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:

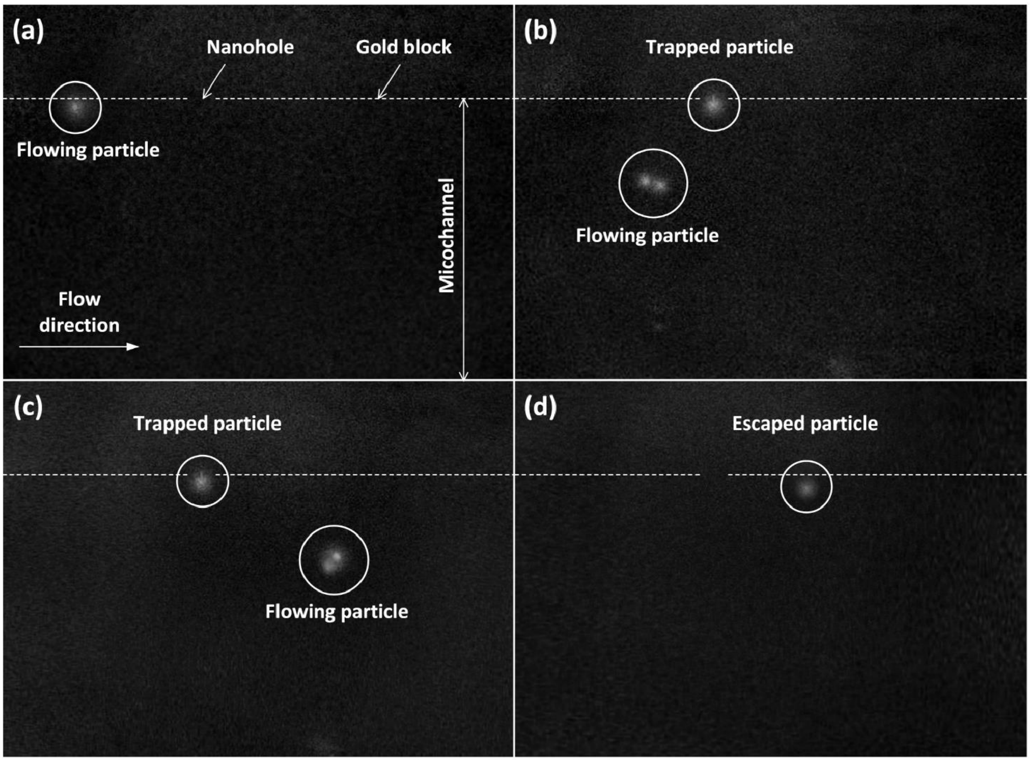
The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55258_R2_RE.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink 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.

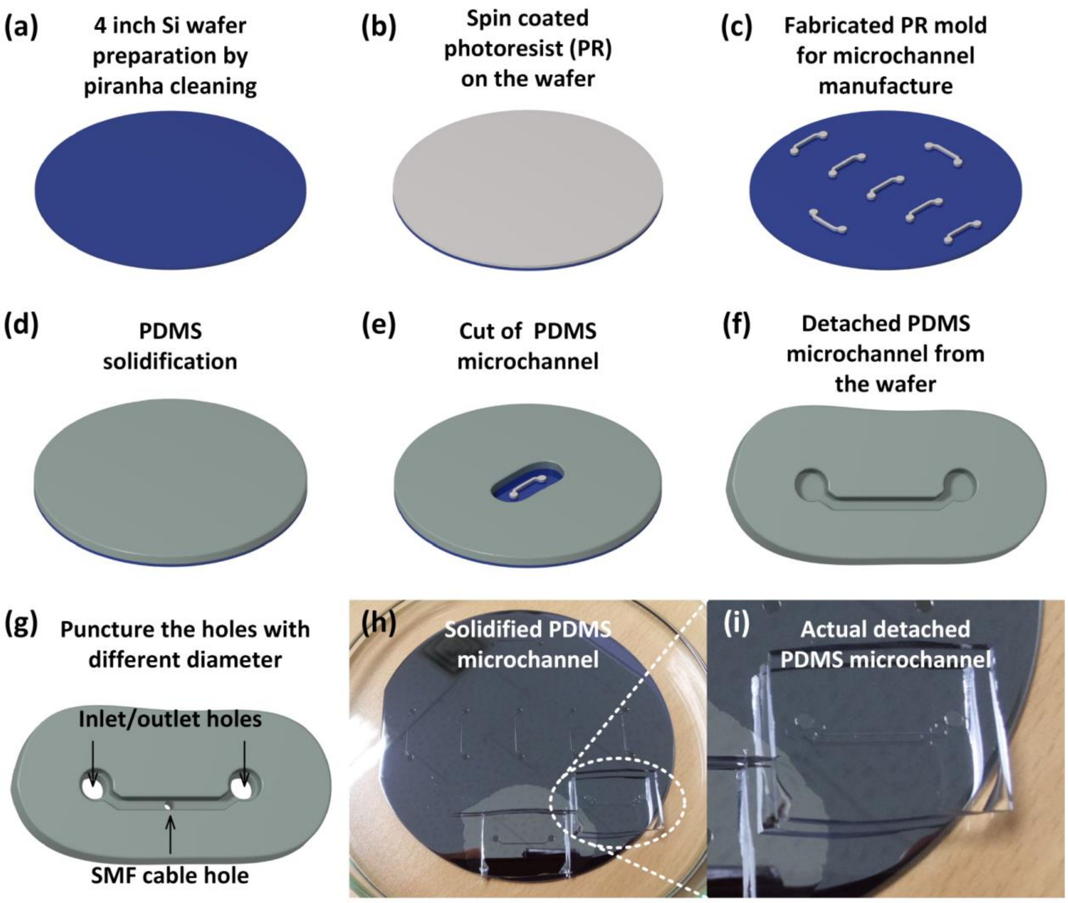
Comment 2) *For Figure 7, please process the image by windowing and leveling the images for higher quality. It is not clear what is being shown.*

→ 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.*

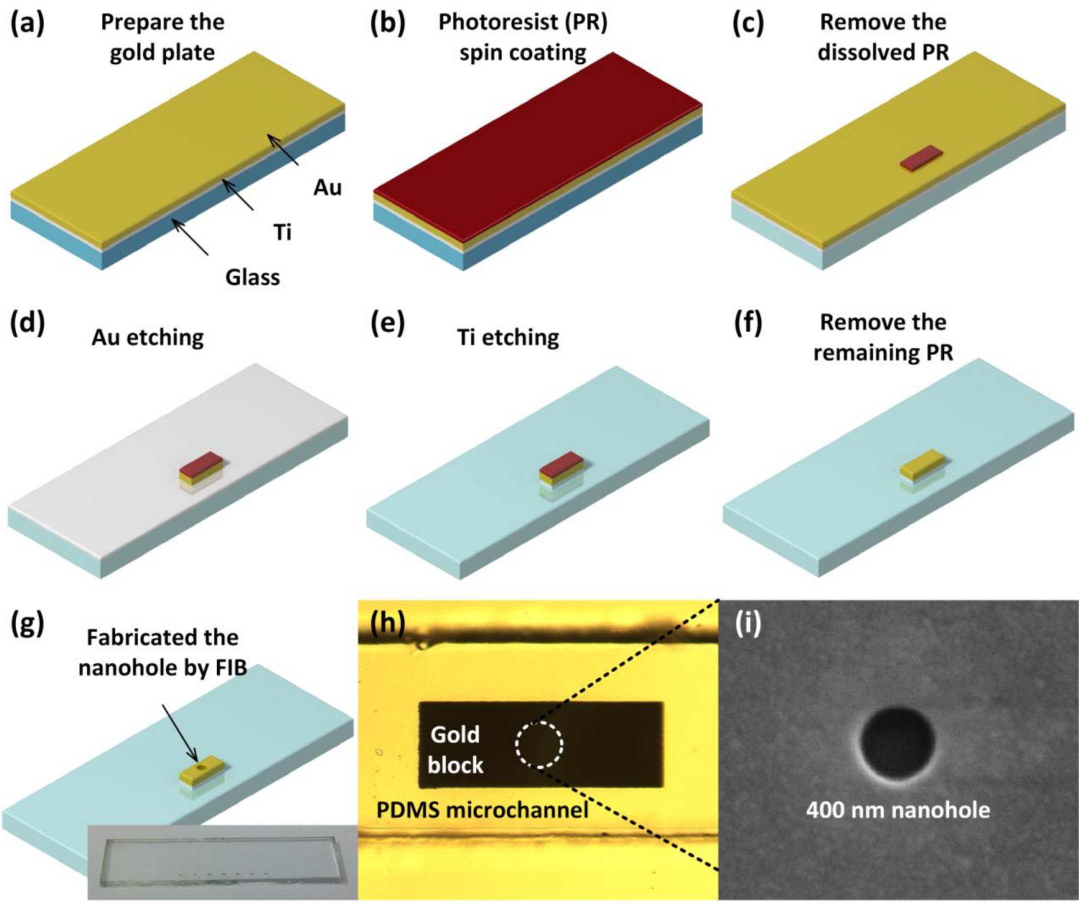
→ We have added the citation for the atmospheric plasma machine.

“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.