used in the case of imaging objects with an optical field that is propagating in the far-field. However, the fundamental constraint of conventional optical microscopy is that the diffraction of light limits the spatial resolution to one-half wavelength, or ~ 200 nm in the visible wavelength region. As problems of interest nowadays push further into the nanometric regime, the importance of imaging techniques that permit sub- diffraction resolution has been increasing steadily. Optical detection and imaging techniques and devices, which can break the diffraction limit, now are either based on bulky and expensive instruments or need the introduction of photonic structures made via complicated nano-fabrication processes. However, optically transparent microspheres with high refractive index show that they can focus the light into a region with waist smaller than the diffraction limit and propagating over several optical wavelengths without significant diffraction. This focused light beam is termed as "photonic nanojet". The properties provided by the photonic nanojet, such as high electromagnetic field intensity, sub-diffraction transverse dimension and high sensitivity to perturbations, make the microspheres very promising for nano-objects detection and imaging. Therefore, using microspheres to develop a compact system for detecting and imaging objects with feature size below the diffraction limit inevitably will be of benefit to the field of life sciences, diagnostics or environmental monitoring.

[0005] The invention relates to optical detection and imaging of sub-diffraction-limited nano-objects based on the phenomenon of the "photonic nanojet" by using a microsphere lens array in a microfluidic format. Co-assigned patents and patent applications relevant to the optical detection devices or sensors in a microfluidic device include U. S. Patent Application Publication No. 201 10291026 (Optically accessible microfluidic diagnostic device"), PCT Publication No. 2014055559 ("Microfluidic sensors with enhanced optical signals"), U. S. Patent Application Publication No. 20140016175 ("Micro-fluidic variable optical device array and method of manufacturing the same"), all of which are hereby incorporated by reference in their entireties. Also incorporated herein by reference is U. S. Patent Application Publication No. 2014004361 1 ("Nanophotonic light-field sensor"), which relates to photonic nanojets generated by microspheres.

[0006] The microfluidic format has proven its great potential in designing small diagnostic devices, as discussed in U. S. Patent Application Publication No. 201 10291026. Especially, the dimension features in the microfluidic device can be precisely controlled during the microfabrication process; this allows structures with optical accessibility to luminous radiations to be integrated into the device for biomolecules detection. The amount of the biomolecules are expressed by the fluorescent signal, and the latter is recorded by a fluorescent microscope. However, only featuring a microfluidic structure incorporated with an optical microscope, the microfluidic diagnostic device neither integrates with components for the enhancement of the optical signal read-out, nor shows possibility to identify single biomolecules or so.

[0007] Further related art is PCT Publication No. 2014055559, which particularly describes the use of a microfluidic device with a nano-sensor at the location of the microfluidic channel for detecting an analyte in a liquid. The nano-sensor comprises metallic nanostructures, and a capture agent is deposited on the surface of the nanostructure, wherein the capture agent specifically binds to the analyte. The nano-sensor can amplify the light signal to and/or from the analyte or a light label attached to the analyte, when the latter is bound or in proximity to the capture agent. Even though the nanostructures for optical signal enhancement are integrated into this microfluidic device, the latter still cannot either directly image the target analyte, or even identify its size, due to the lack of optical components which can realize these functions, such as microlenses.

[0008] Generating a lens array in a microfluidic device is achieved in U. S. Patent Application Publication No. 20140016175, in which a microfluidic variable optical device array and a method of manufacturing the same are described. In the optical device array, an addressing layer comprising an electrode wire arranged in a predetermined pattern, a barrier wall portion to define a plurality of cell regions, a transparent electrode layer covering the plurality of cell regions, as well as non-mixed conductive and nonconductive fluids disposed in each of the cell regions, are integrated. By utilizing the electrowetting technique, a lens array is generated when a voltage is applied between the transparent electrode layer and the addressing layer. However, in contemplating the use of optically transparent microspheres, the design of the microsphere lens array in a microfluidic device has not been proposed. In view of optical detection and/or direct imaging of sub-diffraction-limited nano-objects, the microspheres with high refractive index focus the light into photonic nanojets, the latter holding the basis for nano-objects detection and imaging.

[0009] Also of related interest, U. S. Patent Application Publication No. 2014004361 1 describes a light field sensor that has a layer of nanoscale resonator detector elements, such as silicon nanoshells, below a layer of dielectric microlenses. By taking advantages of photonic nanojets in the microlenses and circulating resonances in the nanoshells, the light field sensor achieves improved sensitivity. However, the device is neither for nano-objects detection, nor integrated with a microfluidic component.

[0010] Accordingly, although there have been advances in the fields of microfluidic devices, lens arrays, microlenses and photonic nanojets, there remains a great need in the art for a system which can achieve detection, sizing and direct imaging of nano-objects that are smaller than the diffraction-limit of the conventional optical microscope.

Summary of invention

[001 1] The invention provides an optical system for use in detection and imaging of a plurality of sub-diffraction-limited nano-objects by using at least a microsphere as a microlens.

[0012] The proposed system comprises the following properties: it incorporates a microsphere lens array over the substrate of a microfluidic device, uses an optical detection method based on the photonic nanojet phenomenon, and transports nano-objects in the microfluidic channel with a depth comparable to the longitudinal dimension of the photonic nanojet.

[0013] Accordingly, the invention provides an optical system for use in detecting and imaging at least a sub-diffraction-limited nano-object, that comprises at least one microfluidic channel adapted to be travelled across by a fluid medium comprising nano-objects, said channel being delimited by at least a bottom wall and a ceiling wall positioned opposite to and facing the bottom wall, said bottom wall comprising a transparent substrate; at least one microsphere used as a microlens arranged as to be fixed on a part of the bottom wall facing the ceiling wall; a light source arranged in such a manner that it illuminates the at least one microsphere through the transparent substrate of the bottom wall and thereby focusses the light as a photonic nanojet inside the fluid medium between the bottom wall and the ceiling wall; means for moving a nano-object comprised within a fluid medium through the photonic nanojet; and light detection means arranged to measure the light scattered or induced by a nano-object hit by a photonic nanojet, the light scattered or induced returning to the light detection means by first passing through the at least one microsphere.

[0014] In a preferred embodiment of the invention, the distance (Tchannei) between the ceiling wall and a point of a periphery of the at least one microsphere, the point being one nearest to the ceiling wall, is equal to or smaller than three times a longitudinal dimension (Lnanojet) of the photonic nanojet.

[0015] In a further preferred embodiment, the ratio between the refractive index of the at least one microsphere (np) and the refractive index of the fluid medium (nm) comprising a nano-objects is between 1.2 to 1.6.

[001 6] In a further preferred embodiment, the at least one microsphere has a mean size comprised between 1 μιτι to 100 μιτι .

[0017] In a further preferred embodiment, the means for moving a nano-object comprised within a fluid medium comprise a pump configured to pump the fluid medium through the microfluidic channel.

[0018] In a further preferred embodiment, when at least a nano-object is coupled to the ceiling wall, the means for moving a nano-object comprised within a fluid medium comprise a translational actuator configured to actuate the ceiling wall in a direction substantially parallel to the bottom wall.

[0019] In a further preferred embodiment, the light source is a laser source, a narrow-band fluorescence source or a broad-band white light source.

[0020] In a further preferred embodiment, the bottom wall of the microfluidic channel comprises at least a microwell of a size suitable to fix the at least one microsphere on it.

[0021] In a further preferred embodiment, the light detection means is an optical microscope integrated with a charge coupled device camera and/or a spectrometer, CMOS (complementary metal-oxide-semiconductor) image sensors, or CMOS-based avalanche photo diodes.

Brief description of drawings

[0022] [Fig. 1] Conceptual diagram of a nano-objects optical system.

[0023] [Fig. 2] Schematic illustrations showing the working principle of the optical system at different conditions.

[0024] [Fig. 2a] The flow inside the microfluidic channel is a pressure-driven fluid motion, therefore, the flow pattern along the depth of the channel has a parabolic fluid velocity profile. The nano-objects present in the fluid medium are transported through the photonic nanojets and detected.

[0025] [Fig. 2b] The ceiling of the microchannel has translational motion with respect to the microspheres, the flow pattern along the depth of the channel has a linear fluid velocity profile. The nano-objects are transported through the photonic nanojets with the fluid medium and detected.

[0026] [Fig. 2c] Sub-diffraction-limited nano-objects are positioned on the ceiling of the microchannel and the latter has translational motion with respect to the microspheres. Therefore, the ceiling with nano-objects can be scanned by the microlens array continuously. By recording the images that corresponds to different locations and using image reconstruction algorithms, a whole image covering the entire sample area can be obtained.

[0027] [Fig. 3] A flow diagram illustration on the process of microfluidic device fabrication and nano-objects detection.

[0028] [Fig. 4] A microscopy image illustrating the enhanced retroreflected light by a nanoparticle hit by a photonic nanojet.

[0029] [Fig. 5] A microscopy image illustrates the enhanced retroreflected light by two 50 nm gold nanoparticles, each being located in a photonic nanojet that is emerging from a microlens with diameter of 3 μιτι.

Description of embodiments

[0030] The present disclosure may be more readily understood by reference to the following detailed description presented in connection with the accompanying drawing figures, which form a part of this disclosure. It is to be understood that this disclosure is not limited to the specific conditions or parameters described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed disclosure.

[0031] The invention provides optical system for use in detection and imaging of sub-diffraction-limited nano-objects by using microspheres as microlenses.

[0032] Conventional optics suffer from the so-called diffraction limit, because they are only capable of transmitting the propagating components emanating from the source. This is because the evanescent waves that carry subwavelength information about the object decay exponentially in a medium with positive permittivity and permeability and are lost before reaching the image plane. The resolution of an optical imaging system is proportional to the size of its objective, and inversely proportional to the wavelength of the light being observed.

[0033] Advanced optical techniques have been proposed for sensitive detection of nano-objects, but required high-end, expensive, and bulky experimental setups. If direct optical detection of nanoparticles in liquids would be possible using a standard microscope, this could lead to more affordable and portable sensing applications. However, as said, conventional optics only permits detecting relatively large objects because the scattered light intensity decreases with decreasing size, and the light collection capability of a standard microscope objective is limited by its numerical aperture (NA). As a consequence, only nanoparticles that are either larger than several hundred nanometers or having extremely enhanced scattering probability can be detected, while particles not fulfilling the aforementioned features (for instance, smaller particles) would not be detected. These nanoparticles are herein referred to as "sub-diffraction-limited nano-objects".

[0034] When a microlens has a refractive index contrast relative to the fluid medium that is less than 2:1 and a diameter between several to tens of wavelengths (λ), a highly focused propagating beam from the shadow-side surface of the microsphere is generated due to constructive interference of the light field. This beam is termed as "photonic nanojet" and has a sub-λ full width at half-maximum (fwhm) transverse dimension and typically is several λ in length. A very interesting predicted property of a photonic nanojet is that the presence of a particle having a mean size much smaller than λ, i.e., sub-diffraction-limited nano-objects, and positioned within the nanojet, significantly enhances backscattering of the light through the microsphere.

[0035] To make sure that the nano-objects pass through the photonic nanojet, the present invention features an optical system (Figs. 1, 3) where at least a microsphere is employed as a microlens and said at least one microsphere is positioned on the bottom wall of a microfluidic channel. Such channel is delimited by at least a bottom wall and a ceiling wall positioned opposite to and facing the bottom wall, whereby the at least one microsphere is at a fixed position in the bottom wall facing the ceiling wall, and the bottom wall comprises a transparent substrate. The microfluidic channel is adapted to be travelled across by a fluid medium that comprise the at least one nano-object. The fluid medium and/or ceiling of the channel have a non-zero translational velocity with respect to the microspheres to ensure that the nano-objects pass though the at least one nanojets.

[0036] Concerning the transportation of the nano-objects in the microfluidic channel, the flow inside the microfluidic channel can either be pressure-driven (Fig. 2a) or shear-driven fluid motion, the latter case corresponding to the situation in which the ceiling of the microchannel has translational motion with respect to the microspheres (Fig. 2b). Correspondingly, the flow pattern along the depth of the microfluidic channel owns either a parabolic or a linear fluid velocity profile. No matter which velocity profile is performed for transportation of the fluid medium, the nano-objects passing through the photonic nanojet will generate an enhanced optical signal for detection. Moreover, due to the well-controlled depth of the microfluidic channel, the size of the nano-objects can be determined precisely despite the variation on the position of the nano-objects along the depth of the microfluidic channel.

[0037] In a further embodiment of the optical system, the sub-diffraction- limited nano-objects are in a fixed positon on the ceiling wall of the microchannel and the latter can have translational motion with respect to the microspheres (Fig. 2c). A continuous acquisition on the information (presence and/or size) of the nano-objects can be achieved as the ceiling moves. In this case, the optical system performs as a scanning microscopic setup with sub-diffraction resolution, and the nano-objects or nano-patterns on the ceiling are detected or imaged via the microlens array. By recording the images that corresponds to different locations and using image reconstruction algorithms, a whole image covering the entire sample area can be obtained.

[0038] In a preferred embodiment according to the invention, the microfluidic half-channel's depth (Tchannei) is equal to or smaller than three times of the longitudinal dimension of the photonic nanojet ($L_{nanojet}$) that is emerging from a microlenS ($T_{channel} \leq 3 \times L_{nanojet}$).

[0039] In some embodiments, the microspheres used in the optical system as microlenses have a mean size that can vary from 1 μιτι to 100 μιτι, and can be made of any suitable transparent material such as plastic polymeric materials, glass and the like.

[0040] In a preferred embodiment, where a multiplicity of microspheres are immobilized on the bottom wall, a specific and well organized pattern is obtained thanks to microfabrication. For example, a hydrophobic layer is deposited on the glass substrate and several microwells are generated in the hydrophobic layer by e.g. standard microfabrication processes. These microwells can be arranged into an array format with precisely controlled position. Dielectric microspheres are patterned inside the hydrophilic microwells due to their selective wettability. As will be evident for a skilled in the art, the size of the so-created microwells will be such that microspheres can be put in them and fixed. Consolidation of the microspheres within the microwells is provided therefore by the dimension of the microwell itself and even, in some cases, by the electrostatic interaction between the microsphere and the microwell. During the microfabrication process, the size of the microwells can be precisely controlled to have a size which is very close to that of the microsphere, i.e. substantially the same, in order to have the possibility to insert and fix the microspheres inside the microwells' recess. The diameter of the microwells for microlens patterning is kept the same as the size of the microsphere, while the thickness of the hydrophobic layer ($T_{microweii}$) can vary from the radius of the microsphere ($R_{microsphere}$) to the diameter of the microsphere ($D_{microsphere}$), SO that for example $R_{microsphere} \leq T_{microwell} \leq D_{microsphere}$-

[0041] The optical system can detect the nano-objects with feature size smaller than the diffraction limit in an optional way when the optical contrast varies from 1.2 to 1.6. For "optical contrast" is herein meant the ratio between the refractive index of the microsphere (np) and the refractive index of the fluid medium (nm), i.e. np/nm. For the nano-objects dispersed in the fluid medium the fluid medium that is transported in the microfluidic channel may

be a gas, a liquid or a combination of gas and liquid as long as the optical contrast varies from 1.2 to 1.6.

[0042] In the optical system a light source is needed to illuminate the microlens array and the nano-objects in the visible spectrum, the illumination may be a broad-band white light source, a narrow-band fluorescence source, or a laser source.

[0043] Once the light of the photonic nanojet is backscattered, an optical signal detection is needed to collect and record the signal; such detection can be achieved with an optical microscope integrated with a commercial CCD (charge coupled device) camera and/or a spectrometer, CMOS (complementary metal-oxide- semiconductor) image sensors, or CMOS-based avalanche photo diodes.

[0044] In the optical system, when a broadband white light source and an optical microscope are used, the detection of nano-objects may be performed in either a bright-field, or a dark-field condition.

[0045] The microfluidic channel for fluid medium and nano-objects transportation is generated by bonding the substrate of the microlens array to a half-channel structure fabricated in a bulk material, the latter may be either a reflective, or a non-reflective material.

[0046] In a preferred embodiment the microfluidic channel may be generated by directly bonding a hard material to the substrate of microlens array, the distance between two layers may be precisely controlled by sandwiching commercially available spacer beads with calibrated size.

[0047] EXAMPLE

[0048] Figure 1 shows a preferred embodiment of the optical system of the invention for exemplary and non-limiting purposes. A glass chip 1 is used as bottom wall. A hydrophobic layer 2 is deposited on the glass substrate. Microwells 3 are generated in the hydrophobic layer by standard microfabrication processes. These microwells are arranged into an array format with precisely controlled position. Dielectric microspheres 4 are patterned inside the hydrophilic microwells due to their selective wettability. Consolidation of the microspheres within the microwells is provided by the dimension of the microwell and the electrostatic interaction between the microsphere and the microwell. During the microfabrication process, the size of the microwells is controlled precisely to have the same size of the microsphere. Therefore, the positioning of single microsphere per microwell is achieved and the microspheres in different microwells creates a well-aligned pattern. A light source 5 is positioned beneath the bottom wall, illuminating the microsphere array. Due to its high refractive index, the microsphere is used as microlens to focus the illumination light into an extremely small region, i.e. a photonic nanojet 6. A fluid medium 7 with dispersed nano-objects 8 is transported within the microfluidic channel 9, the latter is realized by providing the ceiling wall 10 made of a bulk material. The height of the microchannel (Tchannei) 12 is comparable with the length of the photonic nanojet (Lnanojet) 13. When a nano-object passes the photonic nanojet, the intensity of the retroreflected light is highly enhanced. A measurement on the retroreflected light is performed by an optical detection component or sensor 11. The existence and the size of the nano-object are both revealed by the measurement. As shown in Fig. 4, a microscopy image can be easily and reliably obtained from the enhanced retroreflected light of one or a plurality of nanoparticles hit by a photonic nanojet. In the depicted example, a 200 nm gold nanoparticle located in the trajectory of a photonic nanojet emerged from a melamine microsphere with diameter of 3 μιτι can be accurately visualized. Figure 5 shows a plurality of 50nm gold nanoparticles imaged by using the same above-mentioned setting.

Claims

Claim 1. An optical system for use in detecting and imaging at least a sub-diffraction-limited nano-object, characterized in that it comprises:

- at least one microfluidic channel adapted to be travelled across by a fluid medium comprising nano-objects, said channel being delimited by at least a bottom wall and a ceiling wall positioned opposite to and facing the bottom wall, said bottom wall comprising a transparent substrate;
- at least one microsphere used as a microlens arranged as to be fixed on a part of the bottom wall facing the ceiling wall;
- a light source arranged in such a manner that it illuminates the at least one microsphere through the transparent substrate of the bottom wall and thereby focusses the light as a photonic nanojet inside the fluid medium between the bottom wall and the ceiling wall;
- means for moving a nano-object comprised within a fluid medium through the photonic nanojet; and
- light detection means arranged to measure the light scattered or induced by a nano-object hit by a photonic nanojet, the light scattered or induced returning to the light detection means by first passing through the at least one microsphere.

Claim 2. The optical system according to claim 1, wherein the distance $(T_{channel})$ between the ceiling wall and a point of a periphery of the at least one microsphere, the point being one nearest to the ceiling wall, is equal to or smaller than three times a longitudinal dimension $(L_{nanojet})$ of the photonic nanojet.

Claim 3. The optical system according to claims 1 or 2, wherein the ratio between the refractive index of the at least one microsphere (np) and the refractive index of the fluid medium (nm) comprising a nano-objects is between 1.2 to 1.6.

Claim 4. The optical system according to any previous claims, wherein the at least one microsphere has a mean size comprised between 1 μm to 100 μm.

Claim 5. The optical system according to any previous claim, wherein the means for moving a nano-object comprised within a fluid medium comprise a pump configured to pump the fluid medium through the microfluidic channel.

Claim 6. The optical system according to any previous claim, wherein, when at least a nano-object is coupled to the ceiling wall, the means for moving a nano-object comprised within a fluid medium comprise a translational actuator configured to actuate the ceiling wall in a direction substantially parallel to the bottom wall.

Claim 7. The optical system according to any previous claim, wherein the light source is a laser source, a narrow-band fluorescence source or a broad-band white light source.

Claim 8. The optical system according to any previous claim, wherein the bottom wall of the microfluidic channel comprises at least a microwell of a size suitable to fix the at least one microsphere on it.

Claim 9. The optical system according to any previous claim, wherein the light detection means is an optical microscope integrated with a charge coupled device camera and/or a spectrometer, CMOS (complementary metal-oxide-semiconductor) image sensors, or CMOS-based avalanche photo diodes.

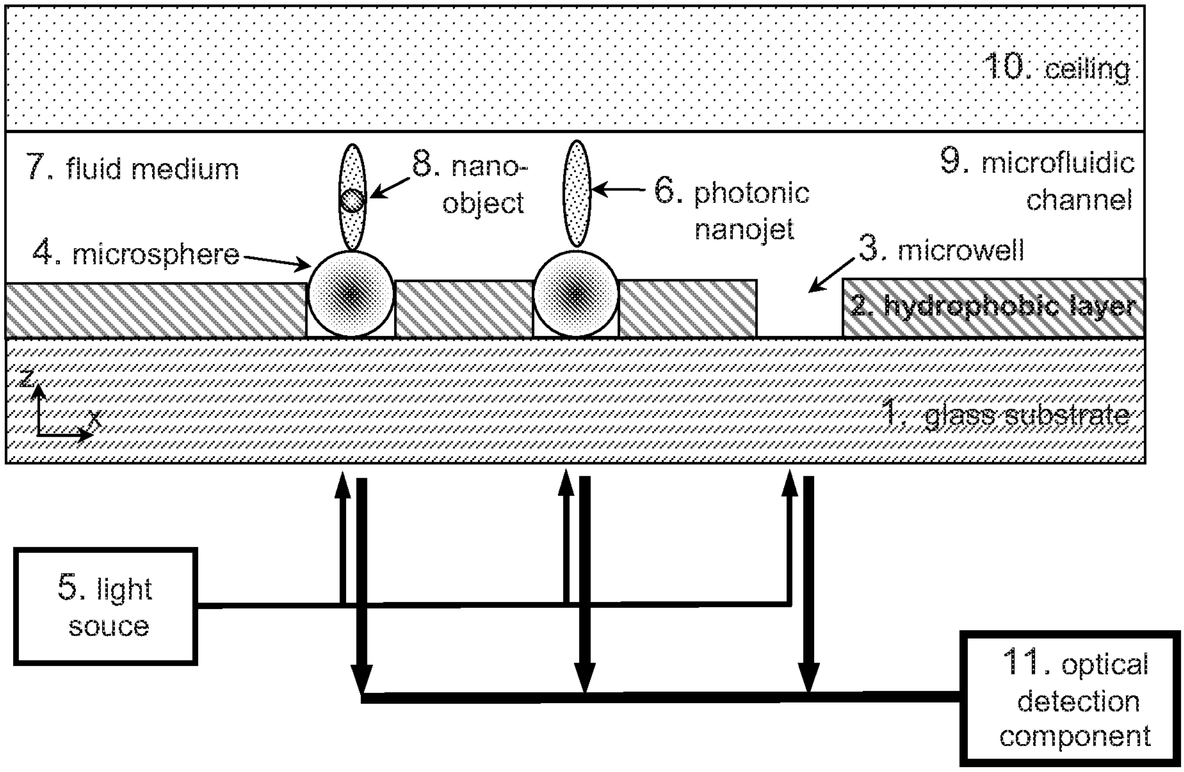
10. ceiling
7. fluid medium
8. nano-object
6. photonic nanojet
9. microfluidic channel
4. microsphere
3. microwell
2. hydrophobic layer
z
x
1. glass substrate
5. light souce
11. optical detection component

FIG.1

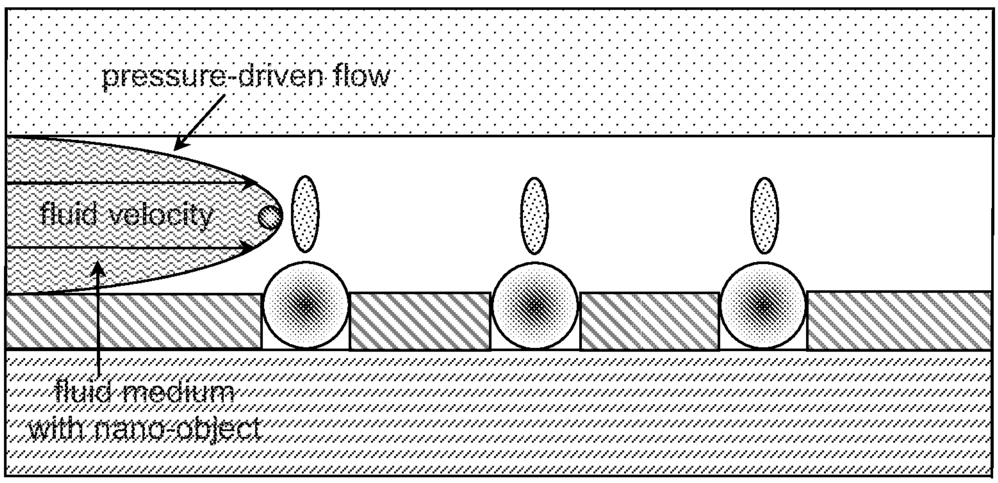
pressure-driven flow
fluid velocity
fluid medium with nano-object

FIG.2a

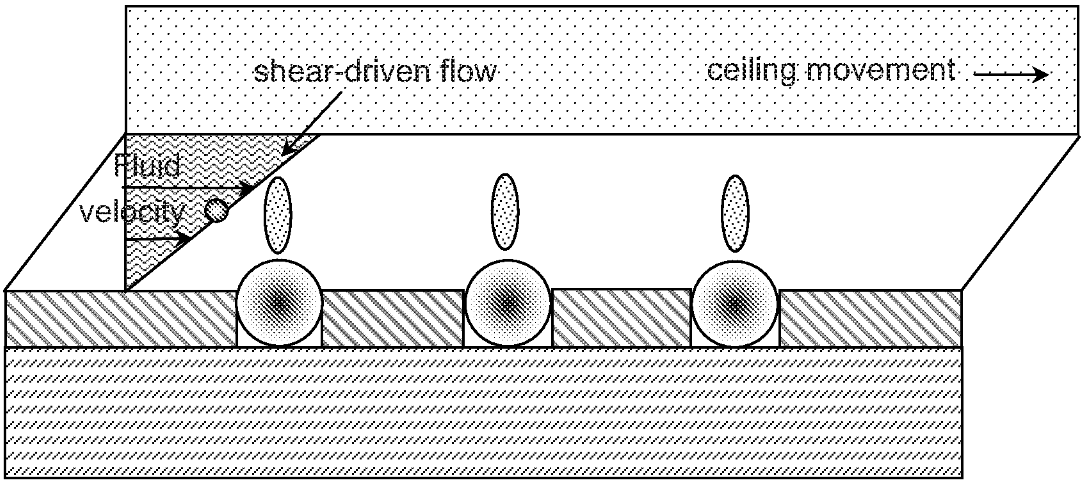
shear-driven flow
ceiling movement
Fluid
velocity

FIG.2b

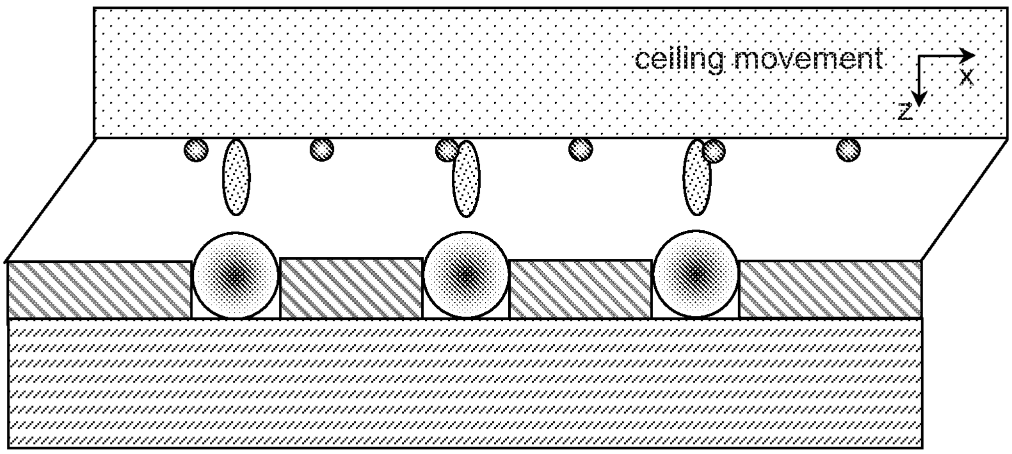
ceiling movement
x
z

FIG.2c

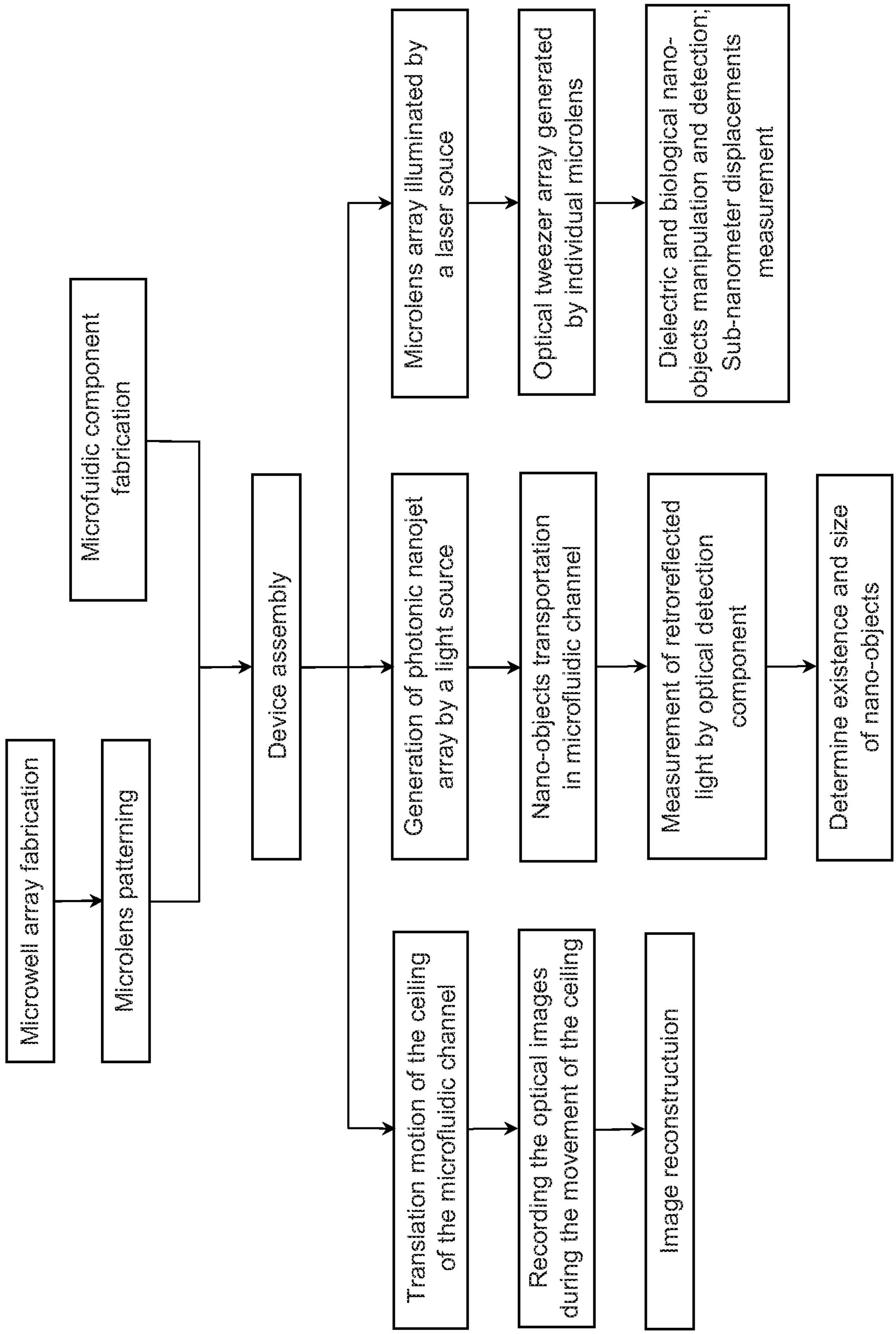
Microwell array fabrication
Microlens patterning
Microfluidic component fabrication
Device assembly
Translation motion of the ceiling of the microfluidic channel
Recording the optical images during the movement of the ceiling
Image reconstructuion
Generation of photonic nanojet array by a light source
Nano-objects transportation in microfluidic channel
Measurement of retroreflected light by optical detection component
Determine existence and size of nano-objects
Microlens array illuminated by a laser souce
Optical tweezer array generated by individual microlens
Dielectric and biological nano-objects manipulation and detection; Sub-nanometer displacements measurement

FIG.3

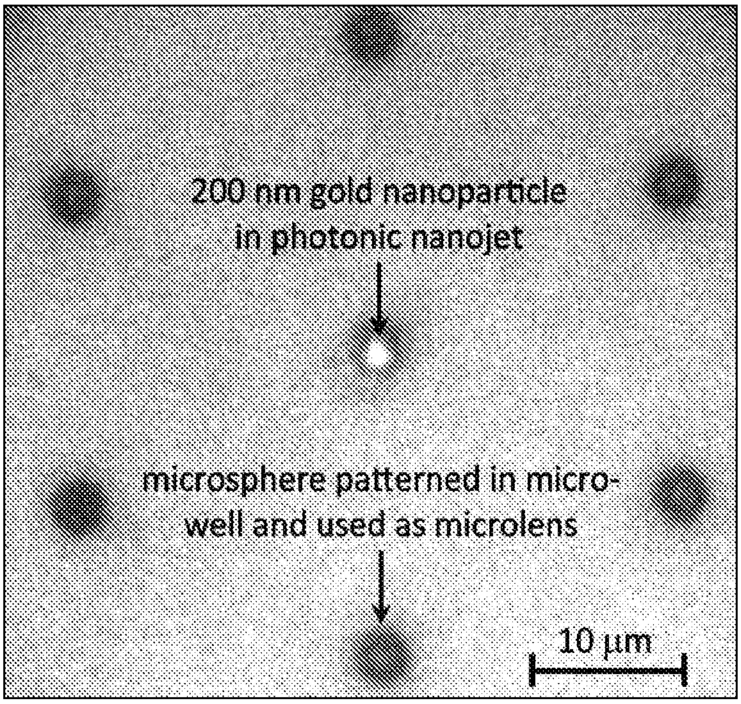
200 nm gold nanoparticle
in photonic nanojet
microsphere patterned in micro-
well and used as microlens
10 µm

FIG.4

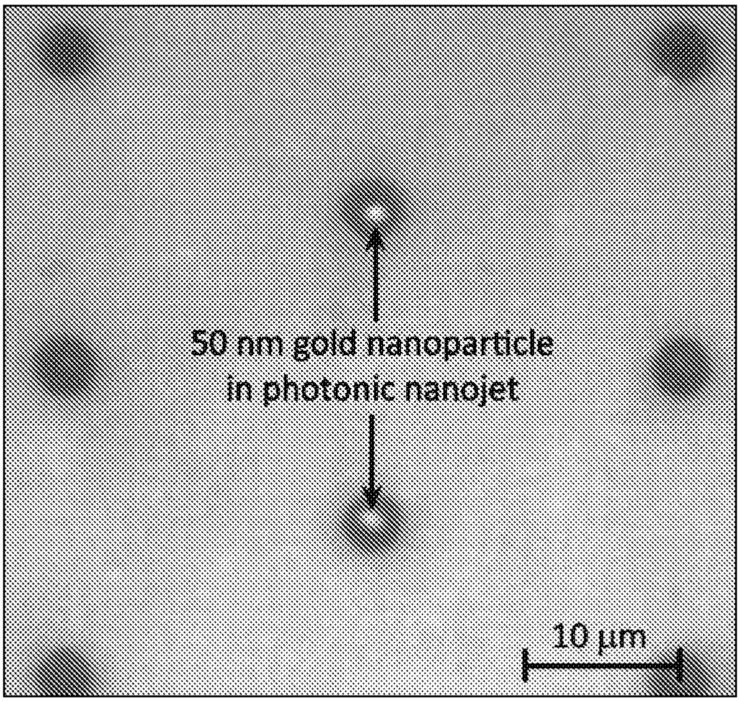
50 nm gold nanoparticle
in photonic nanojet
10 µm

FIG.5

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/055891

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/03 G01N21/05 G01N21/64 B01L3/00 G01N15/14
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)
G01N BOIL G02B

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, COMPENDEX, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	HUI YANG ET AL: "Photonic Nanojet Array for Fast Detection of Single Nanoparticles in a Flow", NANO LETTERS, vol. 15, no. 3, 11 March 2015 (2015-03-11), pages 1730-1735, XP055232129, US ISSN: 1530-6984, D0I: 10.1021/nl5044067 the whole document ----- -/--	1,3-5,7., 8

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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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
1 December 2015	11/12/2015

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JÉROME WENGER ET AL: "Disposable Microscope Objective Lenses for Fluorescence Correlation Spectroscopy Using Latex Microspheres", ANALYTICAL CHEMISTRY, vol. 80, no. 17, 1 September 2008 (2008-09-01), pages 6800-6804, XP055192994, ISSN: 0003-2700, D0I: 10.1021/ac801016z page 6800 - page 6802 page 6804; figure 1 -----	1-9
X	H YANG ET AL: "SELF-ASSEMBLED MELAMINE MICROLENS ARRAYS FOR IMMUNOFLUORESCENCE ENHANCEMENT", 17TH INTERNATIONAL CONFERENCE ON MINIATURIZED SYSTEMS FOR CHEMISTRY AND LIFE SCIENCES, 27 October 2013 (2013-10-27), XP055232265, the whole document figures 1-5 -----	1-9
X	ARASH DARAFSHEH ET AL: "Super-resolution imaging by high-index microspheres immersed in a liquid", 2012 14TH INTERNATIONAL CONFERENCE ON TRANSPARENT OPTICAL NETWORKS (ICTON 2012) : COVENTRY, UNITED KINGDOM, 2 - 5 JULY 2012, IEEE, PISCATAWAY, NJ, 2 July 2012 (2012-07-02), pages 1-3, XP032214563, DOI: 10.1109/ICTON.2012.6254502 ISBN: 978-1-4673-2228-7 the whole document figure 1 -----	1,3,4,7, 9
A	US 5 926 271 A (COUDERC FRANCOIS [FR] ET AL) 20 July 1999 (1999-07-20) column 4, line 57 - column 5, line 27; figure 1A -----	1,7
A	US 2013/228675 Al (CHEN CONG LIANG [US] ET AL) 5 September 2013 (2013-09-05) paragraphs [0079], [0131] - [0134]; figure 15 -----	1

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Information on patent family members

International application No

PCT/IB2015/055891

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