Electronic Supplementary Material (ESI) for RSC Advances.
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Supplementary Information

S1 GLAD-MACE substrate

Si substrate was etched with GLAD-MACE method and the substrate is shown in figure below. Fig. S1A shows a silicon wafer etched to form GLAD-MACE substrate, where the black patterns are where Si nanowires are formed, and the reflective portion of the wafer was cover by photoresist where no etching occurred. Fig. S1B shows the GLAD-MACE integrated in a fluidic chamber. Fig. S1C shows an SEM image of the etching site (black portion on the wafer, indicated by arrow) which is comprised of multiple numbers of nanowires. The wires tend to clump together after being dried, forming clusters on the surface. Fig. S1D is a TEM image shows the middle section of a nanowire with diameter smaller than 100 nm. Typically for GLAD-MACE etched nanowires, the top sections of the nanowires will be of smaller diameter compared to the middle section. Rough nanowire surface can be seen from the TEM image. We have confirmed in another publication[1] that the nanowire surfaces are porous with pore diameter around 6 nm.

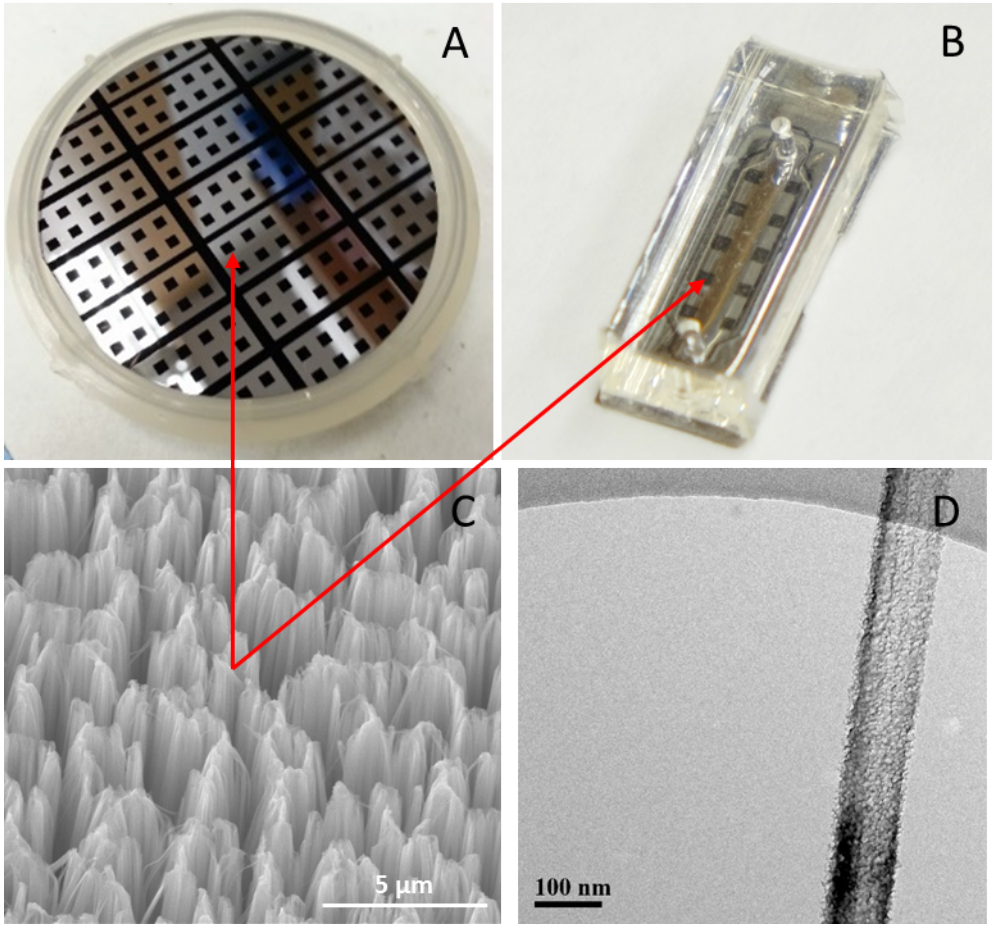

Fig. S1 (A) Si wafer etched with GLAD-MACE method, where black portion on wafer is the etching site. (C)SEM image of the GLAD-MACE detection site and (D) the TEM image of a single nanowire.

Results of flow experiments from microfluidic chamber with 1 mm chamber height

Flow experiment was first carried out on GLAD-MACE substrates with microfluidic chamber with height of 1mm. Free incubation without flow was also performed as control. Target antisense hybridization solution with antisense concentrations of 1µM and 0.1µM were used. Both flow and free incubation experiments were continued for one hour at room temperature; the flow rate was set to 13.3 µL/min, so that one hour corresponds to 4 residence time.

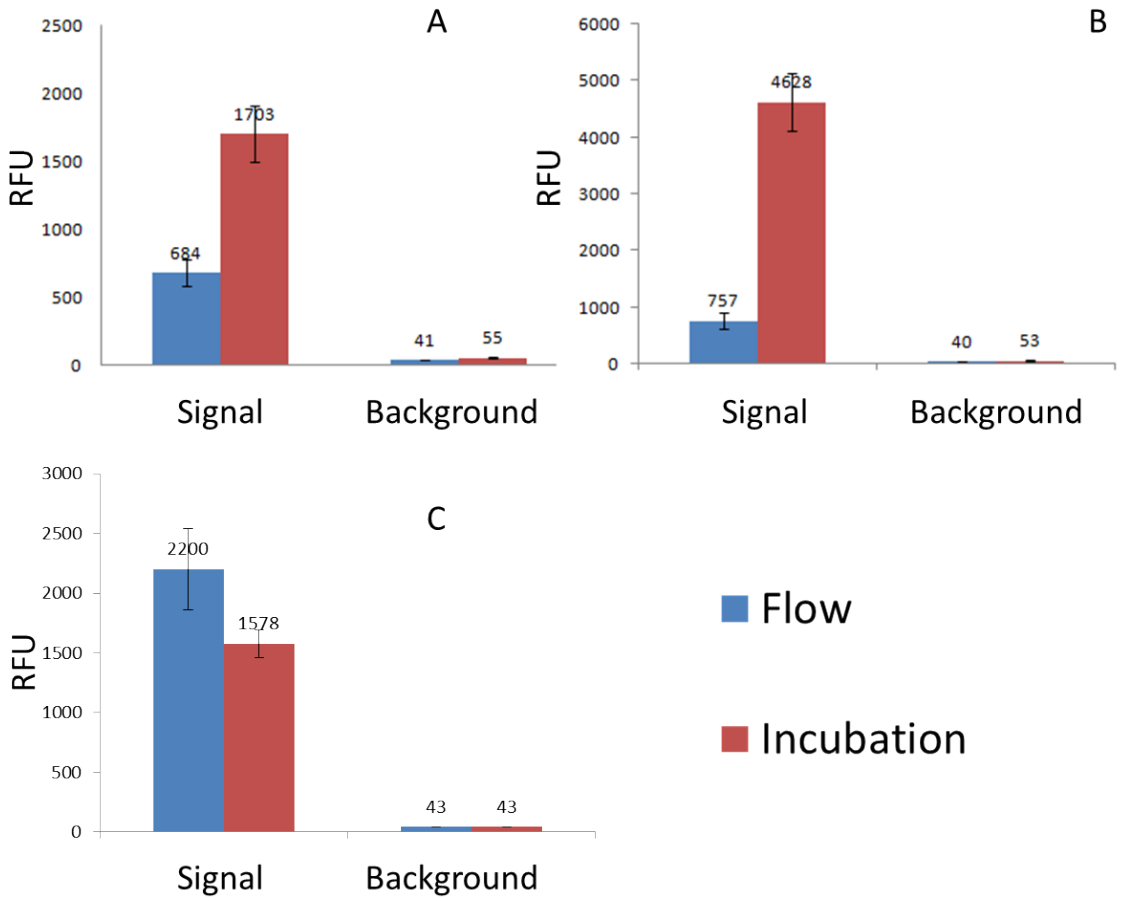

Fig. S2 Flow incubation compared with free incubation (A) Incubation with 0.1 µM target and (B) incubation with 1 µM target both with flow rate of 13.3 µL/min and (C) 0.1 µM target antisense incubation with a slower flow rate of 1.33 µL/min.

Fig. S2A shows the hybridization result with 0.1µM concentration, where the signal from free incubation was significantly higher than that from flow incubation. The same is true for 1µM antisense concentration as shown in Fig. S2B. It might be surprising to observe in 1mm chamber, the flow hybridization produced lower signal compared to free incubation. However, the results can be explained by the slow diffusion of oligonucleotides.

Reynolds number $\mathrm{Re} = \frac{D_H V \rho}{\mu}$ was used to characterize the flow inside fluid chamber. When Re is greater than 3000, turbulent flow will be produced in rectangular duct.[2] However, such a Re is difficult to achieve with our experiment setup, resulting in a laminar flow inside fluidic

chamber. As such, the dimension of the fluidic channel is too large to allow sufficient diffusion to happen before fluid leaves reaction sites, resulting in an ineffective replenishment of target by flow. To allow more time for diffusion, the mean velocity of the flow should be reduced. When transport is the limiting factor,[3] the boundary layer thickness (δ) in a steady state reactor with wall reaction happening only on one side could be approximated to $\delta = \frac{1}{0.67}\sqrt[3]{\frac{DxH}{3U_m}}$, where D is the diffusion constant, x is the distance travelled on reactive surface, H is the height of the microfluidic chamber and U_m is the mean velocity of the flow. Outside the boundary layer, the concentration of the reactants can be regarded as unchanged, inside boundary layer, reactants are consumed.

Fig. S3A shows the sagittal cross sectional view of a chamber and Fig. S3B shows predicted results of reducing mean velocity. Boundary layer increased after velocity decrease, and slower flow enables longer diffusion time, which substantially increased the amount of molecules arriving GLAD-MACE surface. As long as boundary layer thickness is smaller than chamber height, reducing the mean velocity will always result in an increase in arrived molecules.

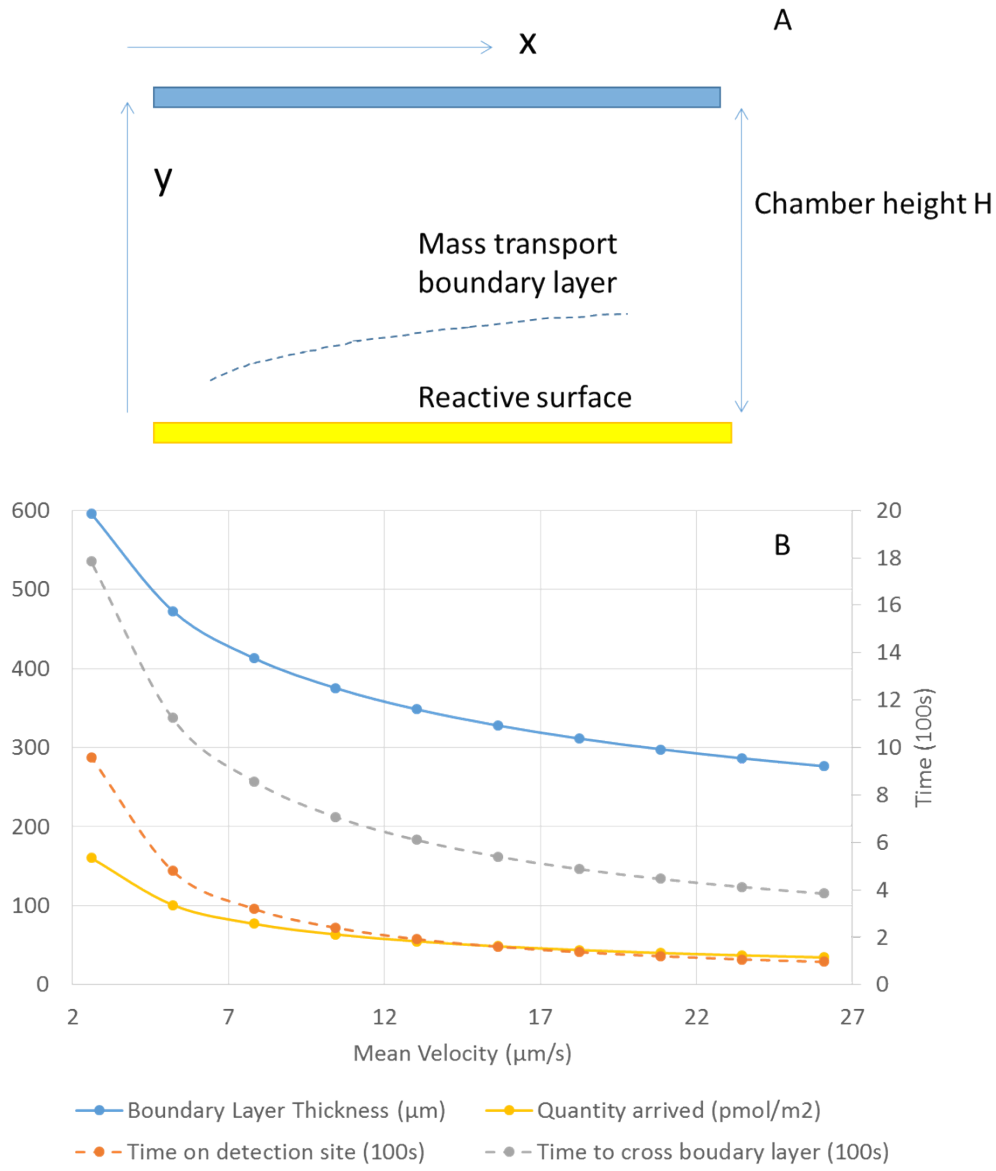

Fig. S3 The effect of mean velocity on boundary layer and target capturing (A) model of the bulk diffusion and (B) the predicted results.

To test our hypothesis of insufficient diffusion, experiment was carried out with 0.1 µM antisense concentration at the flow rate of 1.33 µL/min. The experiment was continued for 400 minutes, which corresponded to 2.67 residence time. As shown in Fig. S2C, compared to previous results obtained with 1 mm chamber at the flow rate of 13.3 µL/min, though less target was used (4 residence time compared to 2.67 residence time), flow hybridization clearly showed an improvement and surpassed that of free incubation. As such, it confirmed our

hypothesis that the lower signal from flow incubation in 1 mm chamber was due to the too fast a flow rate and not enough time to allow diffusion.

References

1. Wu, J.; Zheng, H.; Cheng, H.; Zhou, L.; Leong, K. C.; Rajagopalan, R.; Too, H. P.; Choi, W. K., Thermoporometry characterization of silica microparticles and nanowires. *Langmuir* **2014,** 30, (8), 2206-15.
2. Munson, B. R.; Young, D. F.; Okiishi, T. H., Fundamentals of fluid mechanics. *Fundamentals of fluid mechanics* **1994**.
3. In *PHYSICOCHEMICAL HYDRODYNAMICS - II: 6TH INTERNATIONAL CONFERENCE*, PCH. Physicochemical hydrodynamics, 1987; 1987; pp 419-631.