Electronic Supplementary Information

Sequentially bio-conjugated optofluidic laser for wash-out-free and rapid biomolecular detection

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I. Liquid loading process by capillary action

For the sequentially bio-conjugated FOFL biosensor, the biotinylated HOF continuously absorbs regents into the fiber. A pipette was employed to take the same volume of each reagent, allowing 40 mm liquid length for each. Before sequential bio-conjugation, it is critical to ensure that there were no droplet or liquid plugs in the HOF. First, the pipette absorbed the reagents into the tip (Fig. S1a), and pushed the reagents out to form a droplet on the tip (Fig. S1b). For some reagents, it cannot form a droplet, but it doesn't influence the feasibility of absorbing reagents into HOF via capillary action. Then, the droplet was moved close to the inlet of HOF to enable the absorption of liquid into the HOF via capillary action for incubation (Figs. S1c-1e).

Note that the solution at the inlet of HOF may volatilize during incubation and leave an air gap on the HOF tip, which could be solved in two ways. One is by cutting off a short section of HOF, so that the next solution can be withdrawn into the HOF continuously without any air gaps. The corresponding images are shown in Fig. S2a and S2b. In this structure, the adjacent solution has diffusion and convection due to the concentration gradient. It can be overcome by extending the liquid length of each reagent, which has been investigated in Fig. S4 to Fig. S7. The other method is to leave the short dried section as an air gap for separating the adjacent solutions (Fig. S2c). The length of each air gap should be short enough, otherwise it may affect the total liquid height in the HOF. This strategy can avoid the diffusion and convection between adjacent solutions, therefore, the liquid length of each reagent can be further shortened in this case.

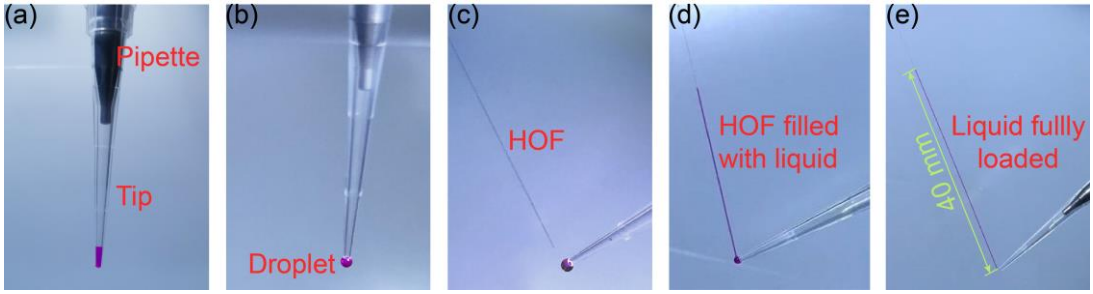

Figure S1 The operation process for the liquid length control in the sequential bio-conjugation. (a) A pipette taking a fixed volume of liquid to precisely control the liquid length in HOF. (b) The droplet formed on the tip. (c) The droplet was moved close to the empty HOF. (d) The liquid was withdrawn into the HOF via capillary action and (e) quickly entered into the HOF completely.

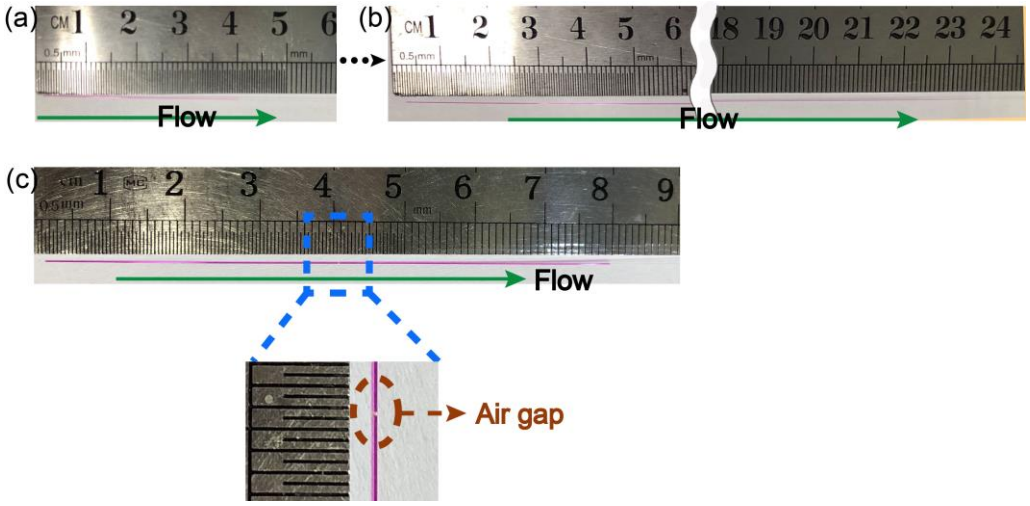

Figure S2 The images for reagents absorbing into the HOF via capillary action. The HOF with a liquid length of (a) 40 mm and (b) 240 mm, in which the solution was withdrawn into the HOF without air gaps. (c) The HOF filled with reagents with a tiny air gap between two sections of liquid. 40 mm of liquid was withdrawn into the HOF for each time. The liquid length of 40 mm, 80 mm, and 240 mm correspond to once, twice, and six times of liquid withdrawal, respectively.

II. Numerical simulation of light distribution

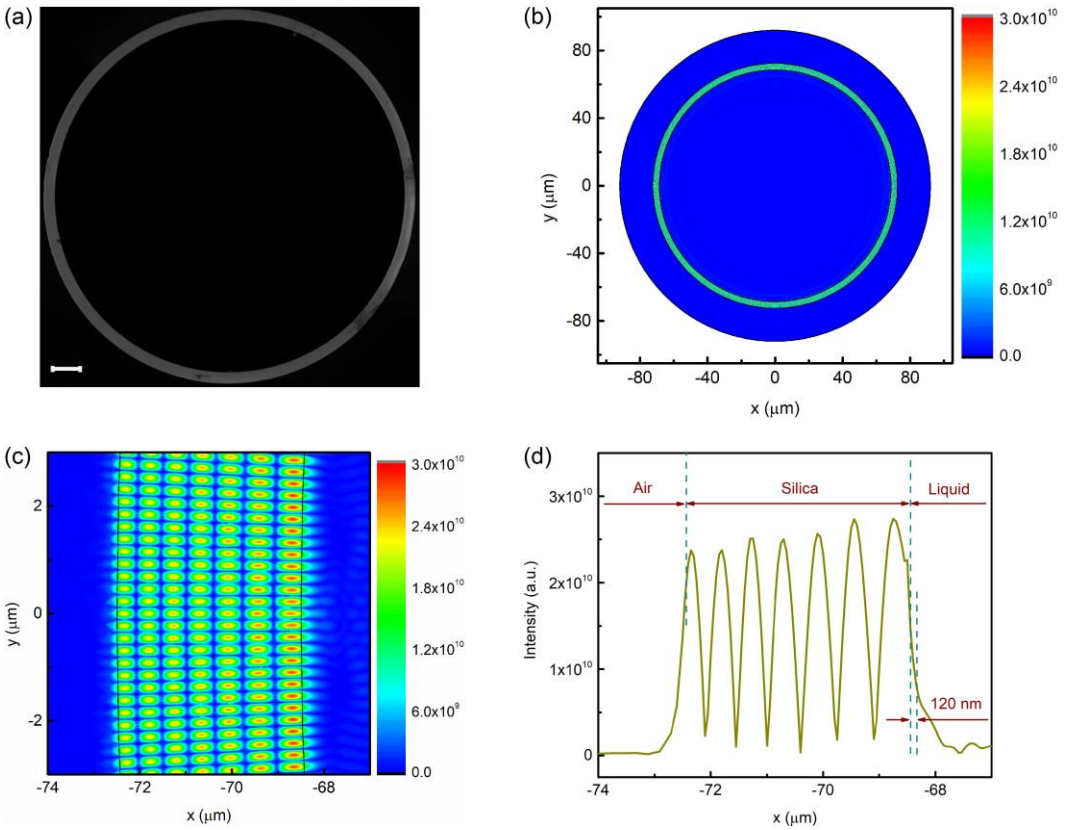

Figure S3 (a) The SEM image of HOF used in this work, showing an outer diameter of 145 μm with a thickness of 4 μm. Scale bar, 10 μm.(b) Numerical simulations of the intensity distribution in the cross-section of the HOF and (c) the enlarged details. (d) Light intensity along the radial direction of HOF (x < 0, y = 0). The evanescent field distribution indicates a penetration depth of 120 nm. In the model, the parameters of HOF geometry are the same as the size of HOF in (a).

III. The impact of diffusion and convection

We evaluated the impact of diffusion and convection on bio-conjugation by fluorescent imaging. In sequential bio-conjugation, the diffusion and convection owing to the concentration gradient of adjacent liquid and the movement of liquid in HOF may influence the concentration of the reagents. It is a critical factor influencing bio-conjugation efficiency except for the binding sites, incubation time, and molecular affinity. To understand the diffusion and convection well, the experiment was performed under different diffusion time and liquid lengths x (Fig. S4). Experimentally, the PBS solution was withdrawn into the cleaned HOF with a length of x, followed by 0.04 mM Rhodamine (RhB) solution with the same length (Fig. S4). The fluorescent intensity along the HOF was recorded by a camera (Canon, 1300D). The distributions of fluorescent intensity under different diffusion time and liquid lengths were extracted from the relevant photograph (Figs. S5a-S5c). The fluorescent intensity can be transferred into the RhB concentration by calibrating the relationship between intensity and concentration (Figs. S6a-S6c).

Figure S7a shows the distribution of RhB concentration along the HOF with a liquid length of 40 mm. L_R denotes the distance from the inlet of the HOF. With the increase of L_R, the RhB concentration keeps at 0.04 mM RhB and gradually decreases. It is attributed to the diffusion and convection between RhB-PBS due to the concentration gradient and the movement of liquid. The unaffected length with no diffusion and convection, L_{ND}, can be employed as the molecular binding area. The unaffected length doesn't change with the diffusion time and fluctuates around 10 mm under the condition of 40 mm liquid length (Fig. S7b). This follows the regular rule of the steady-state convection-diffusion,[1] in which the concentration distribution doesn't change with the diffusion time, making it beneficial to the binding of biomolecules. We further study the influence of liquid length on the unaffected length (Fig. S7c). With the increase of liquid length, the unaffected length increases as the liquid length is longer than 30 mm (Fig. S7d). This is because the concentration gradient decreases to a small value and does not affect the distribution of biomolecules at the location far away from the RhB-PBS interface. Increasing the liquid length can reduce the influence of diffusion and convection, while it also requires a longer HOF length to store the liquid. For the protein molecules such as SAv-Cy3, the molecular weight is much larger than RhB molecules, enabling a slower diffusion and thus relieving the effect on concentration. To compromise all the aspects above, the liquid length of 40 mm was chosen to eliminate the impact of convection and diffusion and provide sufficient molecular binding area. In addition, other methods are helpful to avoid

convection and diffusion, such as introducing a section of an interval solution or air gap.

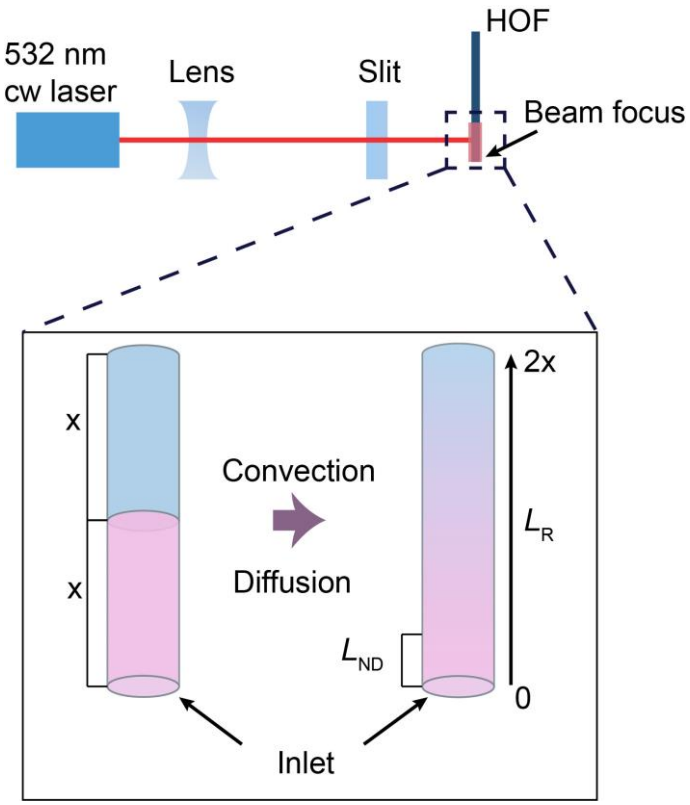

Figure S4 Schematic diagram of fluorescent imaging for the study of convection and diffusion in sequential bio-conjugation. The 532 nm continuous wave (cw) laser is diverged by a concave lens and filtered by two slits to obtain a uniform strip to pump the liquid filled HOF. Both the PBS and RhB are sequentially withdrawn into the HOF from the inlet. x is the liquid length of PBS and RhB. L_R denotes the liquid length from the inlet of the HOF. L_{ND} is the length that isn't influenced by the diffusion and convection.

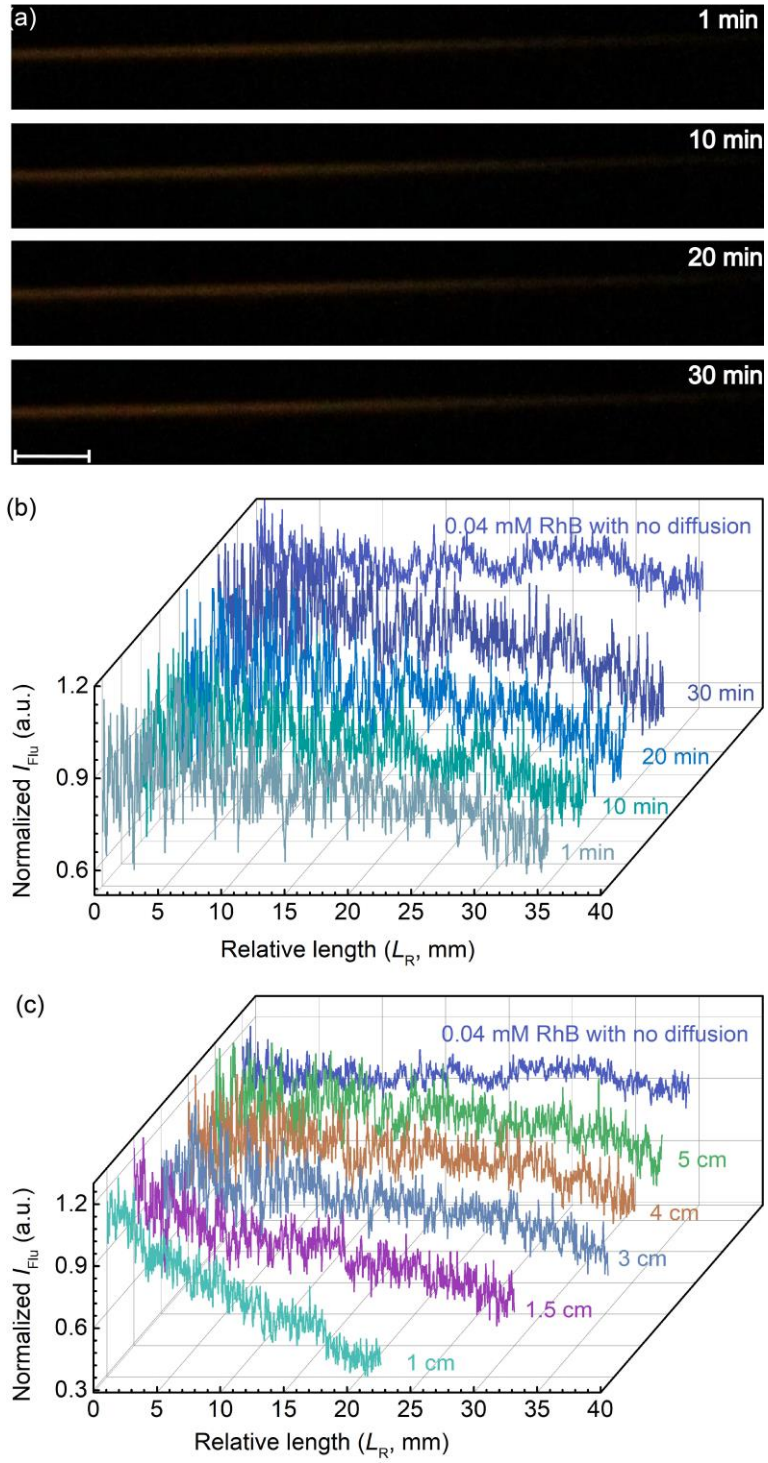

Figure S5 The influence of diffusion and convection in the sequential bio-conjugation. (a) The fluorescent images and (b) the distribution of fluorescence intensity along the HOF at different diffusion time. The liquid lengths of both RhB and PBS are 40 mm. Scale bar, 1 mm. (c) The distribution of fluorescent intensity along the HOF with various liquid lengths.

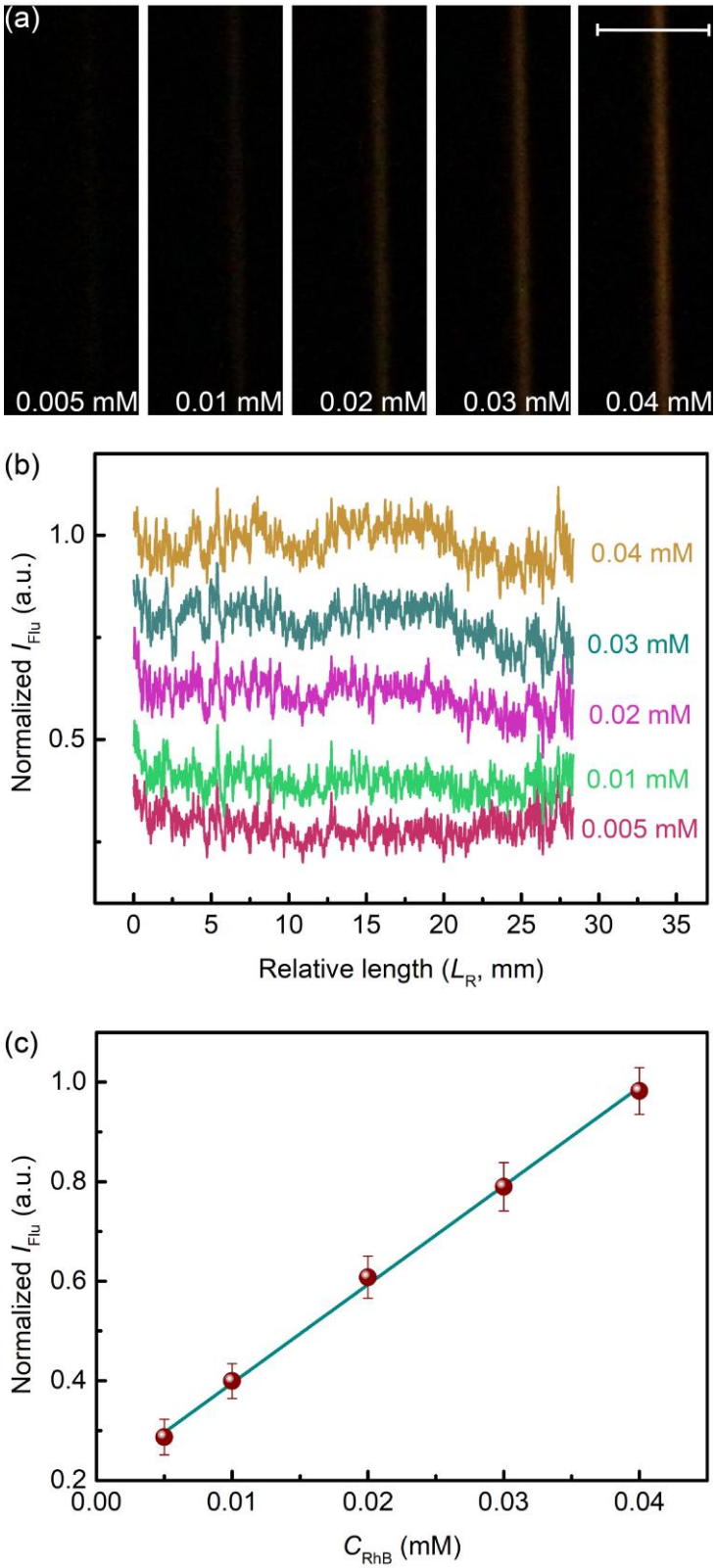

Figure S6 The calibration of RhB concentration with fluorescence intensity. (a) The fluorescent images of HOFs filled with different RhB concentrations. Scale bar, 1 mm. (b) The fluorescent intensity with different RhB concentrations without diffusion and convection. (c) The fluorescent intensity versus RhB concentration.

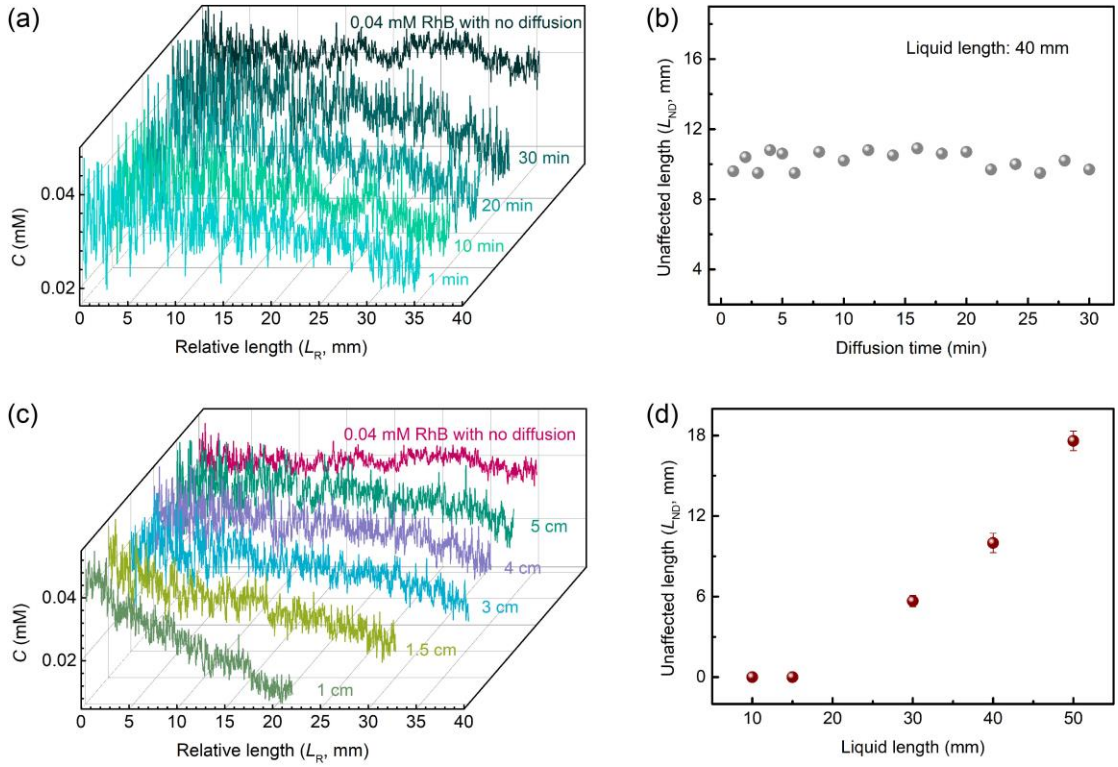

Figure S7 The influence of the diffusion and convection in the sequenced bio-conjugation. (a) The RhB concentration distribution along the HOF at different diffusion time. The liquid lengths of both RhB and PBS both are 40 mm. (b) The unaffected length of RhB concentration varies with the diffusion time, showing no changes and fluctuating around 10 mm. (c) The RhB concentration distribution along the HOF with various liquid lengths. The concentration of RhB keeps at 0.04 mM at the location close to the inlet of HOF, and then gradually decreases at the location close to the interface of RhB-PBS. (d) The relationship between unaffected length and liquid length.

IV. Potential for rapid ELISA assay

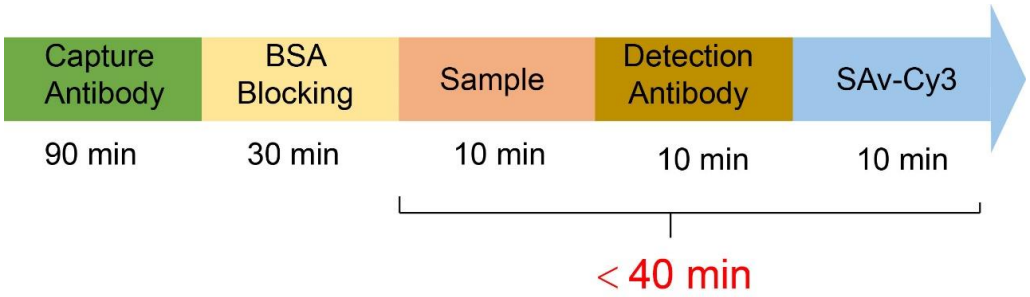

Figure S8 The potential of the sequentially bio-conjugated fiber optofluidic laser for fast assay in ELISA. It can be finished within 40 min, which is ten times faster than that of traditional method (3-5 h).

V. The principle for avidin detection

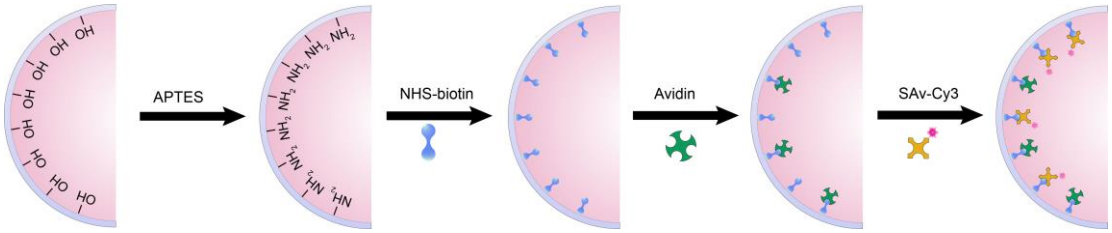

Figure S9 The experimental procedure for avidin detection in the sequentially bio-conjugated FOFL.

VI. Relationship between HOF inner diameter and liquid height

The capillary action induced liquid height in HOF, h, can be theoretically calculated according to Jurin's law,[2]

$$h = \frac{2\gamma \cos\theta}{\rho g r}. \quad (1)$$

Here, γ is the surface tension, θ is the contact angle, ρ is the density of a liquid, g is the local acceleration due to gravity, and r is the radius of the HOF. The liquid height, $h = h(r, \gamma)$, is mainly determined by the radius of the HOF and the surface tension. We assume that the physical and chemical properties of reagents and biosamples are similar to those of water, as deionized water was used as solvents throughout this work. The relationship between the inner radius of HOF and liquid height under different surface tensions is illustrated in Fig. S10. To obtain an increased liquid height by capillary action, a smaller HOF and higher surface tension are more favorable. The HOF used in this work has an inner diameter of 137 μm (Fig. S3a). According to the theory, the maximum liquid height above the liquid level is about 21.7 cm, which gives the great potential of many sequential incubation steps such as those in ELISA. Additionally, the liquid height may also vary with the surface tension. To further enhance the capacity of incubation steps, the surface modification of the HOF, in addition to the insertion depth, can be further optimized.

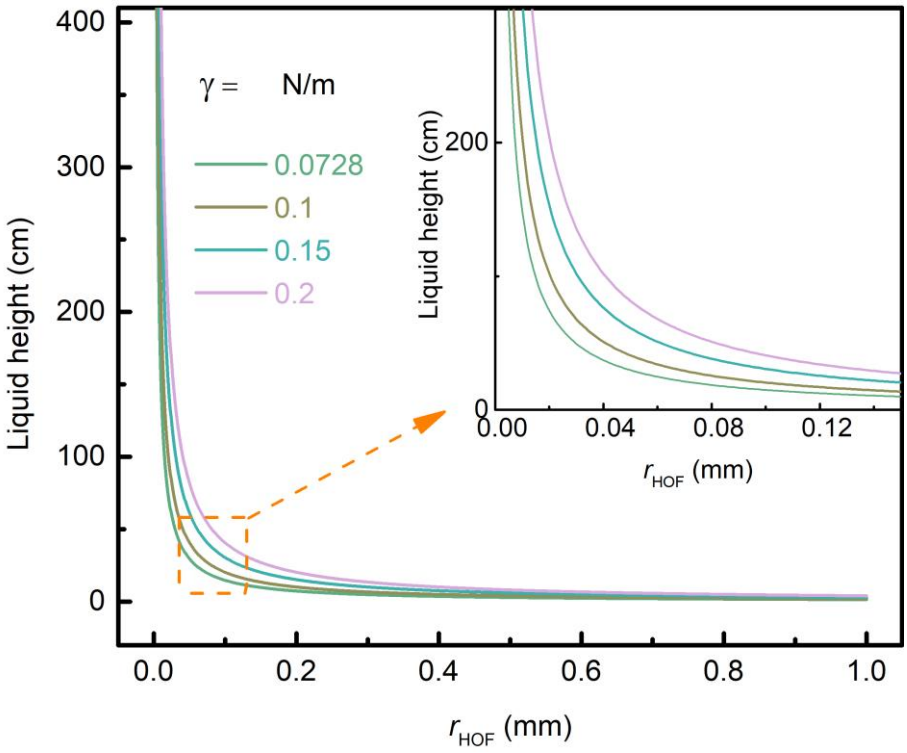

Figure S10 Theoretically calculated liquid height h versus HOF inner diameter r under different surface tensions. The results were simulated according to Jurin's law. In the calculations, θ=0 °, ρ=1000 kg/m^3, g=9.81 m/s^2.

Reference

1 M. Stynes, *Acta Numer.*, 2005, **14**, 445-508.

2 Q. David, Brochard-Wyart Francoise, G. Pierre-Gilles de, *Capillary and Wetting Phenomena*, 2004.