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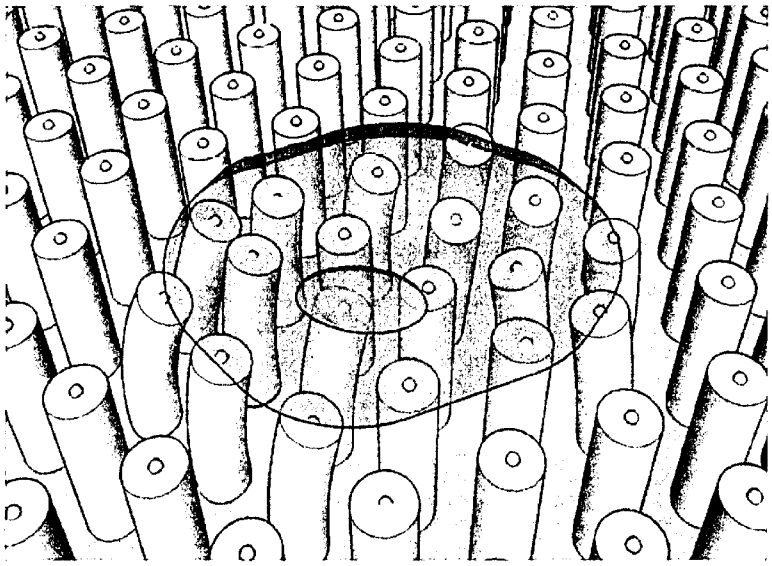

Fig. 1A

(57) Abstract: In various embodiments a plasmonic cell force sensor platform is provided where the platform comprises a plurality of micropillars, where micropillars comprising the plurality of micropillars each have a nanoparticle (e.g., a plasmonic nanoparticle, a fluorescent nanoparticle, etc.) disposed at the tip.

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PLASMONIC MICROPILLAR ARRAY WITH EMBEDDED NANOPARTICLES FOR LARGE AREA CELL FORCE SENSING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of and priority to USSN 62/196,172, filed on July 23, 2015, which is incorporated herein by reference in its entirety for all purposes.

STATEMENT OF GOVERNMENTAL SUPPORT

[0002] This invention was made with government support under GM114188, awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND

[0003] Microfabricated elastic pillar substrates of various geometries have been widely applied to address many fundamental questions in cell biology regarding the mechanotransduction of cell functions. The tip of pillar is usually fluorescently labeled to obtain high contrast between the pillar and the background of the image. An intensity profile of the fluorescent pillar tips is then modeled by a two-dimensional Gaussian fitting to obtain the pillar position and therefore the pillar deflection produced by cells growing on top of the pillars. Local traction force can be calculated as the produced of the amount of pillar deflection and pillar stiffness. Objective lenses with high magnification (*e.g.*, larger than 60x) are usually used to get a good position resolution of (30~50 nm of the pillar. However, the coated fluorescent proteins are often non-uniform on a pillar or across pillars. Furthermore, they can be degraded and/or digested by the cells, and dissolved in the media especially during the media swapping. Therefore the quality of fluorescent image is degraded in a time dependent manner, which will affect the accuracy of dynamic cell force measurement. Besides, for objects larger than the optical diffraction limit of the optical system used for imaging, it's not appropriate to use a simple Gaussian fitting to find the center of a pillar since the profile of pixel intensities cannot be described by a Gaussian function. Such an optical system with high magnification objectives have limited field of view and makes it incapable of monitoring large scale concurrent and instantaneous collective cell behavior.

SUMMARY

[0004] In various embodiments a novel plasmonic cell force sensor platform with superior force sensing accuracy across a large area is provided. In certain embodiments the force sensor platform comprises a plurality of micropillars having a nanoparticle disposed at the tip (e.g., embedded at the tip). In certain embodiments the embedded nanoparticles, provide strong plasmonic scattering which provides a strong signal-to-noise ratio and a point-source-like image pattern for sub-pixel resolution tracing even under a low magnification objective lens.

[0005] Various embodiments contemplated herein may include, but need not be limited to, one or more of the following:

[0006] Embodiment 1: A plasmonic cell force sensor platform, said platform including a plurality of micropillars, wherein micropillars including said plurality of micropillars each have a nanoparticle (*e.g.*, a nanoparticle that can produce an optical signal) disposed at the tip.

[0007] Embodiment 2: The cell force sensor platform of embodiment 1, wherein said nanoparticle is a plasmonic nanoparticle.

[0008] Embodiment 3: The cell force sensor platform according to any one of embodiments 1-2, wherein said micropillars range in average diameter from about 0.1 μm, or about 0.5 μm, or about 1 μm up to about 15 μm, or up to about 10 μm, or up to about 5 μm.

[0009] Embodiment 4: The cell force sensor platform according to any one of embodiments 1-3, wherein the average diameter of said micropillars is about 2 μm.

[0010] Embodiment 5: The cell force sensor platform according to any one of embodiments 1-4, wherein said micropillars range in average length from about 1μm up, or from about 2 μm, or from about 5 μm, up to about 100 μm, or up to about 75 μm, or up to about 50 μm, or up to about 40 μm, or up to about 30 μm, or up to about 20 μm, or up to about 15 μm, or up to about 10 μm.

[0011] Embodiment 6: The cell force sensor platform according to any one of embodiments 1-4, wherein the average length of said micropillars is about 10 μm.

[0012] Embodiment 7: The cell force sensor platform according to any one of embodiments 1-4, wherein the average length of said micropillars is about 7 μm.

[0013] Embodiment 8: The cell force sensor platform according to any one of embodiments 1-7, wherein the spacing between adjacent micropillars ranges from about 0.5 μm up to about 25 μm, or from about 1 μm up to about 20 μm, or from about 2 μm up to about 15 μm, or from about 3 μm up to about 10 μm.

[0014] Embodiment 9: The cell force sensor platform of embodiment 8, wherein the spacing between adjacent micropillars ranges from about 2 μm up to about 5 μm.

[0015] Embodiment 10: The cell force sensor platform of embodiment 8, wherein the spacing between adjacent micropillars is about 4 μm.

[0016] Embodiment 11: The cell force sensor platform according to any one of embodiments 1-10, wherein the size range of said plasmonic nanoparticle ranges from about 20 nm up to about 1000 nm in diameter (or longest dimension).

[0017] Embodiment 12: The cell force sensor platform according to any one of embodiments 1-11, wherein said nanoparticles includes a material selected from the group consisting of ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, gold, copper, titanium, tungsten or an alloy, oxide, or nitride thereof.

[0018] Embodiment 13: The cell force sensor platform of embodiment 12, wherein said nanoparticles comprise gold or silver.

[0019] Embodiment 14: The cell force sensor platform of embodiment 12, wherein said nanoparticles comprise gold.

[0020] Embodiment 15: The cell force sensor platform according to any one of embodiments 1-14, wherein all micropillars comprise the same material nanoparticle.

[0021] Embodiment 16: The cell force sensor platform according to any one of embodiments 1-14, wherein different micropillars comprise different nanoparticle materials to provide different and distinguishable signals.

[0022] Embodiment 17: The cell force sensor platform according to any one of embodiments 1-16, wherein said micropillars are fabricated from a flexible polymer.

[0023] Embodiment 18: The cell force sensor platform of embodiment 17, wherein said micropillars are fabricated from a material selected from the group consisting of polydimethylsiloxane (PDMS), polyolefin plastomer (POP), perfluoropolyethylene (PFPE), polyurethane, polyimides, and cross-linked NOVOLAC® (phenol formaldehyde polymer)

resins, polyolefin plastomers, perfluoropolyethylene, polyurethane, polyimides, cross-linked phenol/formaldehyde polymer resins, and thiol-ene based polymers.

[0024] Embodiment 19: The cell force sensor platform of embodiment 17, wherein said micropillars are fabricated from PDMS.

[0025] Embodiment 20: The cell force sensor platform according to any one of embodiments 1-19, wherein said platform provides a pillar position accuracy of at least about 60 nm, or at least about 50 nm, or at least about 40 nm, or at least about 30 nm, or at least about 20 nm, or at least about 10 nm under a 20x objective lens.

[0026] Embodiment 21: The cell force sensor platform of embodiment 20, wherein said platform provides a pillar position accuracy of about 30nm under a 20x objective lens.

[0027] Embodiment 22: The cell force sensor platform according to any one of embodiments 20-21, wherein said accuracy is under a 20x objective lens with a field of view (FOV) of 450μm × 340μm.

[0028] Embodiment 23: The cell force sensor platform according to any one of embodiments 1-22, wherein said platform provides a force resolution of at least about 400 pN.

[0029] Embodiment 24: The cell force sensor platform according to any one of embodiments 1-23, wherein said platform includes at least 10 micropillars, or at least 20 micropillars, or at least 50 micropillars, or at least 100 micropillars, or at least 250 micropillars, or at least 500 micropillars, or at least 1,000 micropillars, or at least 2,500 micropillars, or at least 5,000 micropillars.

[0030] Embodiment 25: The cell force sensor platform according to any one of embodiments 1-24, wherein said micropillars are disposed over an area ranging up to about 20 cm^2, or up to about 15 cm^2, or up to about 10 cm^2, or up to about 5 cm^2, or up to about 2 cm^2, or up to about 1 cm^2.

[0031] Embodiment 26: A method of fabricating a cell force sensor platform according to any one of embodiments 1-25, said method comprising:

[0032] preparing an array of wells that form a negative mold for a micropillar array;

[0033] depositing a nanofilm (*e.g.*, a metal nanofilm) over said negative mold wherein said nanofilm includes the material (*e.g.*, metal) that will form the nanoparticles;

[0034] removing the nanofilm material on the mold surface leaving microdisks (*e.g.*, metal microdisks) in the bottom of the wells of said negative mold;

[0035] filling the negative mold with a polymer that is to form the micropillars; and melting the microdisks which transfer onto the tips of the micropillars formed by said polymer and form nanoparticles on the micropillars; and

[0036] removing the micropillar array from the negative mold.

[0037] Embodiment 27: The method of embodiment 26, wherein preparing an array of wells includes casting said negative mold using a soft lithography polymer on a positive master mold.

[0038] Embodiment 28: The method of embodiment 27, wherein said soft lithography polymer is PDMS.

[0039] Embodiment 29: The method according to any one of embodiments 27-28, wherein said master mold is formed by etching a silicon substrate.

[0040] Embodiment 30: The method according to any one of embodiments 26-29, wherein said depositing a metal nanofilm includes depositing includes depositing one or more layers of metal including a metal selected from the group consisting of ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, gold, copper, titanium, tungsten or an alloy, oxide, or nitride thereof.

[0041] Embodiment 31: The method according to any one of embodiments 26-30, wherein said depositing a metal nanofilm includes depositing a multi-layer metal nanofilm.

[0042] Embodiment 32: The method of embodiment 31, wherein said multilayer metal nanofilm is an SiO_2/Ti/Au multilayer nanofilm.

[0043] Embodiment 33: The method according to any one of embodiments 26-32, wherein said removing includes using tape to remove metal on the mold surface.

[0044] Embodiment 34: The method according to any one of embodiments 26-33, wherein said melting is by irradiation with laser pulses.

[0045] Embodiment 35: The method of embodiment 34, wherein said melting is by irradiation with nanosecond laser pulses.

[0046] Embodiment 36: A method of measuring the stiffness of cells and/or forces exerted by cells, said method comprising:

[0047] providing cells on a cell force sensor platform according to any one

of embodiments 1-25, wherein said cells are disposed on said platform; and

[0048] determining the deformation of pillars including said platform in response to said cells; and converting the deformation of said pillars into a force exerted by said cells and/or the stiffness of said cells.

[0049] Embodiment 37: A method of delivering an agent into a cell, said method comprising:

[0050] providing cells on a cell force sensor platform according to any one of embodiments 1-25, wherein said cells are disposed on said platform;

[0051] contacting said cells with said agent; and exposing a region of said platform to electromagnetic radiation thereby inducing heating of said nanoparticles where said heating forms bubbles that introduce openings in the membrane of cells in the heated region resulting in the delivery of said agent into those cells.

[0052] Embodiment 38: The method of embodiment 37, wherein said cells are contacted with said agent by providing said agent in culture medium surrounding the cells.

[0053] Embodiment 39: The method according to any one of embodiments 37-38, wherein said exposing includes exposing a region of said platform to a laser pulse.

[0054] Embodiment 40: The method according to any one of embodiments 37-39, wherein said agent is selected from the group consisting of a nucleic acid, a chromosome, a protein, a label, an organelle, and a small organic molecule.

[0055] Embodiment 41: A system for selectively delivering an agent into a cell, said system including: a cell force sensor platform according to any one of embodiments 1-25; and a source of electromagnetic energy capable of heating said nanoparticles.

[0056] Embodiment 42: The system of embodiment 41, wherein said system includes a lens system, a mirror system, or a mask, and/or a positioning system to directing the electromagnetic energy to a specific region of said surface.

[0057] Embodiment 43: The system according to any one of embodiments 41-42, wherein said source of electromagnetic energy is a microwave source or a laser.

[0058] Embodiment 44: The system of embodiment 43, wherein said source of electromagnetic energy is a pulse laser.

BRIEF DESCRIPTION OF THE DRAWINGS

[0059] Figures 1A and 1B illustrate one embodiments of a plasmonic micropillar array. Fig. 1A illustrates a cell disposed on the micropillar array with resulting pillar deformations. Fig. 1B (top) shows a top view of a cell disposed on a micropillar array, and a dark field image of the micropillar array (bottom).

[0060] Figure 2 schematically illustrates a fabrication process to apply laser-induced transfer to embed gold nanoparticles into PDMS micropillar array for high precision pillar position tracing.

[0061] Figure 3A Gold microdisks sandwiched between PDMS pillars and a mold are melted by laser pulses to form nanoparticle and transfer into pillars. Fig. 3B shows images of the pillar immersed in an aqueous solution and imaged with a 20x objective lens in dark field mode. Gold nanoparticle strongly scanner on an otherwise dark background and the PDEMS pillars are invisible. 97.5% of nanoparticles within the FOV of a 20× objective lens are successfully transferred.

[0062] Figure 4 provides SEM and FIB of the pillars showing that each pillar has one gold nanoparticle anchored in the pillar tip.

[0063] Figure 5 illustrates that the nanoparticle on the pillar can be considered a point source when imaged in low magnified imaging system and thus can be fitted by Gaussian point spread function (PSF) with subpixel precision.

[0064] Figures 6, panels A-D, show images under a 20x objective lens. Panel A: Reference image of pillars before cells are plated; Panel B: Pillar images with cells growing on top; Panel C: Fluorescent image of cells stained by WGA 594; Panel D: Superposition of (panel B) and (panel C) to confirm that deflection of pillar is caused by cell force.

[0065] Figure 7, panels A-D, illustrates the precision of the plasmonic micropillars (30 nm resolution under 20x objective lens). Panel A: Reference image; Panel B: Cell image + fluorescence image; Panel C: Same area of pillars in images with and without cells are selected to quantify system precision. Gaussian fitted positions of selected pillars (blue from image (Fig. 6, panel A), red from image (Fig. 6, panel B) are aligned and superpositioned. Panel D: Histogram of apparent displacement of these pillars at rest gives the pillar localization precision of 30 nm.

[0066] Figure 8 illustrates the determination of a cell force map. A reference image and cell image are aligned. The fitted position of corresponding pillar from two images is subtracted to determine deflection of a pillar. Cell force is the product of the pillar deflection and the pillar stiffness.

[0067] Figure 9 . Cell force map imaged by a 20x objective lens. The force vectors are drawn on the fluorescent image of cells. In the illustrated embodiment a force resolution of 400 pN was obtained.

DETAILED DESCRIPTION

[0068] In various embodiments, a novel plasmonic cell force sensor platform is provided that affords superior force sensing accuracy across a large area. In certain embodiments the platform provides 30nm pillar position accuracy under a 20x objective lens with a field-of-view (FOV) of 450μm × 340μm, an area more than 10 times larger than conventional micropillar array approaches that demonstrated similar precision. This high position accuracy comes from the strong plasmonic scattering of plasmonic nanoparticles (*e.g.*, gold nanoparticles) embedded in micropillars comprising the sensor platform. The plasmonic nanoparticles provide a strong signal-to-noise ratio and a point-source-like image pattern for sub-pixel resolution tracing even under a low magnification objective lens.

[0069] In various embodiments the force sensor platform comprises a plurality of micropillars, where micropillars comprising the plurality of micropillars each have a a detectable nanoparticle disposed at the tip, *e.g.*, as illustrated in Figures 1A and 1B (top panel). Illustrative detectable nanoparticle include, but are not limited to plasmonic nanoparticles, fluorescent nanoparticles (*e.g.,* quantum dots), and the like. Figure 1B (bottom panel) illustrates the appearance of the plasmonic nanoparticles at the micropillar tips in dark field microscopy.

[0070] In various embodiments the micropillars range in average diameter from about 0.1 μm up to about 25 μm or up to about 20 μm, or from about 0.1 μm, or from about 0.5 μm, or from about 1 μm up to about 25 μm, or up to about 20 μm, or up to about 15 μm, or up to about 10 μm, or up to about 5 μm. While the micropillars typically have a substantially circular cross-section to provide uniform stiffness (resistance to bending) in all directions, in certain embodiments, the micropillars can be other regular polygons or asymmetric in cross-section. Thus, for example, where the micropillars are rectangular in cross-section they can offer greater stiffness in one direction (*e.g.*, parallel to the long side of the rectangle) as compared to the direction normal to that.

[0071] In various embodiments the micropillars range in average length from about 1μm up, or from about 2 μm, or from about 5 μm, up to about 100 μm, or up to about 75 μm, or up to about 50 μm, or up to about 40 μm, or up to about 30 μm, or up to about 20 μm, or up to about 15 μm, or up to about 10 μm. In certain embodiments the average length of the micropillars is about 10 μm or about 7 μm.

[0072] In various embodiments the spacing between adjacent micropillars ranges from about 0.5 μm up to about 25 μm, or from about 1 μm up to about 20 μm, or from about 2 μm up to about 15 μm, or from about 3 μm up to about 10 μm. In certain embodiments the spacing between adjacent micropillars ranges from about 2 μm up to about 5 μm.

[0073] The micropillars can be fabricated out of essentially any convenient material depending on the pillar dimensions and stiffness desired. In certain embodiments the micropillars are fabricated from a flexible polymer. Illustrative, but non-limiting polymer materials include polydimethylsiloxane (PDMS), polyolefin plastomer (POP), perfluoropolyethylene (PFPE), polyurethane, polyimides, and cross-linked NOVOLAC® (phenol formaldehyde polymer) resins, polyolefin plastomers, perfluoropolyethylene, polyurethane, polyimides, cross-linked phenol/formaldehyde polymer resins, and thiol-ene based polymers, and the like. In certain embodiments the flexible polymer is a polymer used for soft lithography (*e.g.*, PDMS).

[0074] The plasmonic nanoparticles are of a size that permits disposition of the nanoparticle at the micropillar tip and -permits microscopy visualization. In various embodiments the plasmonic nanoparticle range in size from about 20 nm up to about 1000 nm, or up to about 500 nm, or up to about 400 nm in diameter (or largest dimension). The nanoparticle can be fabricated from any material that provides a detectable and localizable signal. In various embodiments the nanoparticles are plasmonic nanoparticles. Suitable materials for such nanoparticle include, but are not limited to metals (*e.g.*, gold, silver, copper, tungsten, platinum, titanium, iron, manganese, and the like, or oxides, nitrides, or alloys thereof), semiconductor materials (*e.g.*, CdSe, CdS, and CdS or CdSe coated with ZnS, and the like), multi-layers of metals and/or metal alloys, and/or metal oxides or nitrides, and the like. In certain embodiments materials comprises one or more of the following: tungsten, tantalum, niobium, Ga, Au, Ag, Cu, Al, Ta, Ti, Ru, Ir, Pt, Pd, Os, Mn, Hf, Zr, V, Nb, La, Y, Gd, Sr, Ba, Cs, Cr, Co, Ni, Zn, Ga, In, Cd, Rh, Re, W, Mo, and oxides, nitrides, alloys, and/or mixtures and/or sinters thereof. Other useful materials

include, but are not limited to ZnS, ZnO, TiO_2, AgI, AgBr, PbS, PbSe, ZnTe, CdTe, In_2S_3, In_2Se_3, Cd_3P_2, Cd_3As_2, InAs, GaAs, and the like. In certain embodiments the nanoparticles comprise a material selected from the group consisting of ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, gold, copper, titanium, tungsten or an alloy, oxide, or nitride thereof. In certain embodiments the nanoparticles comprise gold or silver.

[0075] In various embodiments all the micropillars bearing nanoparticles at the tip comprise nanoparticles formed from the same material (*e.g.*, gold, silver, *etc.*). However in certain embodiments, different micropillars comprise nanoparticles formed from different materials. In such instances the micropillars having different nanoparticles can provide different and distinguishable signals.

[0076] In various embodiments different shaped nanoparticles can be used. Illustrative, but non-limiting nanoparticles (nanoparticle shapes) that can be used in the devices described herein include, but are not limited to a hollow or solid nanosphere, a nanorod, a nanocube, a nano-octahedron, a nano-rhombic dodecahedron, a nanocrescent, a nanowire, a nanotube, a nanopyramid, a nanorod, a nanotoroid, a nanotetrepod, a single- or multi-layered nanodisk, and a nano-octopod nanoparticle. In various embodiments any of the nanoparticles described herein comprise a plasmonic material and/or are a plasmonic nanoparticle.

[0077] Different shapes and/or sizes of gold nanoparticles or other plasmonic nanoparticles give different scattering signals, even when they are all made of the same materials. Accordingly, in certain embodiments, the cell force sensor platform comprises a plurality of micropillars where different micropillars comprise different nanoparticle shapes and/or different nanoparticle sizes to provide different and distinguishable signals. In certain other embodiments all of the plurality of micropillars have the same shape and/or size nanoparticles.

[0078] As noted above, it is believed the force sensor platform(s) described herein provide a substantially higher pillar position accuracy over a larger field of view than has been obtained with any previous micropillar sensor. In certain embodiments the platform provides a pillar position accuracy of at least about 60 nm, or at least about 50 nm, or at least about 40 nm, or at least about 30 nm, or at least about 20 nm, or at least about 10 nm, or at least about 5 nm under an objective lens ranging from about 1x or from about 4x or from about 5x, or from about 10x up to about 40x, or up to about 60x, or up to about 80x, or up to about 100 x. In certain embodiments the platform provides a pillar position accuracy

of about 30nm under a 20x objective lens (*e.g.*,, where the accuracy is under a 20x objective lens with a field of view (FOV) of 450μm × 340μm).

[0079] In various embodiments the platform provides a force resolution of at least about 600 pN, or at least about 500 pN, or at least about 400 pN.

[0080] In various embodiments the force sensor platform comprises at least 10 micropillars, or at least 20 micropillars, or at least 50 micropillars, or at least 100 micropillars, or at least 250 micropillars, or at least 500 micropillars, or at least 1,000 micropillars, or at least 2,500 micropillars, or at least 5,000 micropillars, or at least 10,000 micropillars, or at least 50,000 micropillars, or at least 100,000 micropillars, or at least about 500,000 micropillars, or at least 1,000,000 micropillars, or at least 25,000,000 micropillars, or at least 50,000,000 micropillars, or at least 75,000,000 micropillars, or at least 100,000,000 micropillars. In certain embodiments a 4 cm x 4 cm platform comprises at least 1,000,000 micropillars, or at least 25,000,000 micropillars, or at least 50,000,000 micropillars, or at least 75,000,000 micropillars, or at least 100,000,000 micropillars. In certain embodiments a 4cm x 4 cm platform comprises at least about 100,000,000 micropillars. In certain embodiments the platform comrpsies micropillars at a density of at least about 250,000/cm^2, or at least about 500,000/cm^2, or at least about 750,000/cm^2, or at least about 1,000,000/cm^2, or at least about 2,000,000/cm^2, or at least about 3,000,000/cm^2 or at least about 4,000,000/cm^2, or at least about 5,000,000/cm^2 or at least about 6,000,000/cm^2.

[0081] The area of the force sensor platform is essentially unlimited. In certain embodiments, for example, numerous force sensor platforms can be combined to provide larger platforms. In certain embodiments the pillars comprising the force sensor platform are disposed in an area ranging up to about 20 cm^2, or up to about 15 cm^2, or up to about 10 cm^2, or up to about 5 cm^2, or up to about 2 cm^2, or up to about 1 cm^2. In certain embodiments the force sensor platform is about 1cm x 1 cm, or about 1 cm by 2 cm, or about 2 cm x 2 cm, or about 1 cm x 3 cm, or about 2 cm x 3 cm, or about 3 cm x 3 cm, or about 1 cm x 4 cm, or about 2 cm x 4 cm, or about 3 cm x 4 cm, or about 4 cm x 4 cm, or about 1 cm x 5 cm, or about 2 cm x 5 cm, or about 3 cm x 5 cm, or about 4 cm x 5 cm, or about 5 cm x 5 cm.

[0082] One illustrative, but non-limiting method of fabricating the micropillar sensor is shown schematically in Figure 2. One certain embodiments, the method involves

[0083] i) preparing an array of wells that form a negative mold for a

micropillar array;

[0084] ii) depositing a metal nanofilm over the negative mold where the metal nanofilm comprises the metal that will form the nanoparticles;

[0085] iii) removing the metal on the mold surface leaving metal microdisks in the bottom of the wells of the negative mold;

[0086] iv) filling the negative mold with a polymer that is to form the micropillars; and

[0087] v) melting the microdisks which transfer onto the tips of the micropillars formed by the polymer; and

[0088] vi) removing the micropillar array from the negative mold.

[0089] It will be recognized that where the nanoparticle is not a metal nanoparticle, the nanofilm need not be a metal nanofilm, but simply a nanofilm comprising the material that is to form the nanoparticle.

[0090] The negative mold can be fabricated using a number of methods know to those of skill in the art. In one embodiment, as illustrated in Figure 2, the pillar array is first fabricated on a silicon substrate as a master mold by standard microfabrication techniques (*e.g.*, a patterned photoresist followed by an etching step). A complimentary well array in a negative mold is obtained by casting a soft lithograph material (*e.g.*, PDMS) on the master mold, *e.g.*, as illustrated in Figure 2, panel (f). After silane treatment a metal nanofilm (*e.g.*, an SiO_2/Ti/Au multilayer nanofilm) is deposited all over the negative (*e.g.*, PDMS) mold and metal on the mold surface is later removed, *e.g.*, using tape. Metal microdisks left in the bottom of the wells are then covered by the material that is to form the pillars (*e.g.*, PDMS) and irradiated by a laser (*e.g.*, by nanosecond laser pulses). These metal disks (*e.g.*, gold disks) are melted and evolve, driven by the strong surface tension of the molten metal (*e.g.*, molten gold), into nanoparticles, that are found to be transferred into the tips of PDMS pillars after demolding.

[0091] The shape evolution of a metal microdisk (*e.g.*, a gold microdisk) sandwiched between a PDMS pillar and its mold is shown in Fig. 3A. Nanoparticles within the laser-irradiated area are transferred into micropillars as shown in Fig. 3B. The focused ion beam (FIB) image (Fig. 4) show that the nanoparticles are partially embedded in the tips of these pillars. Under a low magnification optical system, these nanoparticles serve as point-source-like light sources that strongly scatter light under dark field mode microscopy

imaging, and provide superior signal-to-noise ratio to the background pillar and cell scattering signals for high precision position tracing.

[0092] In order to study the position accuracy of the micropillars for force sensing under a 20× objective lens, Madin-Darby Canine Kidney epithelial cells (MDCK cells) were grown on the device. Dark field images shown in Fig. 6, panel A, were first taken before the cells were plated. The undeflected pillar position was determined by Gaussian fitting the image of gold nanoparticles, *e.g.*, as shown in Fig. 5. With cells growing on top, the pillars respond to cell traction forces and bend. This deflection was captured again as shown in Fig. 6, panel B. An area of pillars away from any cell in Fig. 6, panel B can be selected to compare with the same area in Fig. 6, panel A to determine the position accuracy of these micropillars in the imaging and position fitting system (Fig. 7, panel C). In Fig. 7, panel D the histogram of the apparent displacement of these pillars at rest shows that the spatial resolution of our plasmonic micropillar platform is ~ 30nm.

[0093] Figure 8 illustrates the determination of a cell force map. A reference image and cell image are aligned. The fitted position of corresponding pillar from two images is subtracted to determine deflection of a pillar. Cell force is the product of the pillar deflection and the pillar stiffness.

[0094] Cell forces can be calculated (*e.g.*, by home-developed MATLAB codes_ and are usually represented by vectors drawn on each pillar whose length is proportional to the force magnitude. Fig. 9 gives the force map of MDCK cells growing on top of the pillar array.

[0095] In various embodiments this platform can be used to measure the stiffness of cells through the cell force and deformation measurement of the pillars. The differences in stiffness can be used to identify differences in cells. For example, cancer cells have been shown to have very different stiffness compared with normal cells.

[0096] In various embodiments this platform can be used in drug development by measuring cell force or stiffness as altered by treatment with particular drugs (*e.g.*,, candidate anti-cancer drugs).

[0097] In various embodiments this platform can be used for large area cell mechanotype monitoring, for example, during induced epithelial-to-mesenchymal transition in cancer cells, in transdifferentiation from lipofibroblasts to myofibroblasts in lung cells, and the like.

[0098] In certain embodiments the embedded nanoparticles at the top of the pillars can serve as heat sources when irradiated (*e.g.*, via optical irradiation, microwave irradiation, and the like). This permits selective cell ablation at the single cell level or subcellular level. Real time monitoring of cell forces in reaction reaction to photothermal treatment applied by laser irritated nanoparticles is also possible.

[0099] In certain embodiments by irradiating the nanoparticles with a pulse laser, nanocavitation bubbles can be induced at the pillar top which can porate cells cultured on the platform. High throughput, large area intracellular delivery of functional cargo into cells is feasible at the single cell or sub-cellular level resolution.

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[0114] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

What is claimed is:

1. A plasmonic cell force sensor platform, said platform comprising a plurality of micropillars, wherein micropillars comprising said plurality of micropillars each have a plasmonic nanoparticle disposed at the tip.

2. The cell force sensor platform of claim 1, wherein said micropillars range in average diameter from about 0.1 μm, or about 0.5 μm, or about 1 μm up to about 20 μm, or up to about 15 μm, or up to about 10 μm, or up to about 5 μm.

3. The cell force sensor platform according to any one of claims 1-2, wherein the average diameter of said micropillars is about 2 μm.

4. The cell force sensor platform according to any one of claims 1-3, wherein said micropillars range in average length from about 1μm up, or from about 2 μm, or from about 5 μm, up to about 100 μm, or up to about 75 μm, or up to about 50 μm, or up to about 40 μm, or up to about 30 μm, or up to about 20 μm, or up to about 15 μm, or up to about 10 μm.

5. The cell force sensor platform according to any one of claims 1-3, wherein the average length of said micropillars is about 10 μm.

6. The cell force sensor platform according to any one of claims 1-3, wherein the average length of said micropillars is about 7 μm.

7. The cell force sensor platform according to any one of claims 1-6, wherein the spacing between adjacent micropillars ranges from about 0.5 μm up to about 25 μm, or from about 1 μm up to about 20 μm, or from about 2 μm up to about 15 μm, or from about 3 μm up to about 10 μm.

8. The cell force sensor platform of claim 7, wherein the spacing between adjacent micropillars ranges from about 2 μm up to about 5 μm.

9. The cell force sensor platform of claim 7, wherein the spacing between adjacent micropillars is about 4 μm.

10. The cell force sensor platform according to any one of claims 1-9, wherein the size range of said plasmonic nanoparticle ranges from about 20 nm up to about 1000 nm in diameter (or longest dimension).

11. The cell force sensor platform according to any one of claims 1-10, wherein said nanoparticles comprises a material selected from the group consisting of ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, gold, copper, titanium, tungsten or an alloy, oxide, or nitride thereof.

12. The cell force sensor platform of claim 11, wherein said nanoparticles comprise gold or silver.

13. The cell force sensor platform of claim 11, wherein said nanoparticles comprise gold.

14. The cell force sensor platform according to any one of claims 1-13, wherein all of said plurality of micropillars comprise the same material nanoparticle.

15. The cell force sensor platform according to any one of claims 1-13, wherein different micropillars comprise different nanoparticle materials to provide different and distinguishable signals.

16. The cell force sensor platform according to any one of claims 1-15, wherein said nanoparticle comprises a nanoparticle shape selected from the group consisting of a nanosphere, a nanorod, nanocube, octahedron, rhombic dodecahedron, a nanocrescent, a nanowire, a nanotube, a nanopyramid, a nanorod, a nanotetrepod, a single- or multi-layered nanodisk, and an octopod nanoparticle.

17. The cell force sensor platform according to any one of claims 1-16, wherein all of said plurality of micropillars comprise the same shape nanoparticle.

18. The cell force sensor platform according to any one of claims 1-16, wherein different micropillars comprise different nanoparticle shapes to provide different and distinguishable signals.

19. The cell force sensor platform according to any one of claims 1-18, wherein all of said plurality of micropillars comprise the same size nanoparticle.

20. The cell force sensor platform according to any one of claims 1-18, wherein different micropillars comprise different nanoparticle sizes to provide different and distinguishable signals.

21. The cell force sensor platform according to any one of claims 1-20, wherein said micropillars are fabricated from a flexible polymer.

22. The cell force sensor platform of claim 21, wherein said micropillars are fabricated from a material selected from the group consisting of polydimethylsiloxane (PDMS), polyolefin plastomer (POP), perfluoropolyethylene (PFPE), polyurethane, polyimides, and cross-linked NOVOLAC® (phenol formaldehyde polymer) resins, polyolefin plastomers, perfluoropolyethylene, polyurethane, polyimides, cross-linked phenol/formaldehyde polymer resins, and thiol-ene based polymers.

23. The cell force sensor platform of claim 21, wherein said micropillars are fabricated from PDMS.

24. The cell force sensor platform according to any one of claims 1-23, wherein said platform provides a pillar position accuracy of at least about 60 nm, or at least about 50 nm, or at least about 40 nm, or at least about 30 nm, or at least about 20 nm, or at least about 10 nm, or at least about 5 nm under an objective lens ranging from about 1x or from about 4x or from about 5x, or from about 10x up to about 40x, or up to about 60x, or up to about 80x, or up to about 100 x.

25. The cell force sensor platform according of claim 24, wherein said platform provides a pillar position accuracy of at least about 60 nm, or at least about 50 nm, or at least about 40 nm, or at least about 30 nm, or at least about 20 nm, or at least about 10 nm under a 20x objective lens.

26. The cell force sensor platform of claim 25, wherein said platform provides a pillar position accuracy of about 30nm under a 20x objective lens.

27. The cell force sensor platform according to any one of claims 25-26, wherein said accuracy is under a 20x objective lens with a field of view (FOV) of 450μm × 340μm.

28. The cell force sensor platform according to any one of claims 1-27, wherein said platform provides a force resolution of at least about 400 pN.

29. The cell force sensor platform according to any one of claims 1-28, wherein said platform comprises at least 10 micropillars, or at least 20 micropillars, or at least 50 micropillars, or at least 100 micropillars, or at least 250 micropillars, or at least 500 micropillars, or at least 1,000 micropillars, or at least 2,500 micropillars, or at least 5,000 micropillars, or at least 10,000 micropillars, or at least 50,000 micropillars, or at least 100,000 micropillars, or at least about 500,000 micropillars, or at least 1,000,000 micropillars, or at least 25,000,000 micropillars, or at least 50,000,000 micropillars, or at least 75,000,000 micropillars, or at least 100,000,000 micropillars.

30. The cell force sensor platform of claim 29, wherein a 4 cm x 4 cm platform comprises at least 1,000,000 micropillars, or at least 25,000,000 micropillars, or at least 50,000,000 micropillars, or at least 75,000,000 micropillars, or at least 100,000,000 micropillars.

31. The cell force sensor platform of claim 30, wherein a 4 cm x 4 cm platform comprises at least about 100,000,000 micropillars.

32. The cell force sensor platform according to any one of claims 1-31, wherein said micropillars are disposed over an area ranging up to about 20 cm^2, or up to about 15 cm^2, or up to about 10 cm^2, or up to about 5 cm^2, or up to about 2 cm^2, or up to about 1 cm^2.

33. A method of fabricating a cell force sensor platform according to any one of claims 1-32, said method comprising:

preparing an array of wells that form a negative mold for a micropillar array;

depositing a metal nanofilm over said negative mold wherein said metal nanofilm comprises the metal that will form the nanoparticles;

removing the metal on the mold surface leaving metal microdisks in the bottom of the wells of said negative mold;

filling the negative mold with a polymer that is to form the micropillars; and

melting the microdisks which transfer onto the tips of the micropillars formed by said polymer; and

removing the micropillar array from the negative mold.

34. The method of claim 33, wherein preparing an array of wells comprises casting said negative mold using a soft lithography polymer on a positive master mold.

35. The method of claim 34, wherein said soft lithography polymer is PDMS.

36. The method according to any one of claims 34-35, wherein said master mold is formed by etching a silicon substrate.

37. The method according to any one of claims 33-36, wherein said depositing a metal nanofilm comprises depositing comprises depositing one or more layers of metal comprising a metal selected from the group consisting of ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, gold, copper, titanium, tungsten or an alloy, oxide, or nitride thereof.

38. The method according to any one of claims 33-37, wherein said depositing a metal nanofilm comprises depositing a multi-layer metal nanofilm.

39. The method of claim 38, wherein said multilayer metal nanofilm is an SiO_2/Ti/Au multilayer nanofilm.

40. The method according to any one of claims 33-39, wherein said removing comprises using tape to remove metal on the mold surface.

41. The method according to any one of claims 33-40, wherein said melting is by irradiation with laser pulses.

42. The method of claim 41, wherein said melting is by irradiation with nanosecond laser pulses.

43. A method of measuring the stiffness of cells and/or forces exerted by cells, said method comprising:

providing cells on a cell force sensor platform according to any one of claims 1-32, wherein said cells are disposed on said platform; and

determining the deformation of pillars comprising said platform in response to said cells; and

converting the deformation of said pillars into a force exerted by said cells and/or the stiffness of said cells.

44. A method of delivering an agent into a cell, said method comprising:

providing cells on a cell force sensor platform according to any one of claims 1-32, wherein said cells are disposed on said platform;

contacting said cells with said agent; and

exposing a region of said platform to electromagnetic radiation thereby inducing heating of said nanoparticles where said heating forms bubbles that introduce openings in the membrane of cells in the heated region resulting in the delivery of said agent into those cells.

45. The method of claim 44, wherein said cells are contacted with said agent by providing said agent in culture medium surrounding the cells.

46. The method according to any one of claims 44-45, wherein said exposing comprises exposing a region of said platform to a laser pulse.

47. The method according to any one of claims 44-46, wherein said agent is selected from the group consisting of a nucleic acid, a chromosome, a protein, a label, an organelle, and a small organic molecule.

48. A system for selectively delivering an agent into a cell, said system comprising:

a cell force sensor platform according to any one of claims 1-32; and

a source of electromagnetic energy capable of heating said nanoparticles.

49. The system of claim 48, wherein said system comprises a lens system, a mirror system, or a mask, and/or a positioning system to directing the electromagnetic energy to a specific region of said surface.

50. The system according to any one of claims 48-49, wherein said source of electromagnetic energy is a microwave source or a laser.

51. The system of claim 50, wherein said source of electromagnetic energy is a pulse laser.

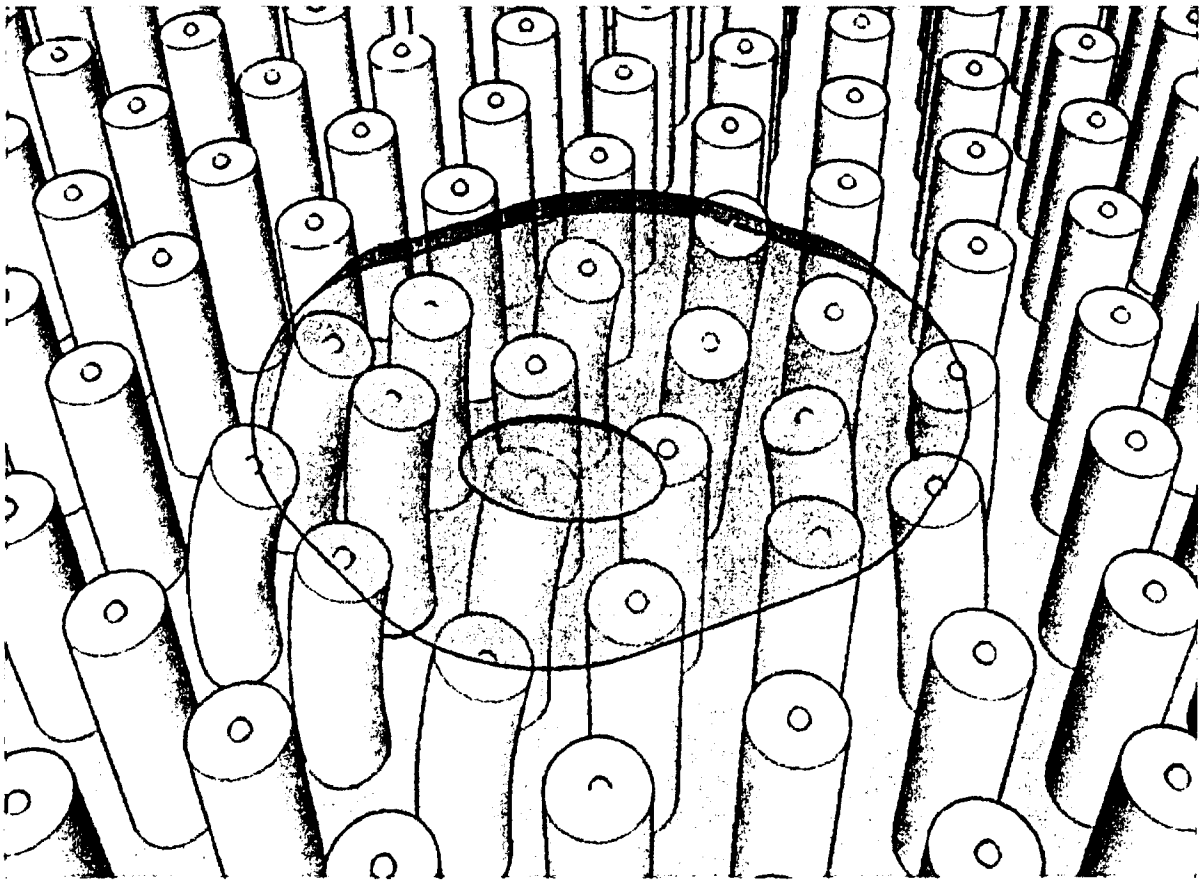
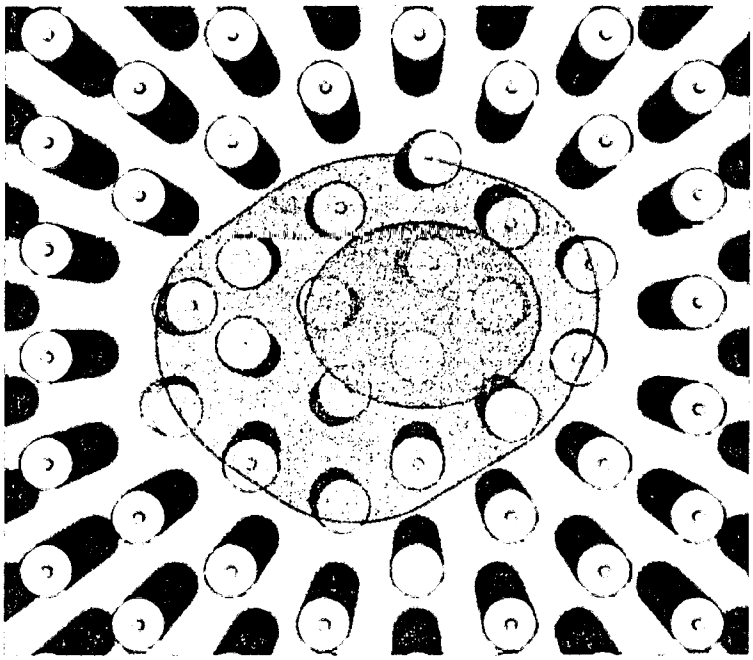


Fig. 1A

Overall View



Dark Field Image

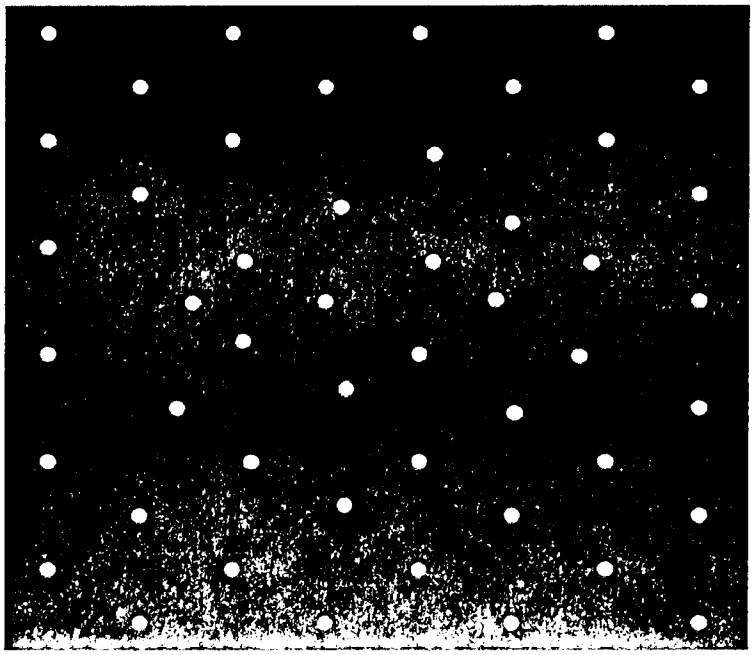


Fig. 1B

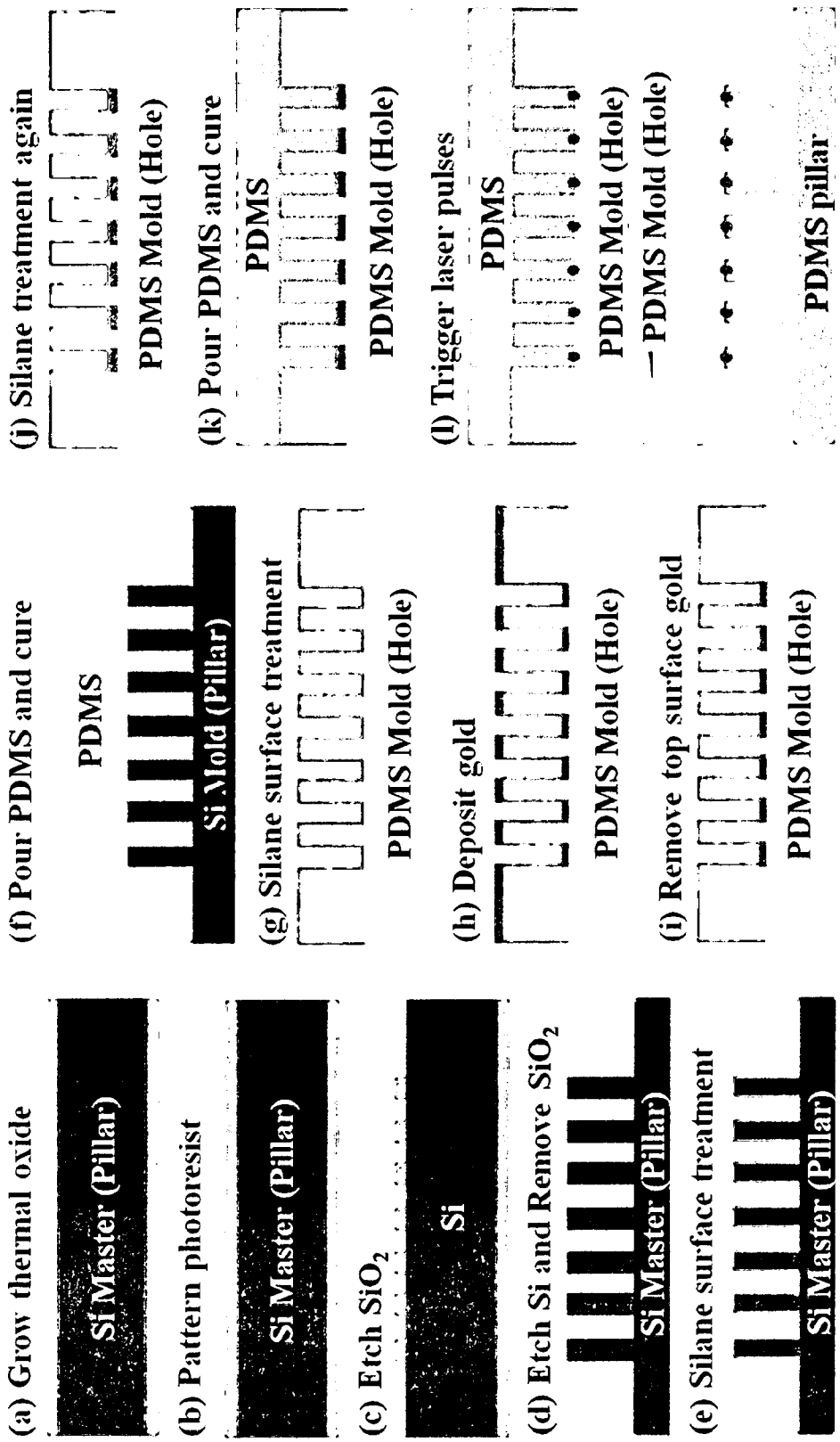

(a) Grow thermal oxide
Si Master (Pillar)
(b) Pattern photoresist
Si Master (Pillar)
(c) Etch SiO_2
Si
(d) Etch Si and Remove SiO_2
Si Master (Pillar)
(e) Silane surface treatment
Si Master (Pillar)
(f) Pour PDMS and cure
PDMS
Si Mold (Pillar)
(g) Silane surface treatment
PDMS Mold (Hole)
(h) Deposit gold
PDMS Mold (Hole)
(i) Remove top surface gold
PDMS Mold (Hole)
(j) Silane treatment again
PDMS Mold (Hole)
(k) Pour PDMS and cure
PDMS
PDMS Mold (Hole)
(l) Trigger laser pulses
PDMS
PDMS Mold (Hole)
PDMS Mold (Hole)
PDMS pillar

Fig. 2

Before demolding

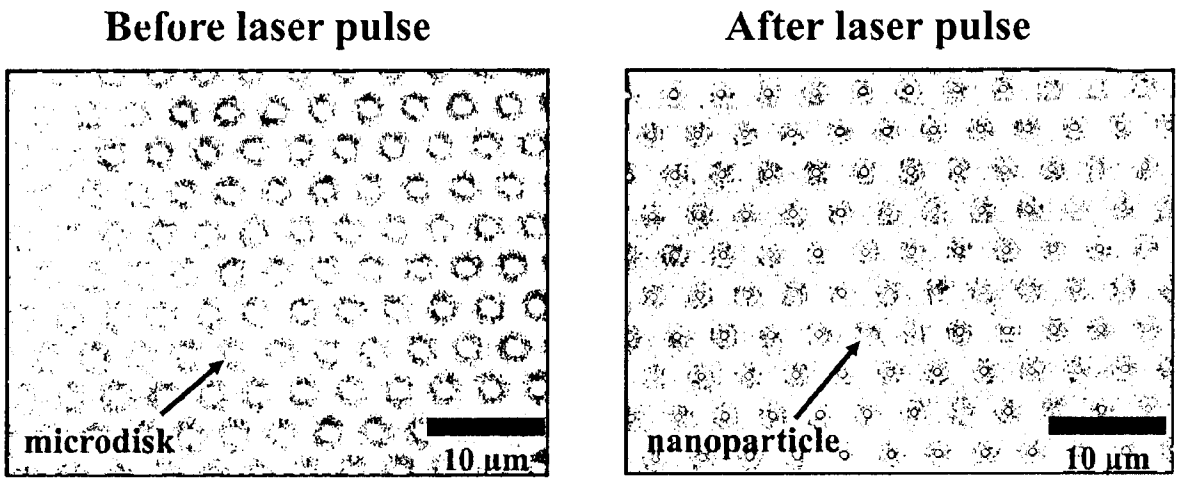


Fig. 3A

After demolding

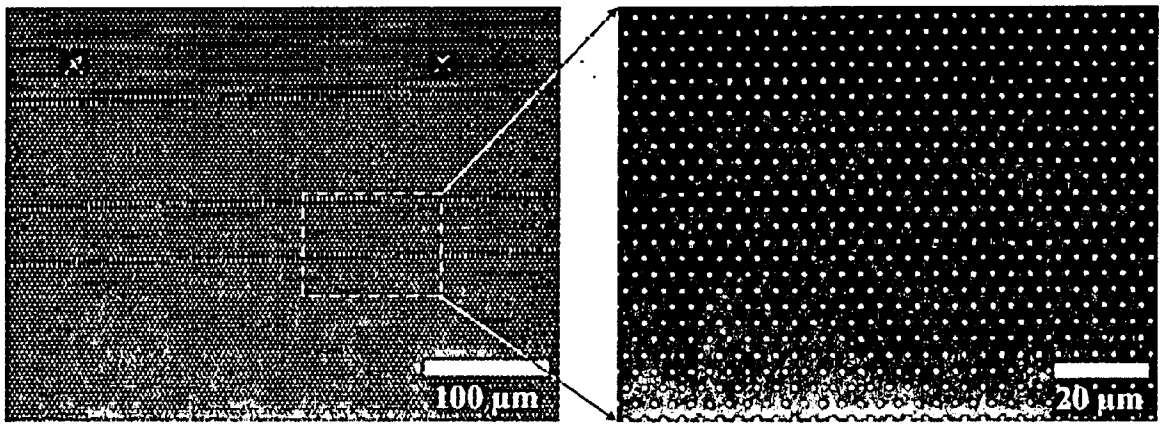


Fig. 3B

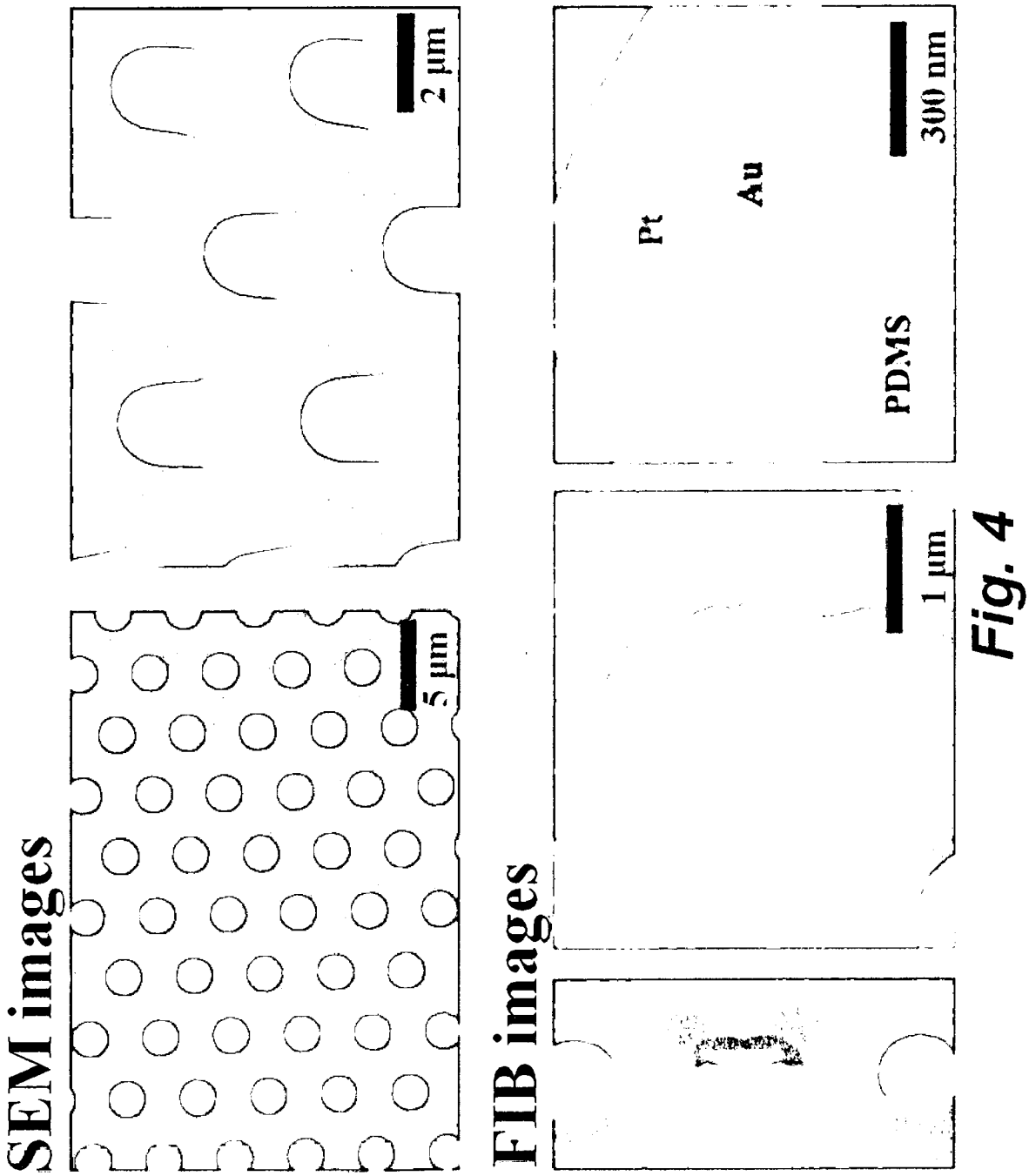
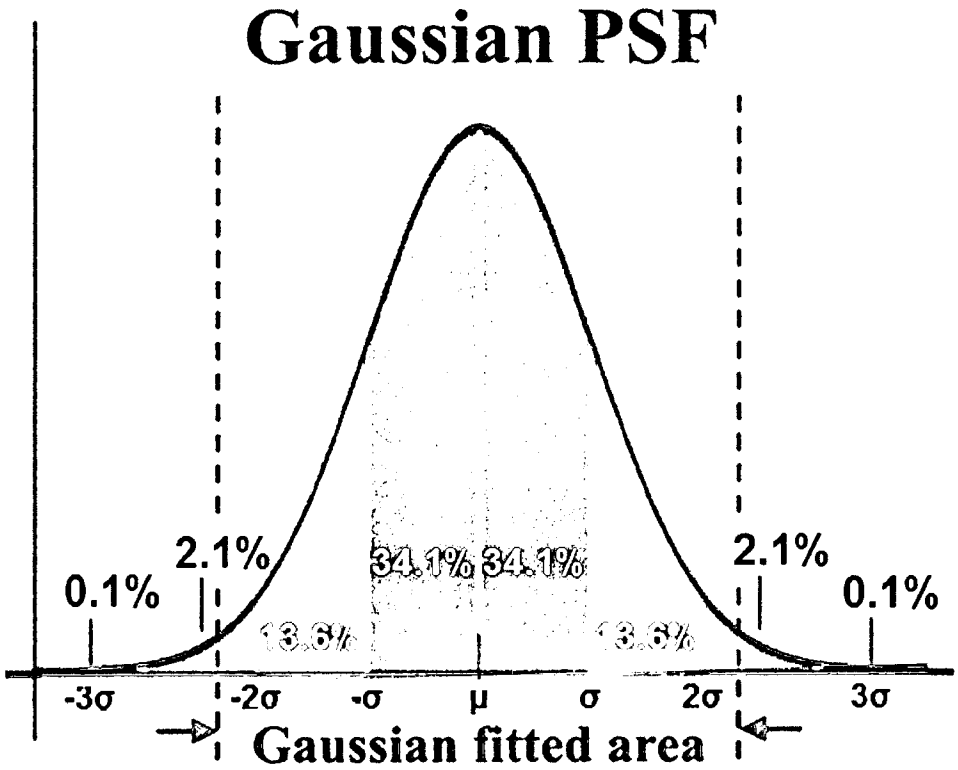


Fig. 4


Gaussian PSF
0.1%
2.1%
34.1%
34.1%
13.6%
13.6%
2.1%
0.1%
-3σ
-2σ
-σ
μ
σ
2σ
3σ
Gaussian fitted area

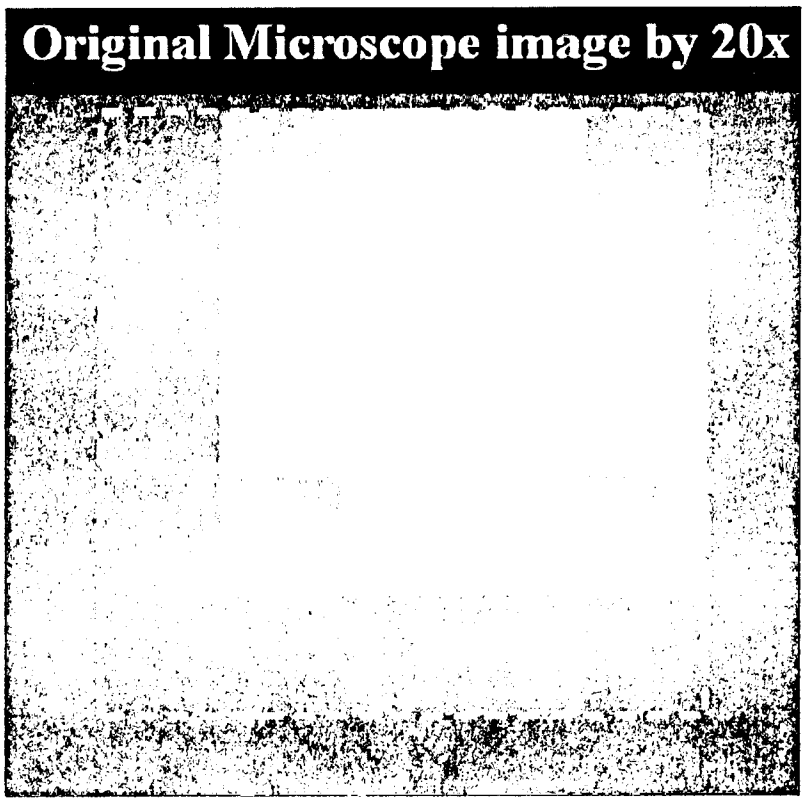
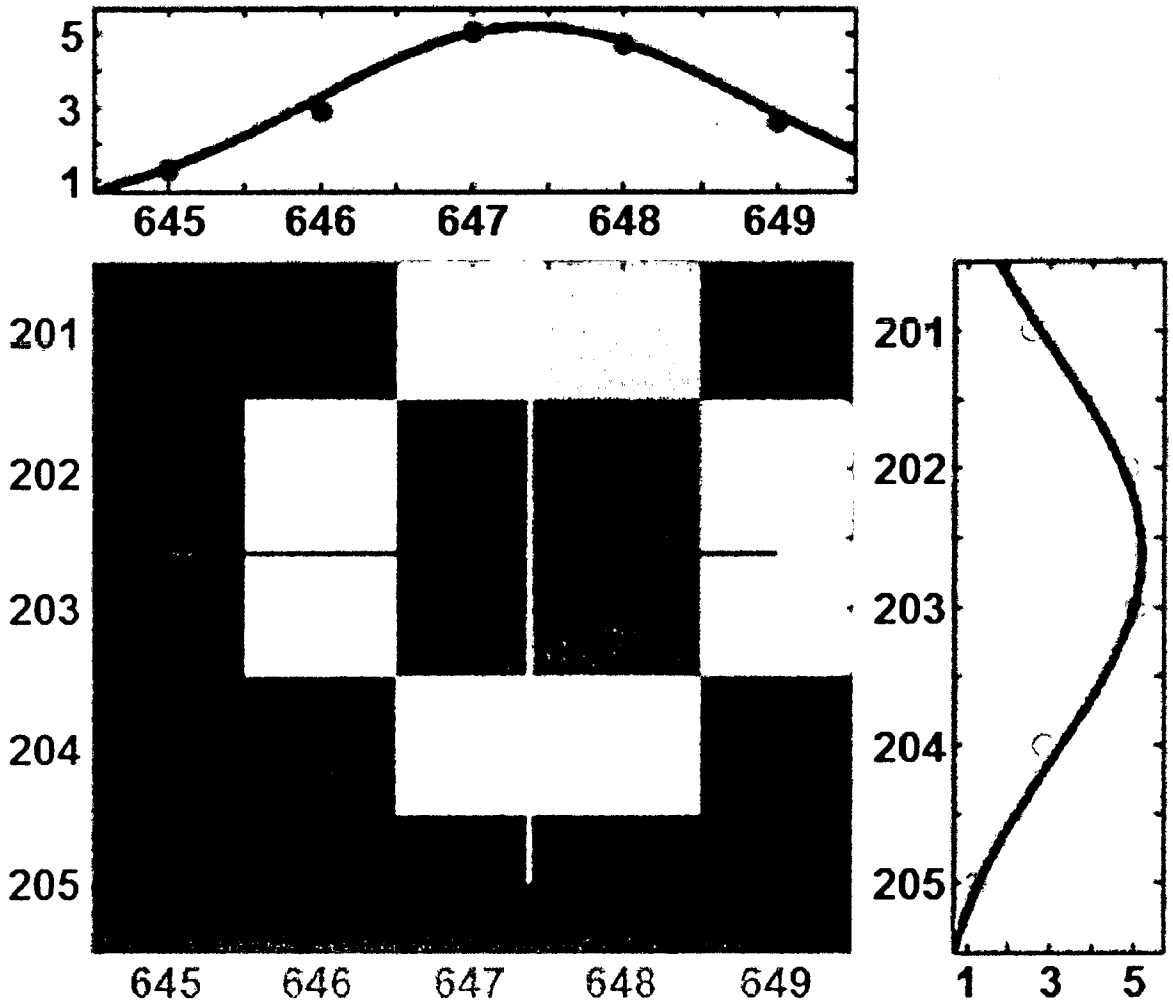

Original Microscope image by 20x

Fig. 5


5
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201
202
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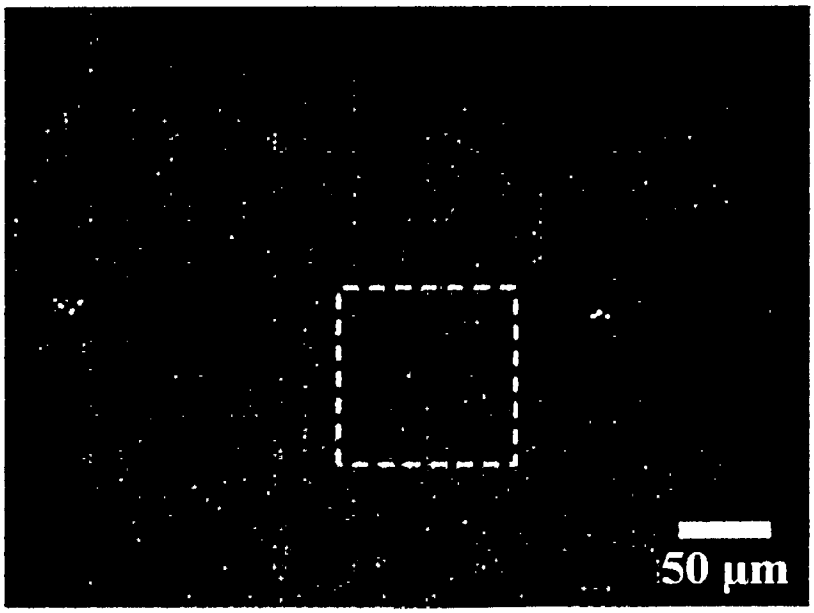
204
205
1
3
5

Fig. 5, cont'd.

A) Reference Image



B) Cell image + Fluorescent image

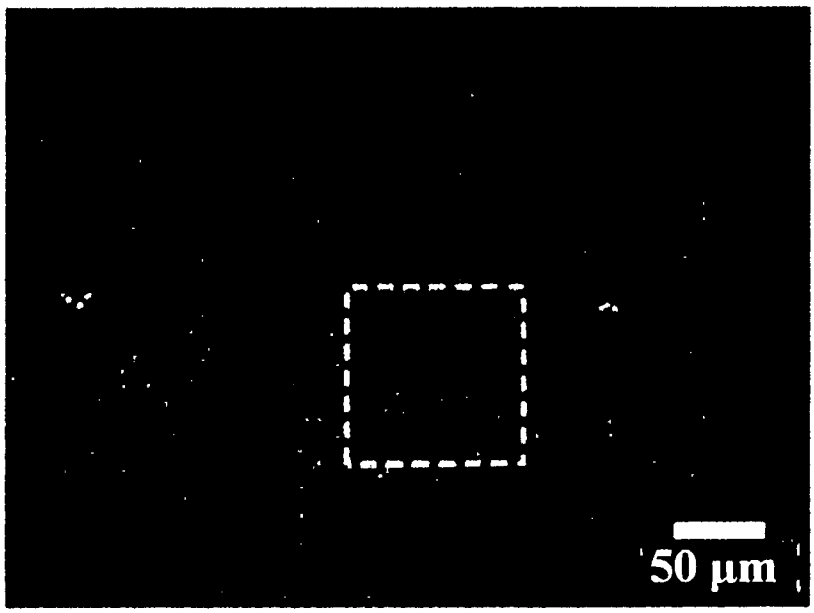
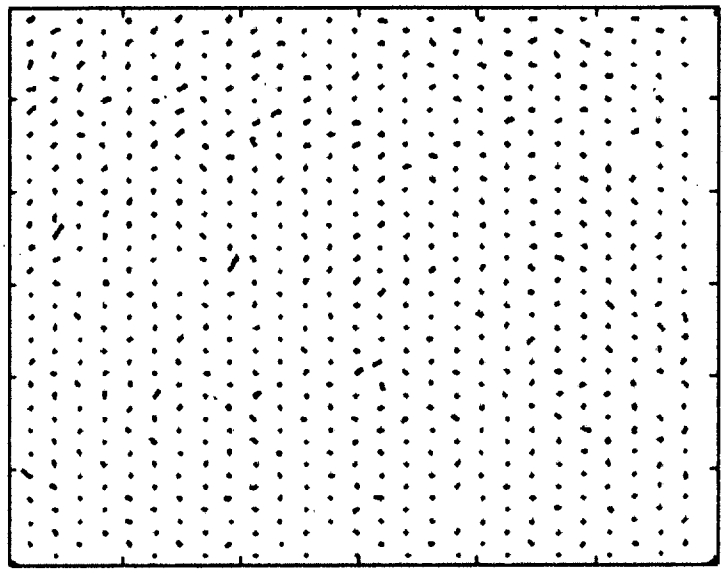


Fig. 6

C) Displacement



D) Histogram of displacement

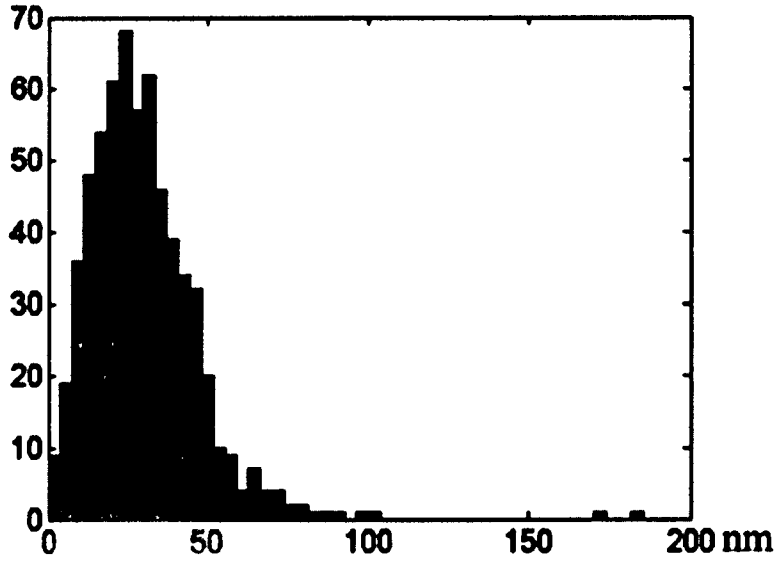
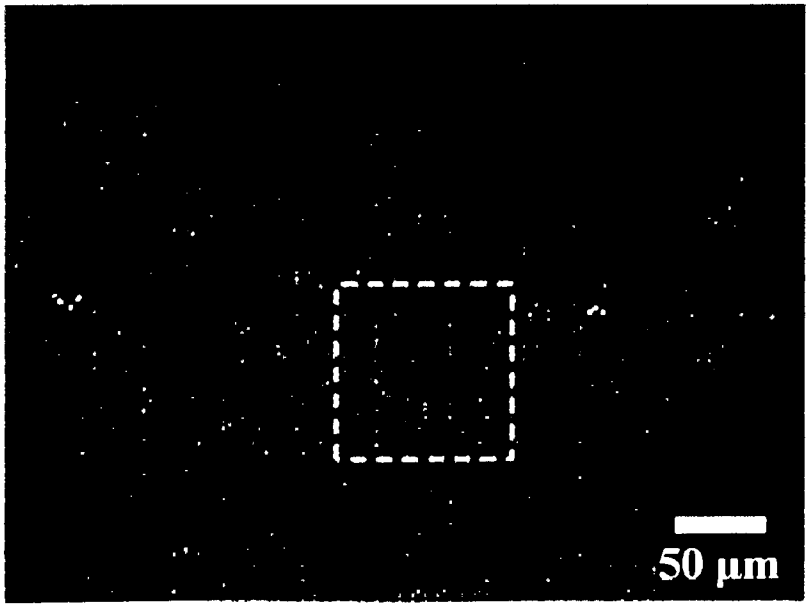


Fig. 6, cont'd.

A) Reference Image



B) Cell image + Fluorsecent Image

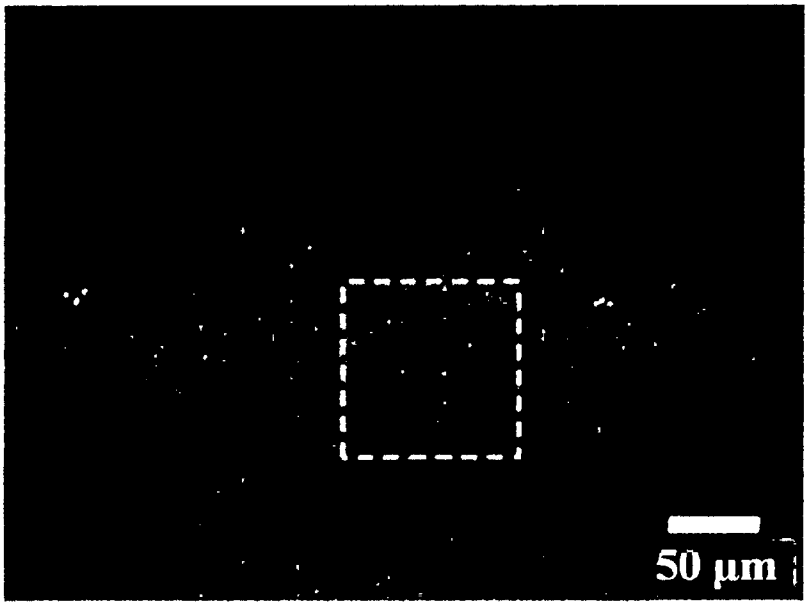
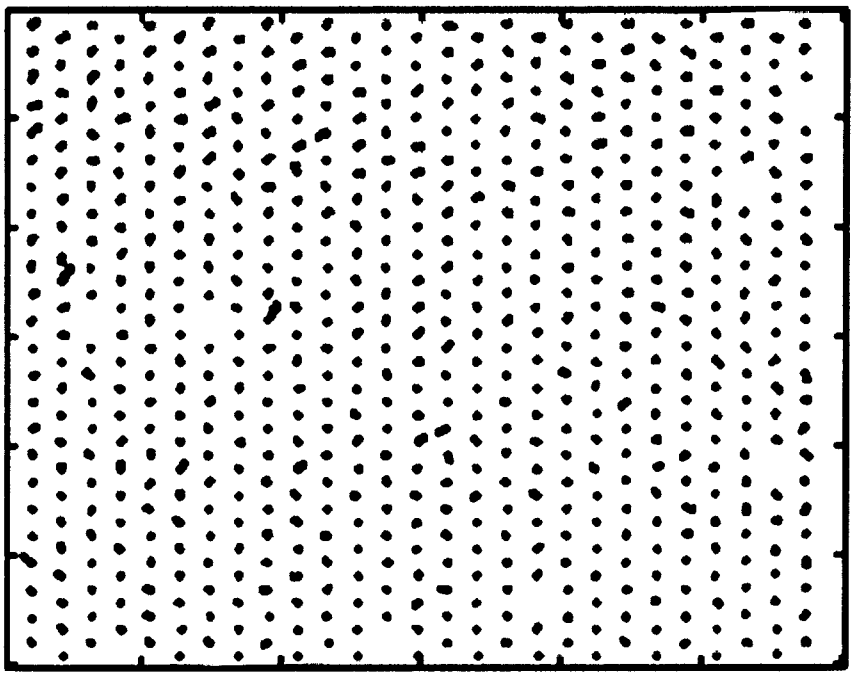


Fig. 7

C) Displacement



D) Histogram of displacement

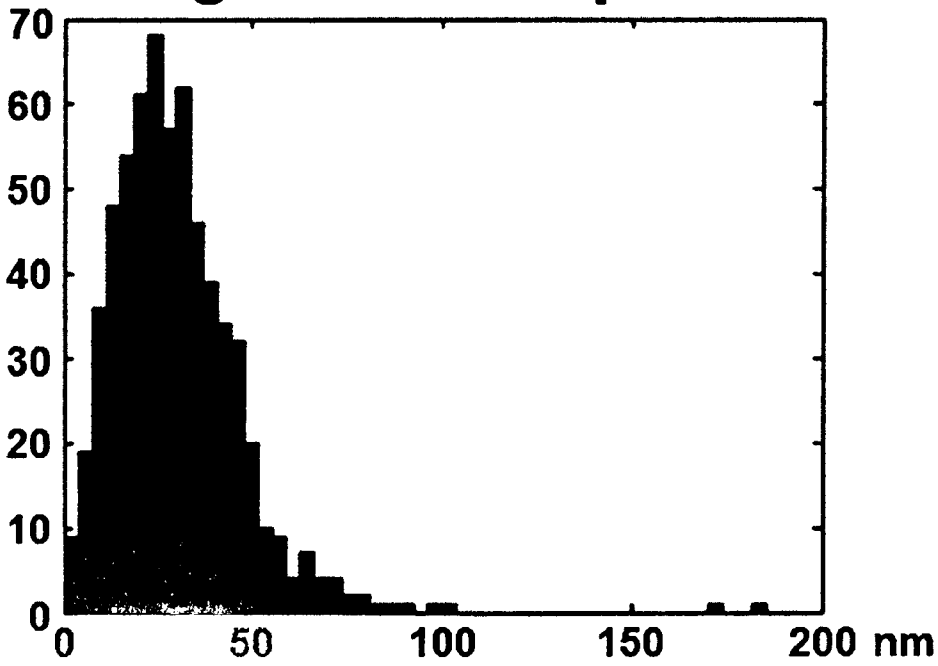
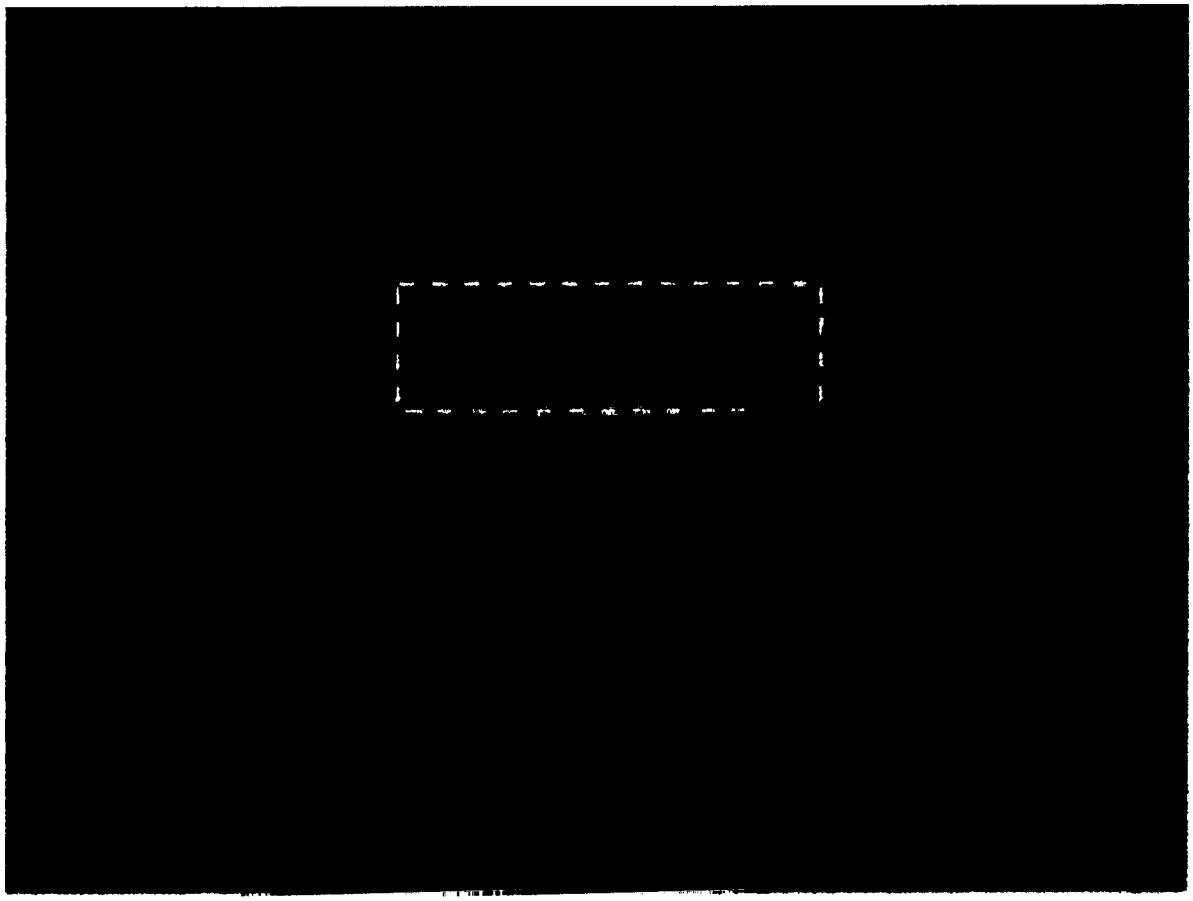


Fig. 7, cont'd.



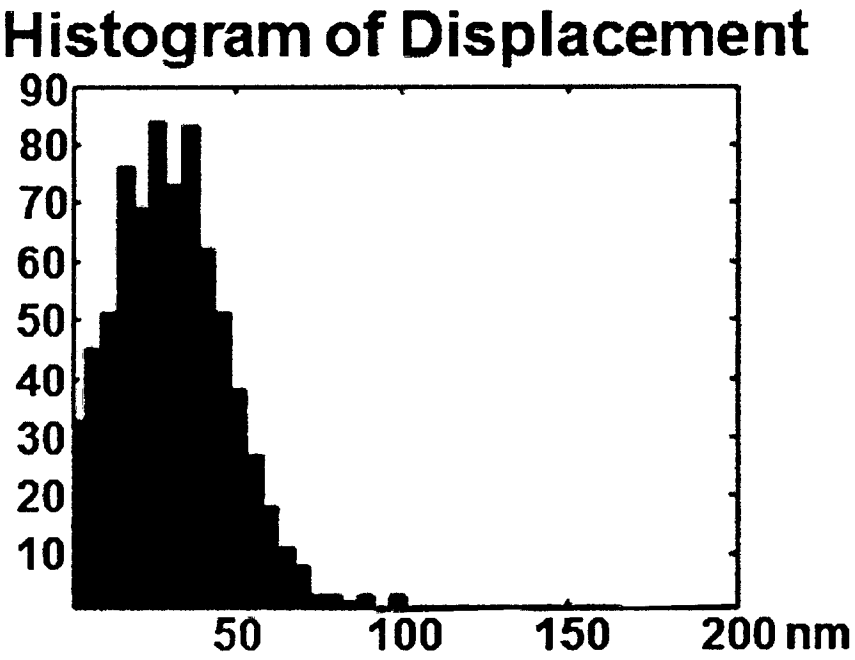

Histogram of Displacement
90
80
70
60
50
40
30
20
10
50
100
150
200 nm

Fig. 8

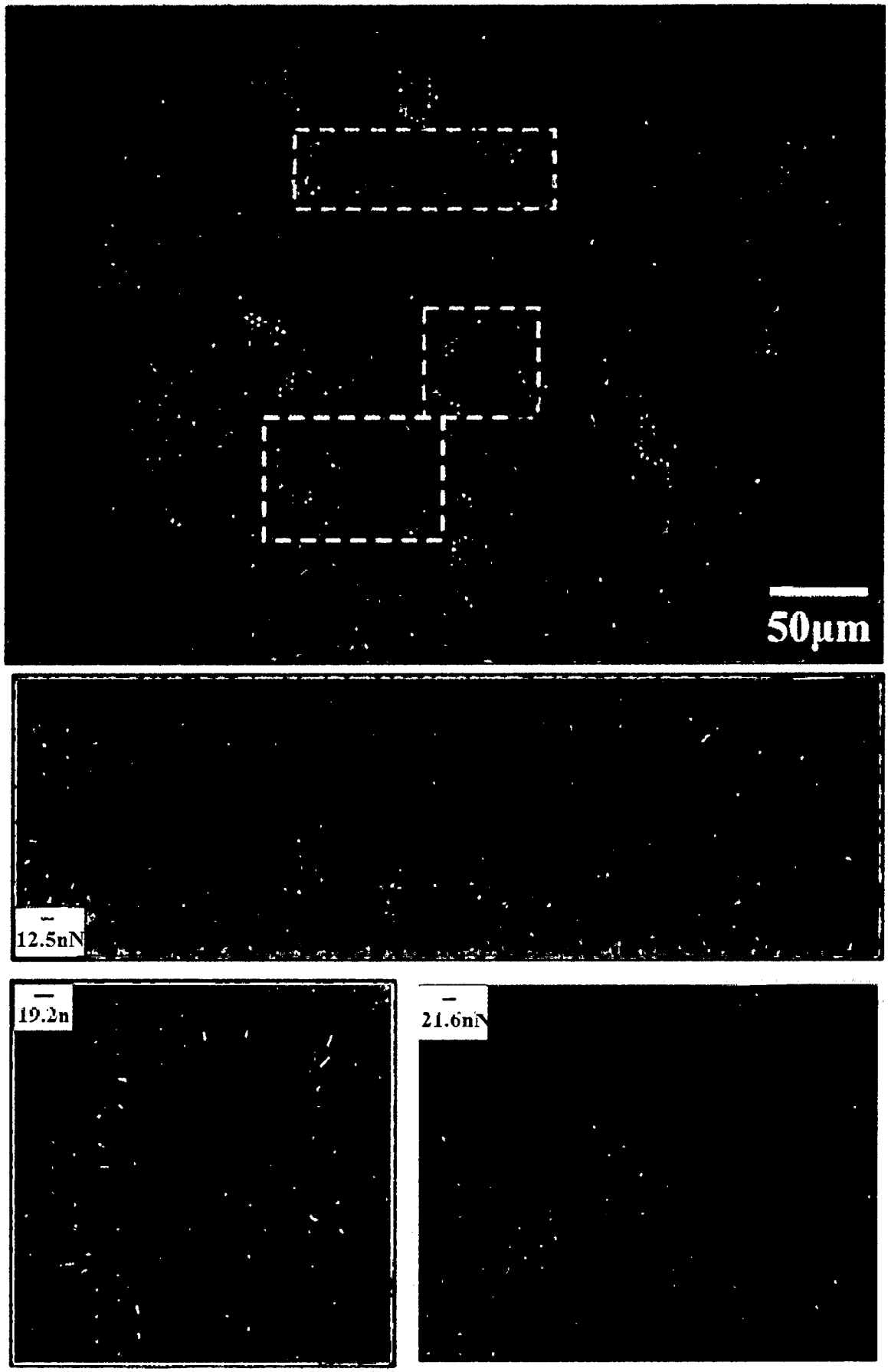


Fig. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/042327

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

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

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

2. ☒ Claims Nos.: 8,9,12,13,22,23,25,26,30,31,34,35,39,42,45,49,51
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 8, 9, 12, 13, 22, 23, 25, 26, 30, 31,34, 35,39, 42, 45, 49 and 51 are regarded as unclear because they refer to multiple dependent claims which do not comply with PCT Rule 6.4(a) (PCT Article 6).

3. ☒ Claims Nos.: 4-7,10,11,14-21,24,27-29,32,33,36-38,40,41,43,44,46-48,50
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/042327

A. CLASSIFICATION OF SUBJECT MATTER

G01N 21/552(2014.01)i, G01N 33/483(2006.01)i

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 21/552; B21D 26/14; C12N 5/00; G01J 3/44; C40B 30/10; C40B 60/12; B05D 5/06; H01F 41/22; C12N 1/20; G01N 33/483

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & keywords: piezoelectric, pressure, cell, micropillar, tip, diameter and plasmonic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011-0128536 A1 (BOND et al.) 02 June 2011 See abstract, paragraphs [0043]-[0072], claims 1-8 and figures 1A-2I.	1-3
A	US 2014-0352382 A1 (WANG et al.) 04 December 2014 See abstract, claims 1-9 and figures 1-4.	1-3
A	US 2009-0186776 A1 (WEBB et al.) 23 July 2009 See abstract, claims 112-123 and figures 1A-7.	1-3
A	US 2012-0092660 A1 (WU et al.) 19 April 2012 See abstract, claims 1-11 and figures 1A-5.	1-3
A	US 2014-0220555 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 07 August 2014 See abstract, claims 1-15 and figures 1a-5.	1-3

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2016 (26.10.2016)	**26 October 2016 (26.10.2016)**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2016/042327

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2011-0128536 A1	02/06/2011	US 2011-0129387 A1	02/06/2011
		US 2014-0333926 A1	13/11/2014
		US 2014-0335269 A1	13/11/2014
		US 8786852 B2	22/07/2014
		US 9080981 B2	14/07/2015
		US 9176065 B2	03/11/2015
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US 2009-0186776 A1	23/07/2009	JP 2005-528582 A	22/09/2005
		US 2003-0124029 A1	03/07/2003
		US 2008-0103059 A1	01/05/2008
		US 7332328 B2	19/02/2008
		WO 03-022421 A2	20/03/2003
		WO 03-022421 A3	20/11/2003
US 2012-0092660 A1	19/04/2012	US 8462333 B2	11/06/2013
US 2014-0220555 A1	07/08/2014	EP 2766473 A1	20/08/2014
		EP 2766473 A4	11/03/2015
		WO 2013-056019 A1	18/04/2013