Journal of Visualized Experiments

Plasmonic trapping and release of nanoparticles in monitoring environment

--Manuscript Draft--

Manuscript Number:	JoVE55258R3
Full Title:	Plasmonic trapping and release of nanoparticles in monitoring environment
Article Type:	Invited Methods Article - JoVE Produced Video
Keywords:	plasmonics plasmonic tweezers optical trapping optical forces microfluidics nanohole immobilization of nanoparticles
Manuscript Classifications:	93.33.55: photoelectric devices; 93.35.43: optical imaging devices (design and techniques); 93.35.44: optical measuring instruments (design and techniques); 93.38.2: clean rooms (general); 93.39.43: structural design; 97.74.13: light absorption; 97.74.30: optics; 97.74.33: photonics; 97.75.6: ion beams (plasma physics); 97.76.31: thin films (theory, deposition and growth)
Corresponding Author:	Yong-Gu Lee, Ph.D. Gwangju Institute of Science and Technology Gwangju, KOREA, REPUBLIC OF
Corresponding Author Secondary Information:	
Corresponding Author E-Mail:	lygu@gist.ac.kr
Corresponding Author's Institution:	Gwangju Institute of Science and Technology
Corresponding Author's Secondary Institution:	
First Author:	Jung-Dae Kim
First Author Secondary Information:	
Other Authors:	Jung-Dae Kim
Order of Authors Secondary Information:	
Abstract:	Plasmonic tweezers use surface plasmon polaritons to confine polarizable nanoscale objects. Many different designs of plasmonic tweezers targeted for strong trapping forces are published in the literature. However, only a few have experimentally measured the trapping force values. Most of the works can only observe immobilization making it difficult to measure the trapping forces. In this paper, we present a new imaging experimental design where the motion of the trapped particle can be monitored in two directions - one parallel and one orthogonal to the plasmonic structure's symmetric axis. Through a novel fluidic channel design, we quantitatively measured the maximal trapping forces in plasmonic tweezers. Furthermore, we were able to witness the unprecedented dramatic motion of the particle in the direction parallel to the laser beam axis.
Author Comments:	Please check an attached rebuttals.
Additional Information:	
Question	**Response**
If this article needs to be "in-press" by a certain date, please indicate the date below and explain in your cover letter.	

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

Manuscript Click here to download Manuscript 55258_R3_RE.docx

TITLE:
Plasmonic trapping and release of nanoparticles in a monitoring environment

AUTHORS:
Jung-Dae Kim
School of Mechanical Engineering
Gwangju Institute of Science and Technology (GIST)
Gwangju, Republic of Korea
kjd@gist.ac.kr

Yong-Gu Lee
School of Mechanical Engineering
Gwangju Institute of Science and Technology (GIST)
Gwangju, Republic of Korea
+82-62-715-2396
lygu@gist.ac.kr

CORRESPONDING AUTHOR:
Yong-Gu Lee
lygu@gist.ac.kr

KEYWORDS:
plasmonics, plasmonic tweezers, optical trapping, optical forces, microfluidics, nanohole, immobilization of nanoparticles

SHORT ABSTRACT:
A microchip fabrication process that incorporates plasmonic tweezers is presented here. The microchip enables the imaging of a trapped particle to measure maximal trapping forces.

LONG ABSTRACT:
Plasmonic tweezers use surface plasmon polaritons to confine polarizable nanoscale objects. Among the various designs of plasmonic tweezers, only a few can observe immobilized particles. Moreover, a limited number of studies have experimentally measured the exertable forces on the particles. The designs can be classified as the protruding nanodisk type or the suppressed nanohole type. For the latter, microscopic observation is extremely challenging. In this paper, a new plasmonic tweezer system is introduced to monitor particles, both in directions parallel and orthogonal to the symmetric axis of a plasmonic nanohole structure. This feature enables us to observe the movement of each particle near the rim of the nanohole. Furthermore, we can quantitatively estimate the maximal trapping forces using a new fluidic channel.

INTRODUCTION:
The ability to manipulate microscale objects is an indispensable feature for many micro/nano experiments. Direct contact manipulations can damage the manipulated objects. Releasing the

previously held objects is also challenging because of stiction problems. To overcome these issues, several indirect methods using fluidic[1], electric[2], magnetic[3], or photonic forces[4-8] have been proposed. Plasmonic tweezers that use photonic forces are based on the physics of extraordinary field enhancement several orders larger than the incident intensity[9]. This extremely strong field enhancement enables the trapping of extremely small nanoparticles. For example, it has been shown to immobilize and manipulate nanoscale objects, such as polystyrene particles[7,10-14], polymer chains[15], proteins[16], quantum dots[17], and DNA molecules[8,18]. Without plasmonic tweezers, it is difficult to trap nanoparticles because they quickly disappear before they are effectively examined or because they are damaged due to the high intensity of the laser.

Many plasmonic studies have used various nanoscale gold structures. We can categorize the gold structures as protruding nanodisk types[12-15,19-21] or suppressed nanohole types[7,8,10,11,22,23]. In terms of imaging convenience, the nanodisk types are more suitable than the nanohole types because, for the latter, the gold substrates can obstruct the observation view. Moreover, the plasmonic trapping occurs near the plasmonic structure and makes observation even more challenging. To the best of our knowledge, plasmonic trapping on nanohole types was only verified using indirect scattering signals. However, no successful direct observations, such as microscopic images, have been reported. Few studies have described the position of trapped particles. One such result was presented by Wang *et al.* They created a gold pillar on a gold substrate and observed the particle motion using a fluorescent microscope[24]. However, this is only effective for monitoring lateral movements not in the direction parallel to the beam axis.

In this paper, we introduce new fluidic microchip design and fabrication procedures. Using this chip, we demonstrate the monitoring of plasmonically trapped particles, both in directions parallel and orthogonal to the plasmonic nanostructure. Furthermore, we measure the maximal force of the immobilized particle by increasing the fluid velocity to find the tipping velocity in the microchip. This study is unique because most studies on plasmonic tweezers cannot quantitatively show the maximal trapping forces used in their experimental setups.

PROTOCOL:

Caution: Please refer to all relevant material safety regulations before use. Several of the chemicals used in microchip fabrication are acutely toxic and carcinogenic. Please use all appropriate safety practices when performing the photolithography and etching processes, including the use of engineering controls (fume hood, hot plate, and aligner) and personal protective equipment (safety glasses, gloves, lab coat, full-length pants, and closed-toe shoes).

1. **Fabrication of the PDMS microchannel**

1.1. **Fabrication of the microchannel mold by the photolithograph process**

1.1.1. Completely remove foreign substances on the 4-inch Si wafer surface with piranha cleaning (**Figure 1a**). Mix sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) at a ratio of 3:1 to make the piranha solution in the dish. Mix by gradually adding small amounts of the strong acid (H_2O_2) to the weak acid (H_2SO_4); reversing this order may cause an explosion because of the highly reactive strong acid.

1.1.2. Immerse the wafer in the piranha solution for 10 min. Subsequently, immerse the wafer in deionized (DI) water for 3 min to remove the remaining piranha solution. Rinse the wafer with flowing DI water for 10 s. Repeat the rinsing procedure 3 times and dry with N_2 gas to remove the remaining DI.

1.1.3. Place the wafer on a hot plate for 20 min at 180 °C to further dehydrate the wafer.

1.1.4. Pour 5 mL of the negative photoresist on top of the wafer and spin coat for 45 s at 1,500 rpm (**Figure 1b**); after spin coating, a photoresist bead is created at the wafer edge because of the relatively high viscosity of the photoresist.

1.1.5. Balance the photoresist-coated wafer by planarization on a leveling stand for 5 h.

1.1.6. Place the photoresist-coated wafer on a hot plate for 12 min at 65 °C, 35 min at 95 °C, and 12 min at 65 °C (soft baking).

1.1.7. Fix the film mask on the mask holder and the soft-baked wafer on the substrate stage of the aligner. Expose to ultraviolet (UV) light for 43 s at 650 mJ/cm^2 to solidify the photoresist.

1.1.8. Place the wafer on the hot plate for 5 min at 65 °C, 15 min at 95 °C, and 5 min at 65 °C (post-exposure baking).

1.1.9. Immerse the wafer in the photoresist developer for 30 min to remove unsolidified photoresist.

1.1.10. Rinse the wafer with isopropyl alcohol (IPA) and dry with N_2 gas to remove the remaining IPA.

1.2. **Manufacture of the PDMS microchannel**

1.2.1. Treat the surface of the wafer and the photoresist mold for 1 min at a power of 200 W using an atmospheric plasma machine[25]; the gas flows of CH_4 and He should be 6 and 30 sccm, respectively. Perform this hydrophobic treatment to easily detach the polydimethylsiloxane (PDMS) microchannel from the surface of the wafer and photoresist mold (**Figure 1c**).

1.2.2. Prepare the PDMS solution by mixing the PDMS base and curing agent at a ratio of 10:1. Stir the mixture for 2 min.

1.2.3. Place the wafer inside a Petri dish (150 mm x 15 mm) and add 100 mL of the PDMS solution. Remove the bubbles that were created from stirring using a desiccator.

1.2.4. Place the Petri dish in the oven for 2 h at 80 °C to solidify the PDMS solution (**Figure 1d** and **h**).

1.2.5. Cut along the contours of the PDMS microchannel with a razor blade and detach it from the wafer; the fabricated PDMS microchannel should have the following dimensions: 13 mm long, 300 µm wide, and 150 µm high (**Figures 1e**, **f**, and **i**).

Note: Two types of holes are produced by a micropuncture to insert the single-mode fiber (SMF) cable and the tubes (inlet and outlet) on the PDMS microchannel (**Figure 1g**). The SMF cable is used to emit the laser beam to the nanohole milled on the gold plate. The tube is used to insert/extract the particle solution to/from the PDMS microchannel.

1.2.6. Puncture 1.5-mm inlet and outlet holes at each end of the PDMS microchannel. Puncture a 0.3-mm SMF cable hole at the center of the PDMS microchannel.

2. **Etching process of the gold plate**

2.1. Prepare a commercially available gold plate with the dimensions of 25 x 6.25 mm^2 (**Figure 2a**).

2.2. Remove any foreign substances on the gold plate with the following cleaning procedures. Clean in the following order by immersing in acetone, methanol, and DI water for 5 min each.

2.3. Rinse the gold plate 3 times with DI water for 10 s and dry the plate with N_2 gas to remove the remaining DI water.

2.4. Place the gold plate on a hot plate for 20 min at 180 °C to completely remove any remaining moisture.

2.5. Pour 0.5 mL of hexamethyldisilazane (HMDS) on the gold plate and spin coat for 40 s at 3,000 rpm.

2.6. Pour 0.5 mL of positive photoresist on top of the spin-coated HMDS and spin coat for 40 s at 3,000 rpm (**Figure 2b**).

2.7. Place the photoresist-coated gold plate on the hot plate for 90 s at 110 °C (soft baking).

2.8. Fix the film mask on the glass wafer and place the soft-baked gold plate on the substrate stage. Expose to UV light for 4.5 s at 64 mJ/cm^2 to dissolve the photoresist.

2.9. Immerse the gold plate in the photoresist developer for 1 min to remove the dissolved photoresist (**Figure 2c**). Rinse the gold plate with DI water and dry with N_2 gas.

2.10. Immerse the gold plate in the Au etchant for 45 s at an etching rate of 28 Å/s to remove the exposed Au (**Figure 2d**). Rinse the gold plate with DI water and dry with N_2 gas.

2.11. Immerse the gold plate in the Ti etchant for 5 s at an etching rate of 25 Å/s to remove the exposed Ti (**Figure 2e**). Rinse the gold plate with DI water and dry with N_2 gas.

2.12. Remove the remaining photoresist on the gold plate by immersing it in acetone, methanol, and DI water for 3 min each (**Figure 2f**); immerse the plate in the written order.

2.13. Rinse the gold plate 3 times with DI water for 10 s. Dry with N_2 gas to remove the DI water.

2.14. Place the gold plate on the hot plate for 3 min at 120 °C to completely remove the moisture; the produced gold block should be 400 x 150 μm^2 (**Figure 2h**).

2.15. Mill a 400-nm nanohole using a focused ion beam (FIB) at the center of the gold block that was fabricated after etching (**Figures 2g** and **i**). Create a 370-nm circle pattern to focus on the gold block with an ion accelerating voltage of 30 kV at 28 pA for 3 s.

3. Assembly of the microchip

3.1. Treat the two surfaces of the PDMS microchannel and gold plate for 1 min with O_2 plasma to attach them together with a plasma system at a power of 80 W and a pressure of 825 mTorr[25].

Note: It is notably difficult to attach them with precision because the gold block and PDMS microchannel are on the micrometer level. Hence, use an aligner with a camera and a manual stage.

3.2. Fix the glass wafer that is used to attach the film mask to the mask holder of the aligner (**Figure 3a**).

3.3. Attach the O_2-plasma-treated PDMS microchannel to the glass wafer; because the PDMS is hydrophilic, it will easily attach to the glass wafer without any adhesion solution. Fix the gold plate on the substrate stage of the aligner (**Figure 3a**).

3.4. Locate the centers of the SMF cable hole and gold block, which are aligned on the same axis, using the camera on the aligner. Lift the manual stage to combine the two parts (**Figures 3b** and **c**).

4. Improvement of the microchip side surface roughness by PDMS coating

Note: The gold plate with fixed dimensions of 400 x 150 μm^2 is relatively more difficult to cut out than the PDMS material. Therefore, to detach the PDMS microchannel from the wafer, a razor blade is used to cut out a larger piece than the gold plate. After combining the two parts, the excess parts of the PDMS relative to the gold plate must then be cut so that the inside of the channel can be observed from the side using a microscope (**Figure 4a**). However, the cut surface, which is used as a window, has a high surface roughness and consequently produces cloudy images of the particles that flow in the channel (**Figure 4b**). Coating with the PDMS solution is performed again to resolve this problem.

4.1. Prepare the PDMS solution by mixing the PDMS base and curing agent at a 10:1 ratio and stir for 2 min.

4.2. Pour 2 mL of the PDMS solution into the Petri dish and perform the spin coating for 30 s at 1,000 rpm (**Figure 4c**).

4.3. Place the microchip surface that is going to be located on the microscope on the Petri dish (**Figure 4d**). Place the Petri dish in the oven for 1 h at 80 °C to solidify the PDMS solution.

4.4. Cut the border of the microchip and PDMS using a razor blade and subsequently detach it from the Petri dish (**Figures 4e, f**).

5. Laser coupling to insert the SMF cable to the microchip

Note: For the plasmonic tweezer system, an optical fiber incident laser with a 1,064-nm wavelength is used. The SMF cable is used because the diameter of the incident laser (5 mm) is too immense to emit the laser beam at the nanohole milled on the gold block (400 x 150 μm^2) in the microchip. The cladding diameter of the SMF cable is 125 µm. Thus, the incident laser and SMF cable must be coupled.

5.1. Connect a 40X objective lens to the microscope objective mount on the SMF coupler. Fix the SMF cable on the fiber clamp of the SMF coupler. Align the incident laser beam to fill in the back aperture of the objective lens.

5.2. Focus the laser beam to the core of the SMF cable by adjusting the three-axis manual stage equipped on the SMF coupler.

5.3. Insert the opposite end of the SMF cable into the SMF cable hole of the microchip. Measure the laser power prior to the insertion at the edge of the fiber cable, because the fixed fiber cable at the microchip cannot be detached.

5.4. Seal the SMF cable hole using epoxy glue to block the leakage of the flowing particle solution from the gap between the SMF cable hole (300 µm) and the cladding of the SMF cable (125 µm); the end of the inserted fiber cable should not enter the microchannel to avoid the fluid flow. Manually align the fiber cable using visual feedback so that it is perpendicular to the gold block that hosts the nanohole.

6. Plasmonic trapping of single fluorescent polystyrene particle in the microchip

6.1. Attach the syringe, which is filled with the particle solution, to a syringe micropump. Place the cover glass on the sample stage of the fluorescent microscope. Connect tubes to the inlet/outlet holes of the microchip. Place the PDMS-coated microchip surface on top of the cover glass.

6.2. Position the microchip orthogonally to the 60X water immersion objective lens by observing the inside of the channel with the camera installed on the fluorescent microscope. Use transparent tape to fix the microchip in place. Connect the inlet tube of the microchip with the syringe needle.

6.3. Insert the particle solution to the microchip by controlling the micropump at 20 µm/s. At this moment, confirm that the fluorescent particle can be observed well in the channel when the fluorescent lamp is turned on.

6.4. Wait until the particle solution exits from the outlet of the microchip. Set the speed to 3.4 µm/s.

6.5. Turn the laser source device so that it emits the laser into the nanohole; the fluorescent particle will be trapped at the rim of the nanohole.

6.6. Ramp the fluid speed in increments of 0.4 µm/s by controlling the micropump until the trapped particle escapes. Measure the fluid speed when the trapped particles escape. Obtain the maximal trapping force for each laser intensity using this measured fluid speed.

REPRESENTATIVE RESULTS:

The fabrication process of the PDMS microchannel and nanohole gold plate is shown in **Figures 1** and **2**. The method to combine the two parts and the actual microchip is shown in **Figure 3**. The PDMS was cut to reveal the inside of the channel from the side of the microchip. However, it was difficult to observe the particles flowing in the channel because of the surface roughness of the cutting plane. Therefore, we introduced the PDMS coating method to solve this problem, as shown in **Figure 4**.

We observed 5-µm, flowing polystyrene particles in the microchip to confirm the effect of the PDMS coating. **Figure 5** shows the actual fabricated microchip and particles observed in the microchip using the microscope. **Figure 5a** and **c** are the before and after appearances of the microchip. **Figure 5b** and **d** are the magnified surfaces of each. **Figure 5e** shows blurred particles flowing, whereas **Figure 5f** shows that the edges of the particles are notably clear and that movements can be monitored. As above, the PDMS coating of the microchip surface is essential to the monitoring of trapped particles.

Figure 6 shows the 100-nm polystyrene particle undergoing plasmonic optical trapping by the plasmonic tweezer system. An SMF cable with a 0.14 numerical aperture (NA) was used. A tube was inserted at the inlet/outlet holes of the microchip channel. A micropump was used to insert and collect the 100-nm fluorescent polystyrene particle solution. To emphasize the interior appearance of the trapped particle by the plasmonic phenomenon, the dotted parts of **Figure 6a** have been enlarged as an inset, **Figure 6b**. The laser delivered by the SMF cable inserted in the microchip was emitted to the nanohole of the gold block, and the particle flowed from left to right. Movement was detected at the nanohole rim, where the field enhancement was the strongest.

Figure 7 shows consecutive images where a 100-nm fluorescent polystyrene particle that flowed in the microchannel was trapped and released at the nanohole at the intensity of 0.42 $mW/\mu m^2$. The particles flowed at a constant speed of 3.4 µm/s in the fluid direction, as shown

in **Figure 7a**. After the laser was turned on, one of the particles was trapped at the nanohole, as shown in **Figure 7b**. On the contrary, another particle flowed into the stream, as shown in **Figure 7c**. Then, the flow speed was increased until the trapped particle escaped. **Figure 7d** shows the particle escaping from the trap. At this moment, we can estimate the trapping force with direct observation by measuring the fluid velocity when the particle escaped. We also worked in the opposite direction. Instead of increasing the fluid velocity, we gradually decreased the laser power in decrements of 1 mW and recorded the intensity when the particle escaped. This laser intensity is defined as the minimum trapping laser intensity and was measured to be 0.24 mW/μm^2.

Figure 1. Fabrication of the PDMS microchannel.
(a) Preparation of the Si wafer. (b) Photoresist spin coating of the wafer. (c) Fabricated microchannel mold by the photolithography process. (d) PDMS solidification using an oven after pouring the PDMS solution on the wafer. (e) PDMS microchannel cutting. (f) PDMS microchannel detachment from the wafer. (g) Inlet/outlet and SMF cable holes punctured on the PDMS microchannel. (h) Actual solidified PDMS on the wafer. (i) Actual detached PDMS microchannel.

Figure 2. Fabrication of the nanohole on the gold plate after the etching process.
(a) Deposition of Au and Ti on the glass. (b) Photoresist spin coating of the gold plate. (c) Dissolved photoresist removal after UV light exposure. (d) Au etching. (e) Ti etching. (f) Remaining photoresist removal. (g) Nanohole milling by a focused ion beam on the gold block. (h) Actual fabricated gold block. (i) Actual milled nanohole on the gold block.

Figure 3. Assembly process of the microchip.
(a) Fix the PDMS microchannel and gold plate on the mask holder and substrate stage, respectively, equipped on the aligner. (b) Combination of the PDMS microchannel part and the gold plate after surface treatment with O_2 plasma. (c, d) Assembled microchip after combination. (e) Removal of excess amount of the PDMS microchannel.

Figure 4. Process of surface roughness improvement by PDMS coating.
(a) Remove excess amount using a razor blade after combining the two parts. (b) High surface roughness of the microchip after cutting. (c) PDMS solution spin coating in a Petri dish. (d) Dipping the window surface of the microchip into the spin-coated PDMS solution. (e) PDMS-coated microchip detachment from the Petri dish. (f) Improvement of surface roughness by PDMS coating.

Figure 5. Assembled microchip and observation of 5-μm polystyrene particles in the microchannel before and after PDMS coating.
(a, b) Microchip before PDMS coating and magnified view. (c, d) Microchip after PDMS coating and magnified view. (e, f) Observation of particles in the microchannel before and after PDMS coating.

Figure 6. Designed plasmonic tweezer system.

(a) Schematic of the plasmonic tweezer system. (b) Trapping of a 100-nm fluorescent polystyrene particle at the rim of the nanohole in the microchip.

Figure 7. Trapping and releasing of a 100-nm fluorescent polystyrene particle in the microchannel.
(a) The microchannel with a particle flowing into the stream. (b, c) Trapped particle at the nanohole compared to another particle. (d) Particle that escaped from the trap due to the increased fluid force.

DISCUSSION:
The SMF cable was inserted in the SMF cable hole on the microchip, as shown in the rectangular dot of **Figure 6a**. Because the SMF cable hole is larger than the cable diameter, epoxy glue was used to seal the gap to block the leakage of the flowing particle solution. Before the application of epoxy glue, the gold block and cable edge should be coaxially aligned by hand using a microscope. Although it is ideal for the inserted cable edge and the nanohole to be coaxially aligned, a slight misalignment can be tolerated because the laser beam diverges once it is emitted from the end of the 0.14 NA SMF cable edge, and the beam affects a much larger region. Because the microchip was configured to be perpendicular to the optical axis of the microscope, we could not directly observe the location of the nanohole. The location of the nanohole can only be indirectly determined by observing the location of the plasmonically trapped particle at the nanohole. A solution can be provided by installing a camera at the fiber cable and using it to monitor the gold block.

The distinctive feature of the microchip is its ability to monitor particle motion near the plasmonic nanohole in real time. The motion of the particle follows the scenario described below. When the fluid streams the particles forward, some particles move toward the gold block. In some cases, a particle gets notably close to the rim of the nanohole due to attraction to the nanohole and eventually becomes immobilized. At this moment, the optical force exerted on the particle exceeds the fluid force. Subsequently, the immobilized particle escapes from the nanohole rim when the fluid velocity increases; thus, the fluid force becomes stronger than the optical force. The maximal trapping force can be measured from this terminal fluid velocity. However, the conventional drag force equation cannot be used because the particle is in physical contact with the gold wall at the nanohole. To consider the surface effect of the gold wall, we used the finite-element method, which considers the fluid motion near the surface, and obtained the fluid force.

We have introduced a new plasmonic tweezer setup that enables the monitoring of particle dynamics along the laser beam axis. In contrast, previous studies have only introduced particle movement in the plane perpendicular to the laser beam axis, such as with the nanoblock[12], nanodisk[13,14,19,21], nanostick[20], and nanopyramid[18]. Furthermore, in the case of nanohole types, trapping can only be witnessed by monitoring the scattering signal, and not by visual monitoring[10,11,23]. However, we could not precisely measure the particle position because of the limited capabilities of current imaging techniques. The imaging quality should be further improved to confirm the exact dislocation measurements. This technique can be applied in the

characterization and biosensing of a single molecule.

ACKNOWLEDGMENTS:
This work was supported in part by the ICT R&D program of MSIP/IITP (R0190-15-2040, Development of a contents configuration management system and a simulator for 3D printing using smart materials).

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:
1. Crane, N.B., Onen, O., Carballo, J., Ni, Q., Guldiken., R. Fluidic assembly at the microscale: progress and prospects. *Microfluid. Nanofluid.* **14**, 383-419, doi:10.1007/s10404-012-1060-1 (2013).
2. Yao, B., Luo, G.-A., Feng, X., Wang, W., Chen, L.-X., Wang, Y.-M. A microfluidic device based on gravity and electric force driving for flow cytometry and fluorescence activated cell sorting. *Lab Chip* **4**, 603-607, doi:10.1039/B408422E (2004).
3. Zhang, K., *et al*. On-chip manipulation of continuous picoliter-volume superparamagnetic droplets using a magnetic force. *Lab Chip* **9**, 2992-2999, doi:10.1039/B906229G (2009).
4. Park, I.-Y., Sung, S.-Y., Lee, J.-H., Lee, Y.-G. Manufacturing micro-scale structures by an optical tweezers system controlled by five finger tips. *J. Micromech. Microeng.* **17**, N82-N89, doi:10.1088/0960-1317/17/10/N03 (2007).
5. Kim, J.-D., Hwang, S.-U., Lee, Y.-G. Traceable assembly of microparts using optical tweezers. *J. Micromech. Microeng.* **22**, 105003, doi:10.1088/0960-1317/22/10/105003 (2012).
6. Kim, J.-D., Lee, Y.-G. Construction and actuation of a microscopic gear assembly formed using optical tweezers. *J. Micromech. Microeng.* **23**, 065010, doi:10.1088/0960-1317/23/6/065010 (2013).
7. Kim, J.-D., Choi, J.-H., Lee, Y.-G. A measurement of the maximal forces in plasmonic tweezers. *Nanotechnology* **26**, 425203, doi:10.1088/0957-4484/26/42/425203 (2015).
8. Kim, J.-D., Lee, Y.-G. Trapping of a single DNA molecule using nanoplasmonic structures for biosensor applications. *Biomed. Opt. Express* **5**, 2471-2480, doi:10.1364/BOE.5.002471 (2014).
9. Quidant, R. Plasmonic tweezers – the strength of surface plasmons. *MRS Bull.* **37**, 739-744, doi:10.1557/mrs.2012.172 (2012).
10. Juan, M.L., Gordon, R., Pang, Y., Eftekhari, F., Quidant, R. Self-induced back-action optical trapping of dielectric nanoparticles. *Nat. Phys.* **5**, 915-919, doi:10.1038/nphys1422 (2009).
11. Pang, Y., Gordon, R. Optical trapping of 12 nm dielectric spheres using double-nanoholes in a gold film. *Nano Lett.* **11**, 3763-3767, doi:10.1021/nl201807z (2011).
12. Tanaka, Y., Kaneda, S., Sasaki, K. Nanostructured potential of optical trapping using a plasmonic nanoblock pair. *Nano Lett.* **13**, 2146-2150, doi:10.1021/nl4005892 (2013).
13. Kang, J.-H., *et al*. Low-power nano-optical vortex trapping via plasmonic diabolo nanoantennas. *Nat. Commun.* **2**, 582, doi:10.1038/ncomms1592 (2011).

14. Roxworthy, B.J., *et al*. Application of plasmonic bowtie nanoantenna arrays for optical trapping, stacking, and sorting. *Nano Lett.* **12**, 796-801, doi:10.1021/nl203811q (2012).
15. Shoji, T., Tsuboi, Y. Plasmonic optical tweezers toward molecular manipulation: tailoring plasmonic nanostructure, light source, and resonant trapping. *J. Phys. Chem. Lett.* **5**, 2957-2967, doi:10.1021/jz501231h (2014).
16. Pang, Y., Gordon, R. Optical trapping of a single protein. *Nano Lett.* **12**, 402-406, doi:10.1021/nl203719v (2012).
17. Tsuboi, Y., *et al*. Optical trapping of quantum dots based on gap-mode-excitation of localized surface plasmon. *J. Phys. Chem. Lett.* **1**, 2327-2333, doi:10.1021/jz100659x (2010).
18. Shoji, T., *et al*. Permanent fixing or reversible trapping and release of DNA micropatterns on a gold nanostructure using continuous-wave or femtosecond-pulsed near-infrared laser light. *J. Am. Chem. Soc.* **135**, 6643-6648, doi:10.1021/ja401657j (2013).
19. Grigrenko, A.N., Roberts, N.W., Dickson, M.R., Zhang, Y. Nanometric optical tweezers based on nanostructured substrates. *Nat. Photonics* **2**, 365-370, doi:10.1038/nphoton.2008.78 (2008).
20. Righini, M., *et al*. Nano-optical trapping of rayleigh particles and escherichia coli bacteria with resonant optical antennas. *Nano Lett.* **9**, 3387-3391, doi:10.1021/nl803677x (2009).
21. Chen, K.-Y., Lee, A.-T., Hung, C.-C., Huang, J.-S., Yang, Y.-T. Transport and trapping in two-dimensional nanoscale plasmonic optical lattice. *Nano Lett.* **13**, 4118-4122, doi:10.1021/nl4016254 (2013).
22. Berthelot, J., *et al*. Three-dimensional manipulation with scanning near-field optical nanotweezers. *Nat. Nanotechnol.* **9**, 295-299, doi:10.1038/nnano.2014.24 (2014).
23. Chen, C., *et al*. Enhanced optical trapping and arrangement of nano-objects in a plasmonic nanocavity. *Nano Lett.* **12**, 125-132, doi:10.1021/nl2031458 (2011).
24. Wang, K., Schonbrun, E., Steinvurzel, P., Crozier, K.B. Trapping and rotating nanoparticles using a plasmonic nano-tweezer with an integrated heat sink. *Nat. Commun.* **2**, 469, doi:10.1038/ncomms1480 (2011).
25. Byun, D., Cho, S. J., Kim, S. Fabrication of a flexible penetrating microelectrode array for use on curved surfaces of neural tissues. *J. Micromech. Microeng.* **23**, 125010, doi:10.1088/0960-1317/23/12/125010 (2013).

Figure 1

Click here to download Figure JOVE_figure 1.tif

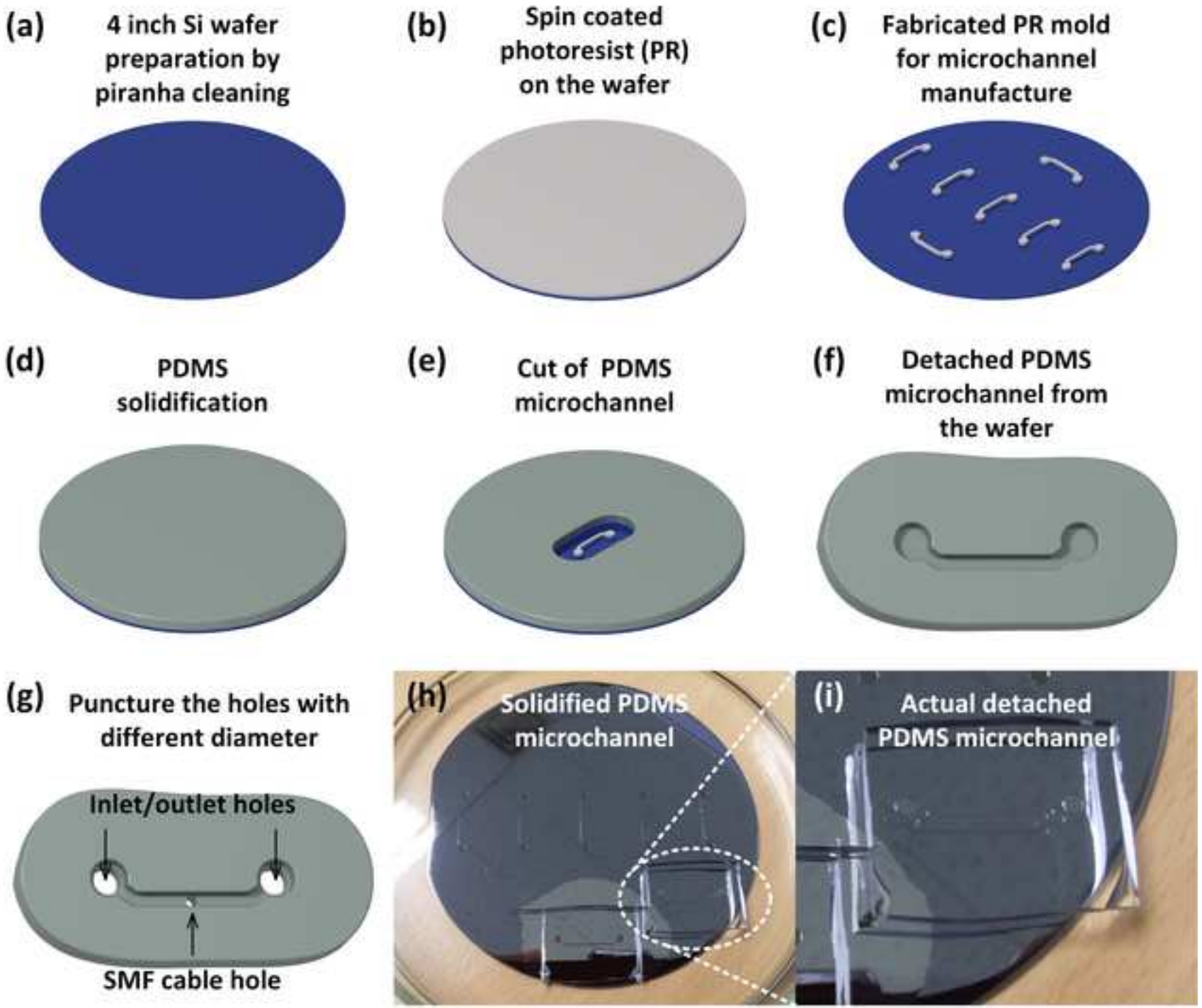


Figure 2

Click here to download Figure JOVE_figure 2.tif

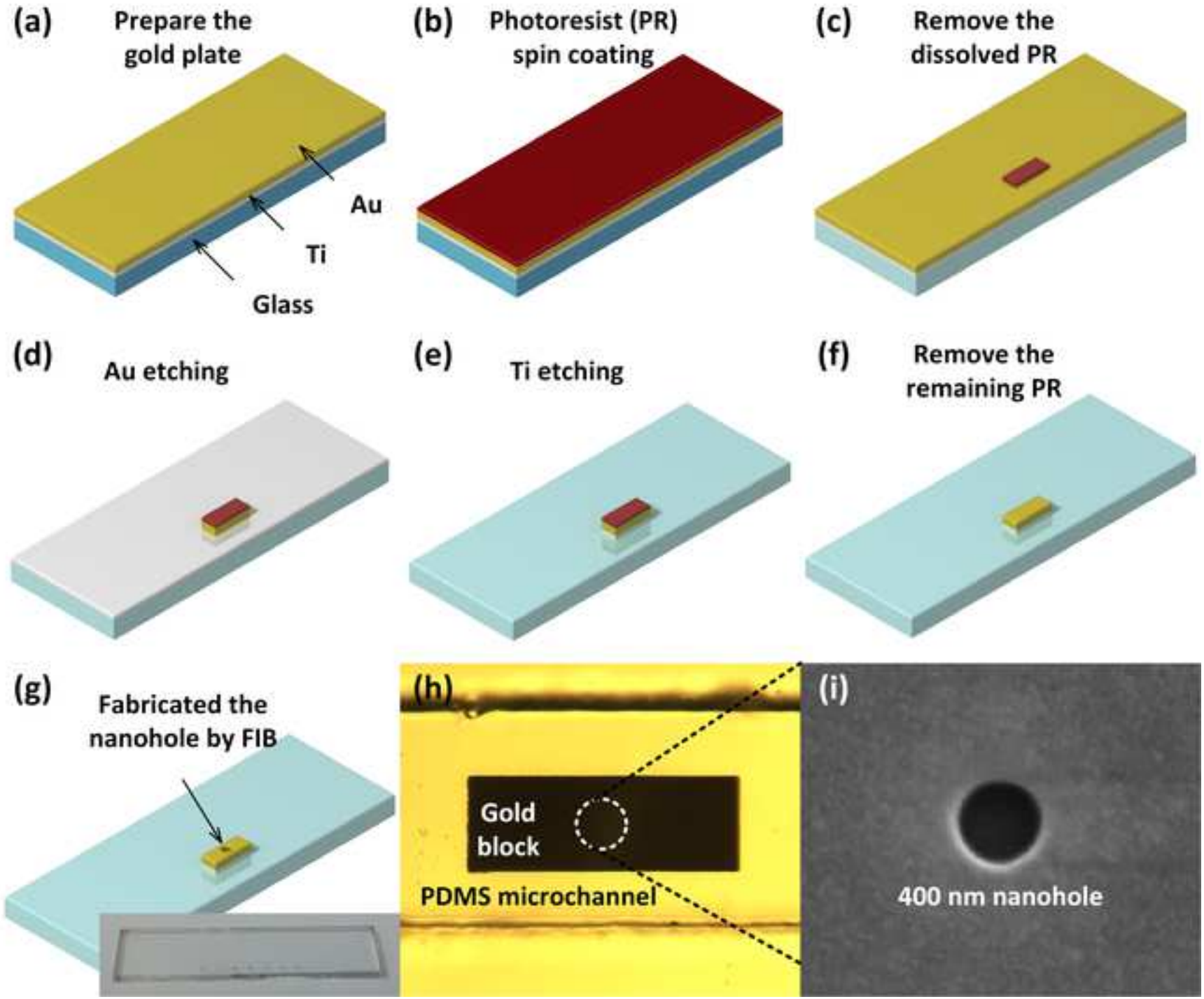


Figure 3

Click here to download Figure JOVE_figure 3.tif

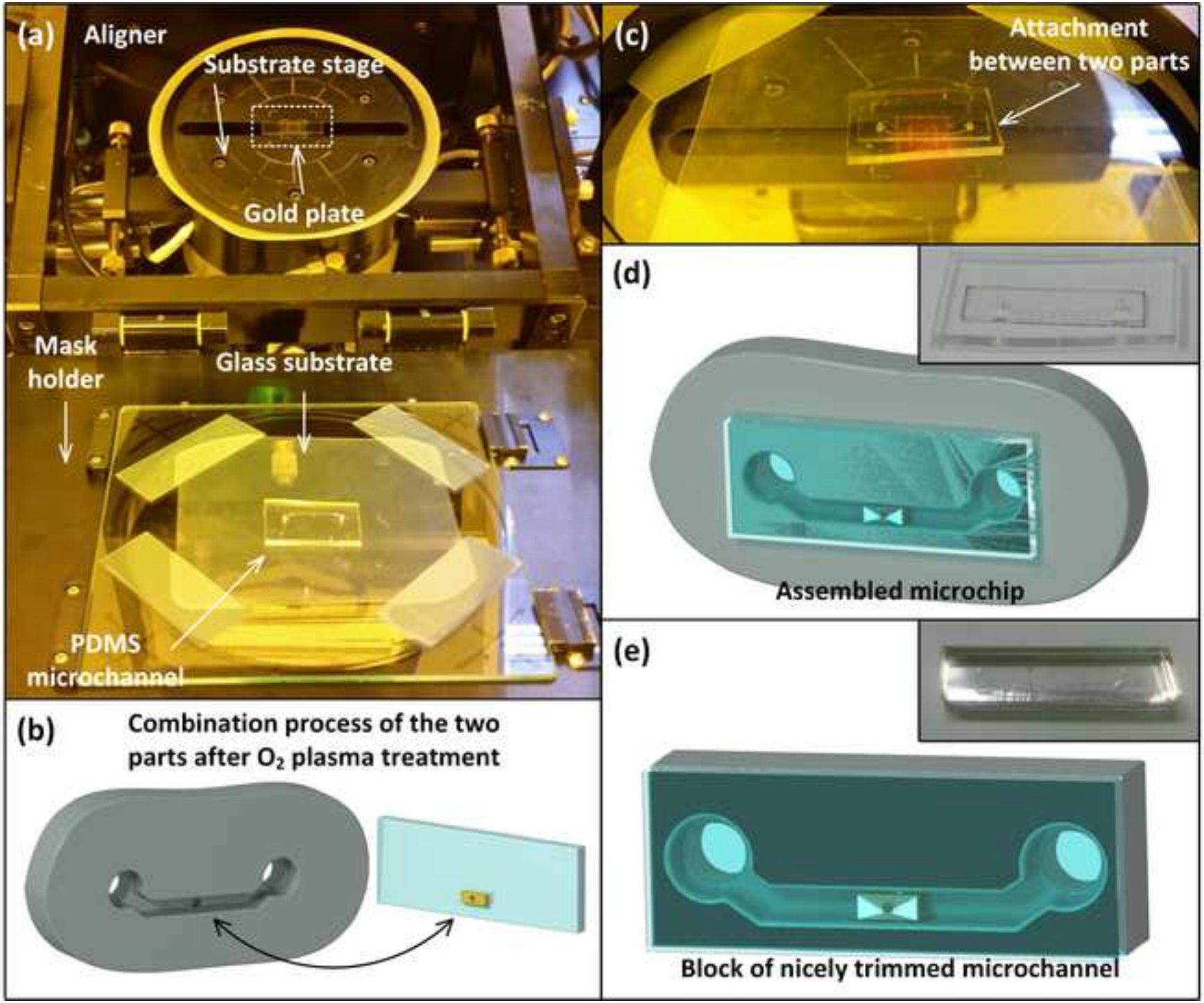


Figure 4

Click here to download Figure JOVE_figure 4.tif

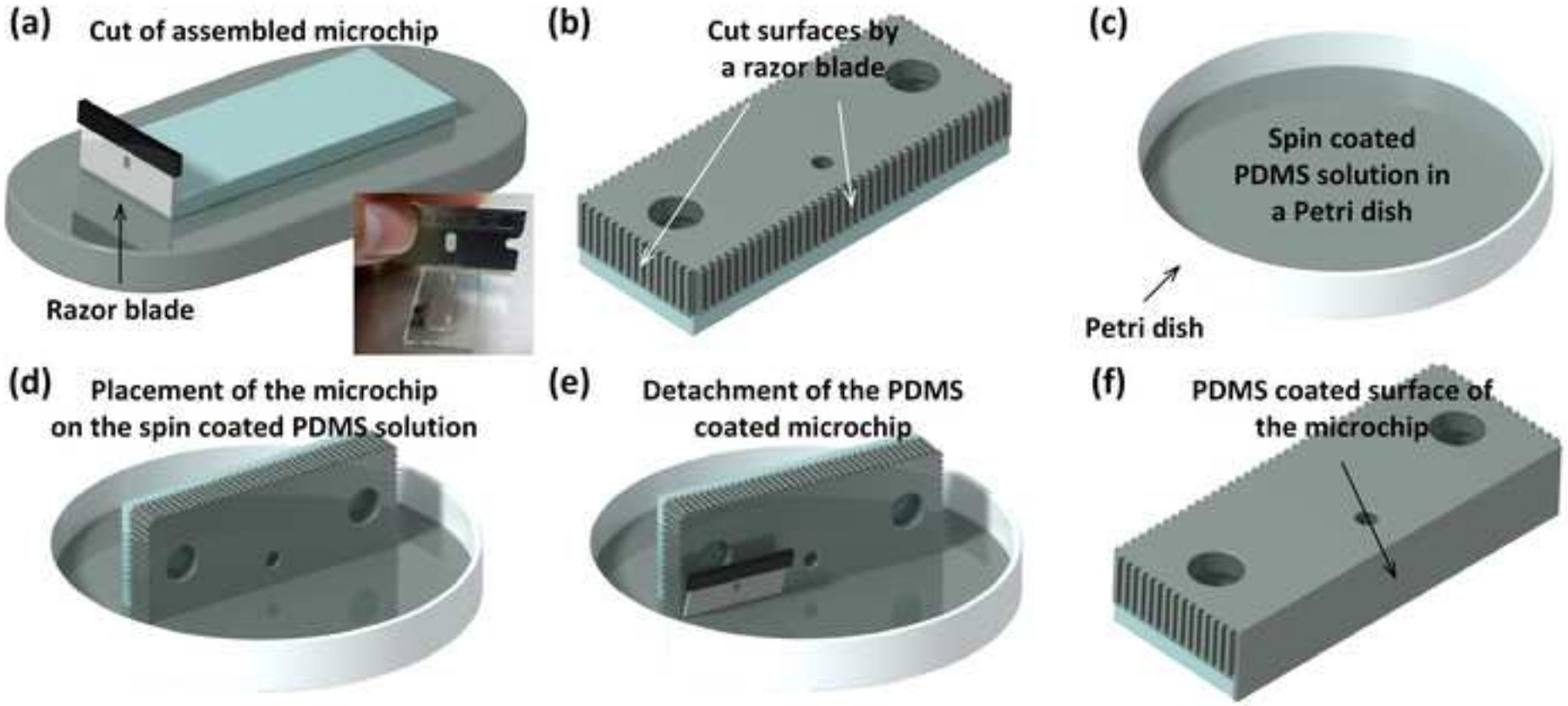


Figure 5

Click here to download Figure JOVE_figure 5.tif

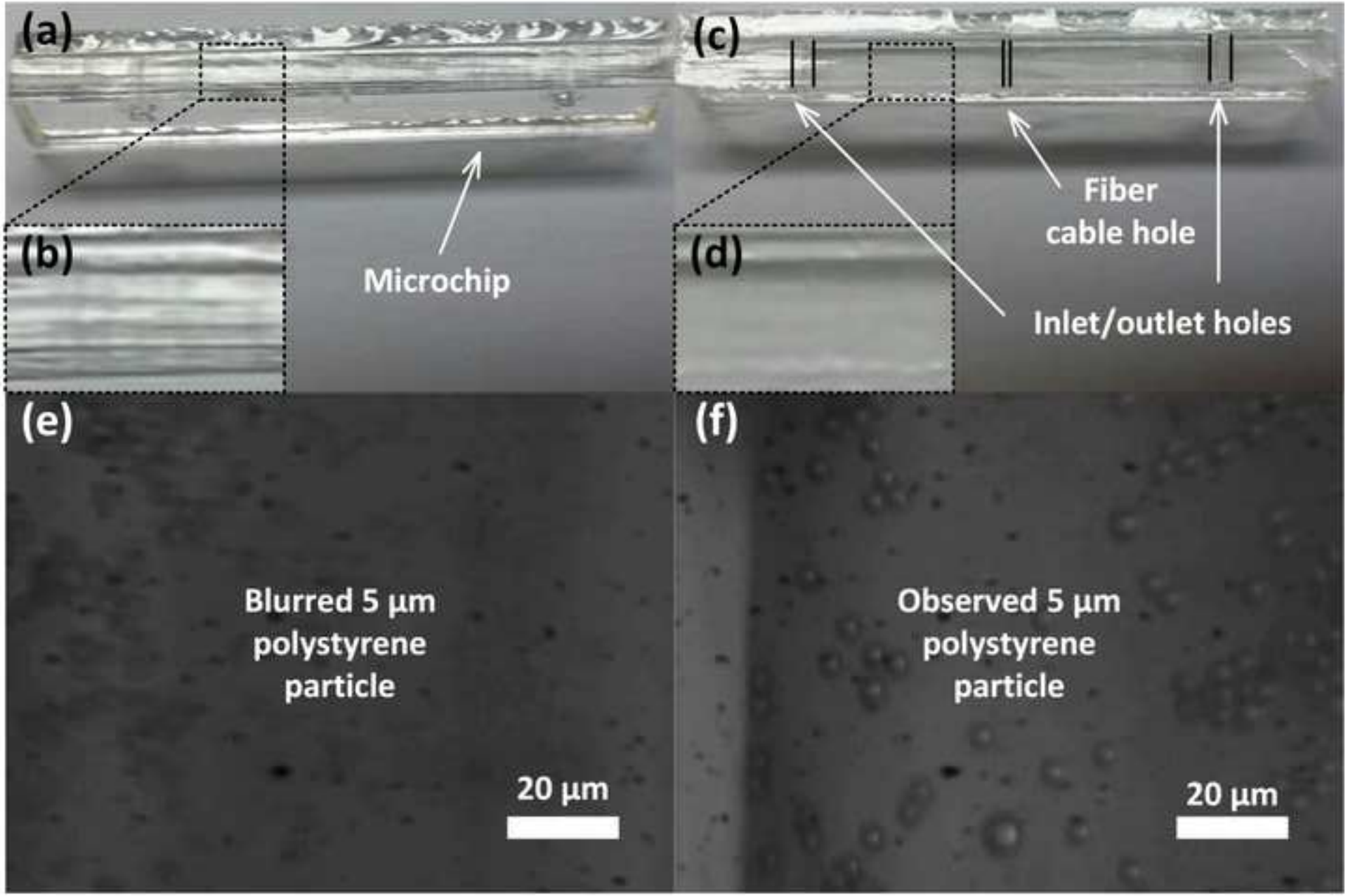


Figure 6

Click here to download Figure JOVE_figure 6.tif

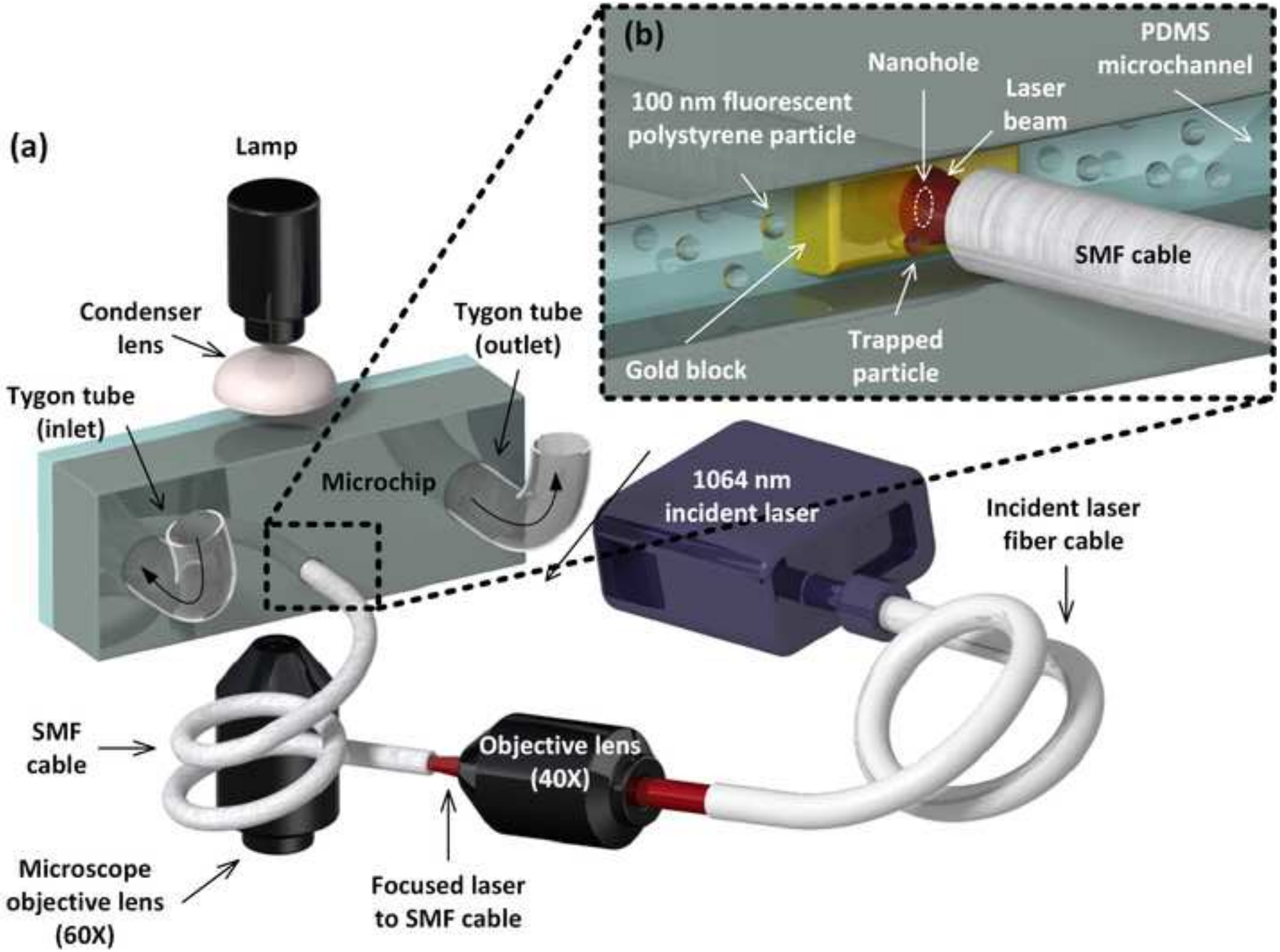
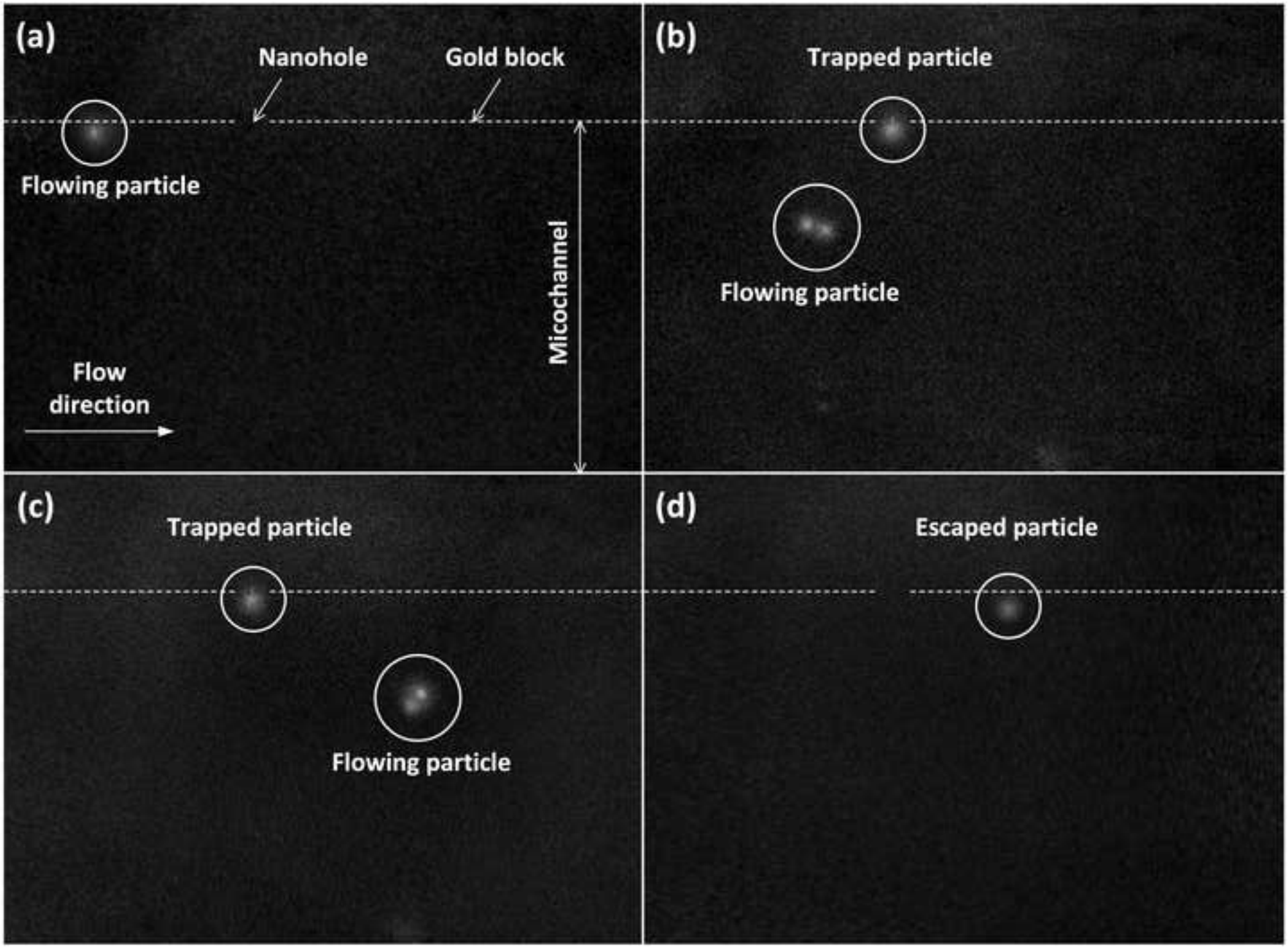


Figure 7

Click here to download Figure JOVE_figure 7.tif



Excel Spreadsheet- Table of Materials/Equipment

Click here to download Excel Spreadsheet- Table of Materials/Equipment 20161124 Materials(JoVE).xls

Name of Reagent/ Equipment	Company	Catalog Number	Comments/Description
Negative photoresist	MicroChem	SU-8 2075	
Developer	MicroChem	SU-8 Developer	
Positive photoresist	Merck Ltd.	AZ GXR-601	
AZ Photoresist Developers	Merck Ltd.	AZ 300 MIF	
HMDS	Merck Ltd.	AZ Adhesion Promoter	
Aligner	Midas System	MDA 400M	
Atmospheric plasma machine	Atmospheric Process Plasma Co.	IDP-1000	
Polydimethylsiloxane (PDMS)	Dow Corning	Sylgard 184 A/B	
Gold coated test slides	EMF Co.	TA124(Ti/Au)	
Au etchant	Transene Inc.	TFA	
Ti etchant	Transene Inc.	TFT	
40X objective lens	Edmund Optics	40X DIN	
60X water immersion objective lens	Olympus	LUMPLFLN 60XW	
Optical fiber incident laser	IPG Photonic	YLR 10	
SMF coupler	Thorlabs	MBT612D/M	
Syringe micropump	Harvard	PC2 70-4501	
Fluorescent microscope	Olympus	IX-51	
Plasma system	Femto Science Inc	CUTE-MPR	

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Plasmonic trapping of nanoparticles and evaluation of the maximal forces with monitoring

Author(s): Jung-Dae Kim, Yong-Gu Lee

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via: ☑ Standard Access ☐ Open Access

Item 2 (check one box):

☑ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name: Yong-Gu Lee

Department: School of Mechanical Engineering

Institution: Gwangju Institute of Science and Technology

Article Title: Plasmonic trapping of nanoparticles and evaluation of the maximal forces with monitoring

Signature: yongGus Date: 2016.07.20

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Rebuttal Comments
Click here to download Rebuttal Comments 20161124 Rebuttals.pdf

Reply to the Reviewer's comments

We would like to express our thanks to the Topical Editor and reviewers for their interest in our manuscript and constructive suggestions, and we are more than happy to satisfy the conditions and queries marked in the comments. In what follows, we elaborate in detail on how the manuscript has been revised to fully address the issues raised in the reviews. In the following, the reviewers' comments are given in italics and our responses to the comments are provided in a different font. The modified contents in the manuscript are showed here with a red color font with corresponding page and line numbers.

Editorial comments:

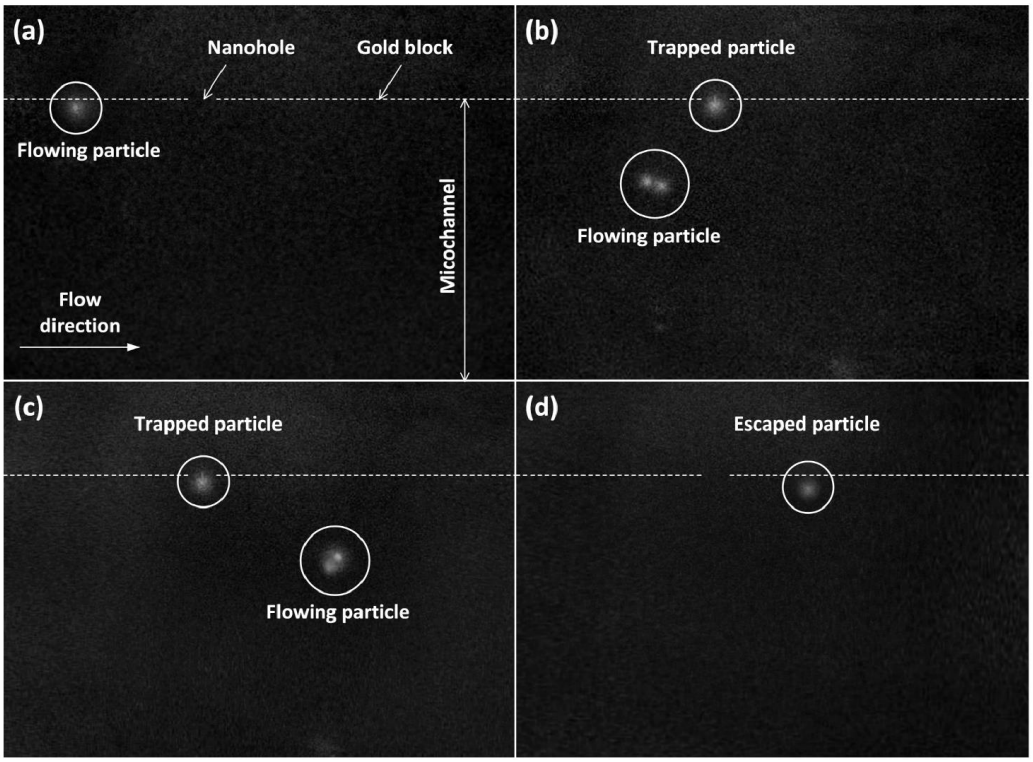
The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55258_R2_RE.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.

Comment 1) *Please use professional copy-editing services as the language still is not publication grade. There continues to be awkward phrases throughout the manuscript.*

→ A native English speaker has edited the manuscript.

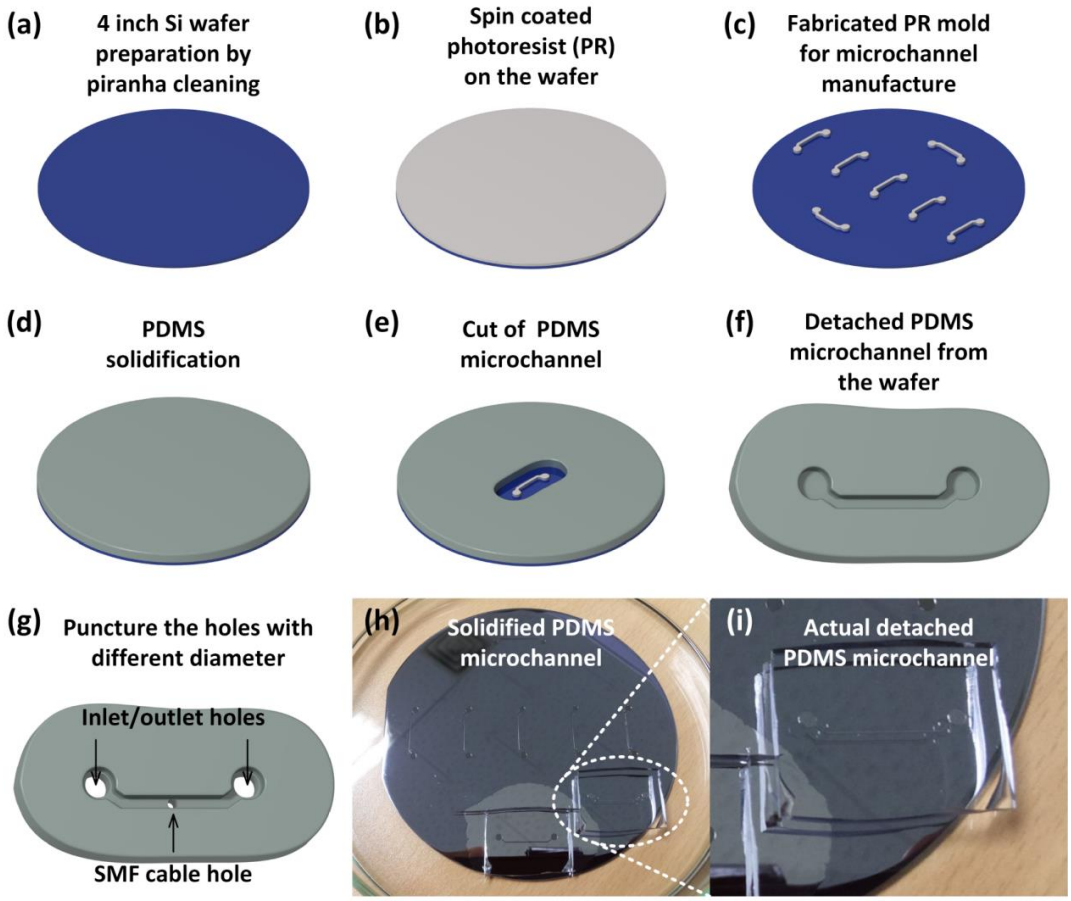
Comment 2) *For Figure 7, please process the image by windowing and leveling the images for higher quality. It is not clear what is being shown.*

→ We have improved images of Fig. 7 as the editor has suggested.



Comment 3) *For Figure 1b and 1c, please rename the SU-8 as the photoresist to be consistent with the protocol text in the manuscript.*

→ We have changed all occurrences of "SU-8" to "photoresist (PR)".



Comment 4) *Some additional details are required:*

→ We have provided additional details as follows.

Comment 4a) *1.1.1: Please provide the piranha solution composition. Clean by immersion?*

→ We have added the composition of the piranha solution. The cleaning was done by immersing and this is explained explicitly.

"Page 2, line 40" ~ "Page 3, line 5"
1.1.1. Completely remove the foreign substances on the 4-inch Si wafer surface by piranha cleaning (**Figure 1a**). Mix sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) at a ratio of 3:1 to make the piranha solution in the dish. Mix by gradually adding small amounts of the strong acid (H_2O_2) to the weak acid (H_2SO_4). The reverse order of adding may cause sudden an explosion because of the highly reactive strong acid.

1.1.2. Immerse the wafer in the piranha solution for 10 min. Subsequently, immerse the wafer in a deionized (DI) water for 3 min to remove the remaining piranha solution on the wafer. Rinse the wafer with flowing DI water for 10 s. Repeat the rinsing procedure 3 times and dry with N_2 gas to remove the remaining DI.

Comment 4b) *1.1.8: Expose for how long?*

→ We have inserted an exposure time at each step.

"Page 3, line 21"
1.1.8. Expose to ultraviolet (UV) light for 43 s at 650 mJ/cm^2 to solidify the photoresist.

"Page 4, line 40"
2.9. Expose them to UV light for 4.5 s at 64 mJ/cm^2 to dissolve the photoresist.

Comment 4c) *1.2.1: Please provide a citation here for the atmospheric plasma machine.*

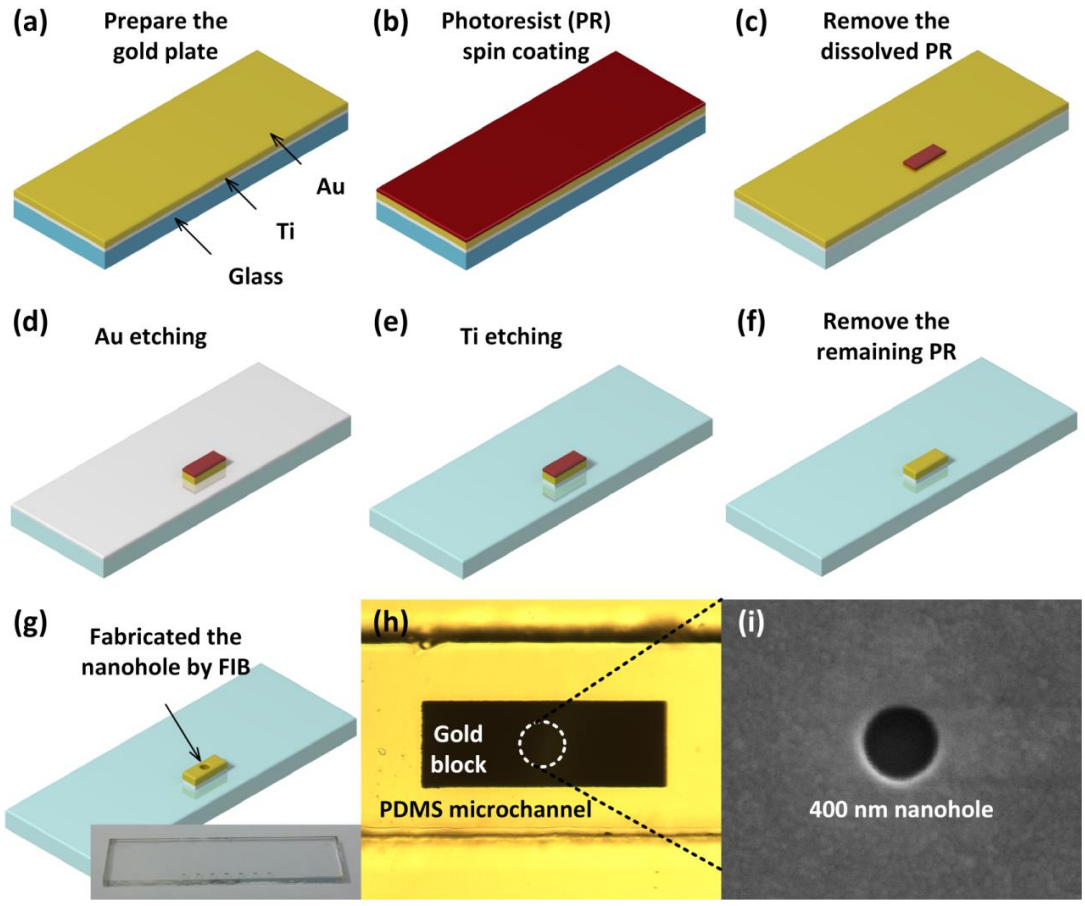
→ We have added the citation for the atmospheric plasma machine.

"Page 3, line 33" ~ "Page 3, line 37"
1.2.1 Treat the surface of the wafer and the photoresist mold for 1 min at a power of 200 W using an atmospheric plasma machine[25]. Here, gas flows of CH_4 and He are 6 and 30 sccm, respectively. This hydrophobic treatment is performed to easily detach the polydimethylsiloxane (PDMS) microchannel from the surface of the wafer and photoresist mold (**Figure 1c**).

Comment 4d) *2.1: How is the sputter coater used? What are the parameters used here?*

→ In this paper, we have only used a commercially available gold plate to fabricate the microchip. Therefore, we have removed the descriptions related to the sputter to avoid any possible confusions. Furthermore, we have modified the caption of Fig. 2(a).

"Page 4, line 17" ~ "Page 4, line 18"
2.1. Prepare a commercially available gold plate with the dimensions of 25 x 6.25 mm^2 (**Figure 2a**).



Comment 4e) *2.2: What are the general cleaning procedures used here? Is it the immersion in acetone, methanol, and DI water?*

→ We have modified the sentences to avoid the confusion.

"Page 4, line 20" ~ "Page 4, line 21"
2.2. Remove any foreign substances on the gold plate with the following cleaning procedures. Clean in the following order by immersing in acetone, methanol, and DI water for 5 min each.

Comment 4f) *3.1: Treat with O_2 plasma how? What settings are used? For how long?*

→ We have added a citation and additional explanations in the manuscript.

"Page 5, line 27" ~ "Page 5, line 29"
3.1. Treat the two surfaces of the PDMS microchannel and gold plate for 1 min with O_2 plasma to attach them together by a plasma system at a power of 80 W and a pressure of 825 mTorr[25].

Comment 4g) *4.2: How much PDMS solution is used here?*

→ We have modified the sentence.

"Page 6, line 15" ~ "Page 6, line 16"
4.2. Pour 2 mL of the PDMS solution in the Petri dish and perform the spin coating for 30 s at 1000 rpm (**Figure 4c**).

Comment 5) *What is Figure 9? Did you mean Figure 7?*

→ We are very sorry for our mistake. We have changed "Figure 9" to "Figure 7".

"Page 8, line 11" ~ "Page 8, line 22"
Figure 7 shows consecutive images where a 100 nm fluorescent polystyrene particle that flowed in the microchannel was trapped and released at the nanohole at the intensity of 0.42 mW/μm^2. The particles flowed at a constant speed of 3.4 μm/s in the fluid direction, as shown in **Figure 7a**. After the laser was turned on, one of the particles was trapped at the nanohole, as shown in **Figure 7b**. On the contrary, another particle flowed into the stream, as shown in **Figure 7c**. Then, the flow speed was increased until the trapped particle escaped. **Figure 7d** shows the particle escaping from the trap. At this moment, we can estimate the trapping force by measuring the fluid velocity when the particle escaped with direct observation. We also worked in the opposite direction. Instead of increasing the fluid velocity, we gradually decreased the laser power in decrements of 1 mW and recorded the intensity when the particle escaped. This laser intensity is defined as the minimum trapping laser intensity and was measured to be 0.24 mW/μm^2.