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ORIGINAL PAPER

Rapid, label-free, and sensitive point-of-care testing of anti-SARS-CoV-2 IgM/IgG using all-fiber Fresnel reflection microfluidic biosensor

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Abstract

The ongoing global pandemic of SARS-CoV-2 has promoted to develop novel serological testing technologies since they can be effectively complementary to RT-PCR. Here, a new all-fiber Fresnel reflection microfluidic biosensor (FRMB) was constructed through combining all-fiber optical system, microfluidic chip, and multimode fiber bio-probe. The transmission of the incident light and the collection and transmission of Fresnel reflection light are achieved using a single-multi-mode fiber optic coupler (SMFC) without any other optical separation elements. This compact design greatly simplifies the whole system structure and improves light transmission efficiency, which makes it suitable for the label-free, sensitive, and easy-to-use point-of-care testing (POCT) of targets in nanoliter samples. Based on Fresnel reflection mechanism and immunoassay principle, both the SARS-CoV-2 IgM and IgG antibodies against the SARS-CoV-2 spike protein could be sensitively quantified in 7 min using the secondary antibodies-modified multimode fiber bio-probe. The FRMB performs in one-step, is accurate, label-free, and sensitive in situ/on-site detection of SARS-CoV-2 IgM or IgG in serum with simple dilution only. The limits of detection of SARS-CoV-2 IgM and SARS-CoV-2 IgG were 0.82 ng/mL and 0.45 ng/mL, respectively. Based on our proposed theory, the affinity constants of SARS-CoV-2 IgM or IgG antibody and their respective secondary antibodies were also determined. The FRMB can be readily extended as a universal platform for the label-free, rapid, and sensitive in situ/on-site measurement of other biomarkers and the investigation of biomolecular interaction.

Keywords SARS-CoV-2 · Fresnel reflection biosensor · Immunoglobulin G · Immunoglobulin M · Point-of-care testing

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a newly emerged coronavirus, triggers the pandemic of coronavirus disease 2019 (COVID-19) [1, 2]. Timely and accurate detection of the virus nucleic acid and antibody is essential for the outbreak containment, antiviral treatment, and vaccine research [3–5]. The virus nucleic acid real-time polymerase chain reaction (RT-PCR) testing is the golden standard for clinical diagnosis of COVID-19 patients [1, 3, 6, 7]. However, they suffered several limits such as long turn-around times, expensive equipment, false negatives, and un-suitable for on-site testing [8, 9]. During a pandemic, the false negative results contribute to the rapid spread of the virus and prevent proper outbreak curb [9, 10]. Serological assays can verify the immune response to SARS-CoV-2, identify sero-conversion, and characterize the virus course. Because of its high sensitivity, specificity, and stability [9, 11, 12], the

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serological testing of SARS-CoV-2 immunoglobulin G (IgG) and/or immunoglobulin M (IgM) induced by coronavirus infections is an effective additional method in COVID-19 diagnosis. After viral infections, IgM provides the first defense line and can be detected in patient blood at 3~6 days. IgG, response for long-term immunity and immunological memory, can be detected at 7~10 days [13]. Therefore, serological testing is helpful for determining the number of infections and evaluating immunization state of the population to curb the virus spread and is also especially useful for return-to-work screening tests and vaccine applications [11, 12].

Several SARS-CoV-2 IgM/IgG testing kits, such as chemiluminescence enzyme immunoassay (CLIA), enzyme-linked immune sorbent assay (ELISA), and lateral flow immunochromatographic assay (LFIA), have been commercially available [8, 11, 14, 15]. Long et al. first applied CLIA to determine the diagnostic value of SARS-CoV-2 IgM/lgG antibodies using a multi-center cross-sectional analysis [11]. Using an indirect ELISA, the SARS-CoV-2 IgM and IgG antibodies were observed in 82.7% and 64.7% of 173 COVID-19 patients, respectively [16]. Although they are powerful tools for high-through detection of antibodies, they require expensive and bulky instrument, time-consuming operation, and trained professional, thus making them be difficult for point-of-care testing (POCT). LFIA is a POCT method that rapidly detects anti-SARS-CoV-2 antibodies in various fluid samples [17]. However, it is a qualitative method with a limited sensitivity [8, 16, 18]. Therefore, it is urgent to develop rapid, cheap, easy-to-use, and sensitive POCT serological technology to deal with this massive attack [19].

Here, we at the first time reported a novel all-fiber Fresnel reflection microfluidic biosensor (FRMB) for the label-free, rapid, easy-to-use, and sensitive detection of SARS-CoV-2 IgG/IgM antibody against the SARS-CoV-2 spike protein, which could bind to their cellular receptors [20–22]. In this system, a single-multi-mode fiber optic coupler (SMFC) was used for the transmission of both incident light and the collection and transmission of Fresnel reflection light. This optical structure not only simplified the structure of the whole system because of no requirement of optical separation elements, but also greatly improved light transmission efficiency, thus increasing the sensitivity of the FRMB. One multimode fiber probe modified by the secondary antibodies was regarded as biorecognition element as well as transducer. The Fresnel reflection light intensity on the biosensing interface decreased with the binding reaction between SARS-CoV-2 IgG or IgM antibodies and their respective antibodies. The quantitative detection of SARS-CoV-2 IgG or IgM antibody could be achieved according to the relationship between Fresnel reflection light intensity and the concentration of SARS-CoV-2 IgG or IgM antibody, respectively. Compared with the single mode fiber (φ4 μm)-based Fresnel reflection sensor [23], the interaction area of biomolecules in the FRMB increased 4 grades higher due to the use of the multimode fiber bio-probe (φ600 μm), thus resulting in a higher sensitivity. Different from the label-free surface plasmon resonance (SPR) biosensor [24], the FRMB has no requirement of the expensive precious metal thin film. Moreover, its SiO_2-based biosensing surface is easier to modify various biorecognition molecules, which greatly reduces the testing cost and allows it to be suitable for POCT. The binding kinetics between the SARS-CoV-2 IgG or IgM antibody and their respective secondary antibodies are also determined using the FRMB.

Experimental section

Reagents and chemicals

Bovine serum albumin (BSA), 3-Mercaptopropyl-trimethoxysilane (MTS), N-(4-maleimidobutyryloxy)succinimide (GMBS) were purchased from Sigma-Aldrich (Steinheim, Germany). The phosphate-buffered saline (PBS) solution and the antibody dilution buffer were prepared, respectively. The sodium dodecyl sulfate (SDS, 0.5%, pH =1.9) was used to regenerate the bio-probe. All other reagents, unless specified, were supplied by Beijing Chemical Agents (Beijing, China).

Anti-SARS-CoV-2 spike protein IgM antibody (SARS-CoV-2 IgM), anti-SARS-CoV-2 spike protein IgG antibody (SARS-CoV-2 IgG), and the secondary antibodies (goat anti-SARS-CoV-2 IgG antibody and goat anti-SARS-CoV-2 IgM antibody) were purchased from Beijing Biodragon Immunotechnologies Co., Ltd. (Beijing, China). Normal human IgG antibody were purchased from Wuhan Fine Biotech Co. Ltd. (Wuhan, China). The antibody stock solution and various standard solutions were prepared using the PBS. The healthy human serum samples were provided by the Beijing Institute of Radiation Medicine (Beijing, China).

Functionalization of fiber bio-probe

The silica multimode fiber with a diameter of 600 μm (NA = 0.22, 3.0 cm length), whose distal end surface was smoothed and polished, was regarded as the bio-probe after covalently modified by the goat anti-SARS-CoV-2 IgG or IgM antibody as previous report with minor adjustment [25] (Fig. S1).

Design of all-fiber Fresnel reflection microfluidic biosensor

Figure 1 shows the schematic and key elements of the FRMB system, and its photo is shown in Fig. S2. The 635-nm laser (Beijing Xianfengzhihui Sci. & Tech. Co. China) was selected as incident light sources because of its stability and compactness, which connected with the single mode fiber of the

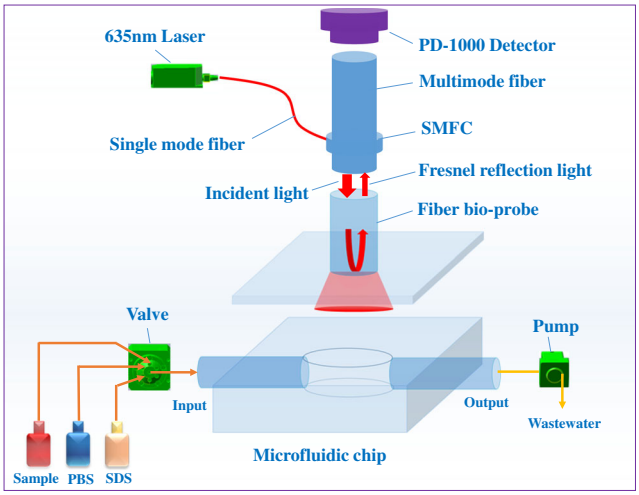


Fig. 1 The scheme of the FRMB system, including the all-fiber optical system, the microfluidic chip, fiber bio-probe, and the fluidics (pump and valve). The incident light couples into the multi-mode fiber bio-probe through the multi-mode fiber of the SMFC, and at the distal end surface of bio-probe, part of incident light reflects back to the bio-probe based on Fresnel reflection mechanism and is detected by the PD-1000 detector. Various solutions are introduced into the microfluidic cell under the control of six-channel valve

SMFC. The single mode fiber with the diameter of about 4 μm only transmits one mode light, and the light in it can rarely loss due to twist in use. The incident light then coupled into the multi-mode fiber bio-probe with the diameter of 600 μm and numerical aperture of 0.22 through the multi-mode fiber of the SMFC. At the distal end surface of bio-probe, part of incident light reflected back to the bio-probe based on Fresnel reflection mechanism. The reflection light was collected by the SMFC and detected by the photodiode detector (PD-1000) through transmission of the multimode fiber of the SMFC. The use of the SMFC greatly simplified the optical structure of the FRMB because no optical alignment and other optical separated elements (e.g., lens, dichroic mirror) were required. This design significantly increased transmission efficiency of the incident light and reflection light, thus improving the sensitivity of the FRMB. Based on Fresnel reflection mechanism, the reflection light intensity changed with the local refractive index (RI) on the bio-probe surface. When the captured molecules (e.g., antibody) are immobilized onto the bio-probe surface, the RI of bio-probe surface increases with increase of the target biomolecules bound with the captured molecules, which results in the decrease of reflection light intensity. Therefore, the label-free detection of target molecules can be achieved according to the relationship between their concentration and reflection light intensity.

The bio-probe was perpendicular to the microfluidic chip and immersed into the microfluidic cell (Fig. 1). The size of microfluidic cell wasφ600 × 300 μm and had an effective volume of about 300 nL. Various fluids, including buffer, samples, and regeneration solution, were sequentially pumped into the cell under the controlment using a multi-position solenoid valve (VICI Valco, Interchim). The Fresnel reflection light intensity was real-time recorded.

Immunoassay of SARS-CoV-2 IgG or IgM using the FRMB

Figure 2A and B illustrate the immunoassay principle of the FRMB for SARS-CoV-2 IgG and IgM and their typical signal profiles, respectively. Based on Fresnel reflection mechanism and immunoassay principle, the one-step label-free detection of the SARS-CoV-2 IgG and IgM in samples can be achieved using goat anti-SARS-CoV-2 IgG and IgM antibody modified fiber bio-probe. One detection cycle includes four steps as the following. First, the PBS solution is introduced into the microfluidic cell to obtain the stability baseline (phase I in Fig. 2A). Second, the sample containing various concentrations of SARS-CoV-2 IgG or IgM is pumped into the cell. The SARS-CoV-2 IgG or SARS-CoV-2 IgM, if present, can specifically bind to goat anti-SARS-CoV-2 IgG or anti-SARS-CoV-2 IgM antibody immobilized onto the biosensing surface to form the antibody complex, respectively. The formation of these antibody complex increases the local RI of the biosensing surface, which leads to the decrease of the Fresnel reflection light intensity (phase II in Fig. 2A). The higher the concentration of SARS-CoV-2 IgG or SARS-CoV-2 IgM is added, the more the complex forms on the biosensing surface, thus resulting in a lower Fresnel reflection light intensity. Third, after several minutes' reaction, the bio-probe surface is regenerated using 0.5% SDS solution (pH 1.9) for 180 s (phase III in Fig. 2A) and washed with PBS solution (phase IV in Fig. 2A) to reuse.

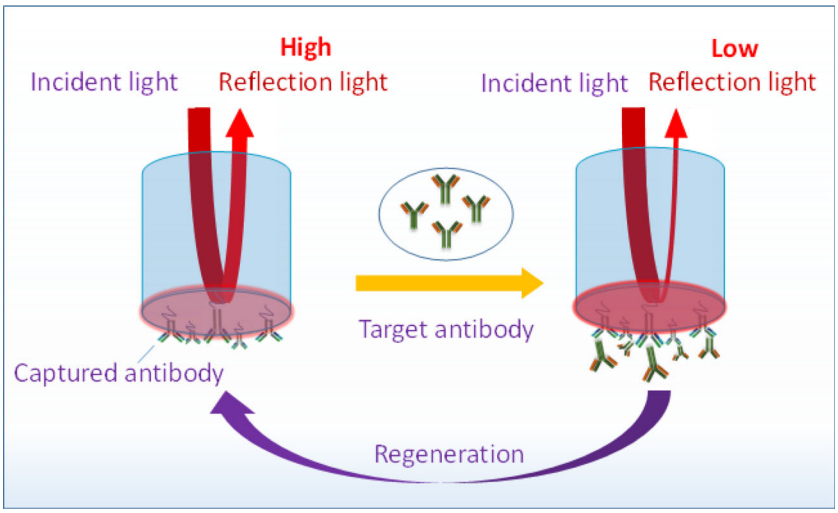
Figure 2B shows the representative real-time signal traces for one analysis cycle. For each assay, the decrease value (ΔI) of Fresnel reflection light intensity for subsequently determining dose-response relationships are calculated by subtracting from the baseline signal value (I_b) according to Eq. 1:

$$\Delta I = \text{Signal value at the baseline} - \text{Signal value after the reaction} \quad (1)$$

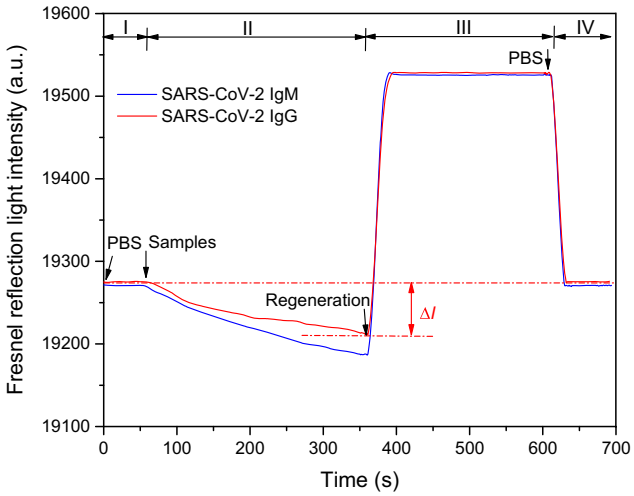
To reduce the influence of incident light intensity fluctuation, the Fresnel reflection light intensity of each standard point was normalized as the ratio to the Fresnel reflection light intensity of the blank sample containing no SARS-CoV-2 IgG or SARS-CoV-2 IgM according to Eq. 2.

$$I_n = \frac{\Delta I}{I_b} \cdot 10^6 \quad (2)$$

where 10^6 is the normalization factor.



(A)



(B)

Fig. 2 Immunoassay of SARS-CoV-2 IgG or IgM using the FRMB. **A** Schematic of immunoassay principle of SARS-CoV-2 IgG or IgM using the FRMB based on Fresnel reflection mechanism and immunoassay. The target antibody (SARS-CoV-2 IgG or IgM antibody) binds with the captured antibody (goat anti-SARS-CoV-2 IgG or IgM antibody) immobilized on the fiber bio-probe reduces the reflection light intensity. **B** Representative signal profile for SARS-CoV-2 IgG or IgM detection using the FRMB (reaction time is 300 s, the concentrations of SARS-CoV-2 IgG and IgM are 50 ng/mL and 100 ng/mL, respectively)

Measuring affinity constants of biomolecules

Measuring the kinetic constants of biomolecule interactions is greatly important for characterizing the bio-specific affinity in solution and developing novel biosensor [26]. Although various methods have been applied for detecting affinity constant of biomolecules, label-free biosensors have their unique features because they do not require the labeling of biomolecules [27]. To demonstrate the capability of the FRMB determining the kinetic constant of SARS-CoV-2 IgG or IgM and their respective antibody, a theoretical model was built to fit kinetic data. We assumed that the biosensing surface was homogenously covered by the captured antibody monolayer and each SARS-CoV-2 IgG or IgM bound with one goat anti-SARS-CoV-2 IgG or IgM antibody immobilized on the bio-probe, respectively. In these experiments, the SARS-CoV-2 IgG or IgM concentration in solution was much higher than those of goat anti-SARS-CoV-2 IgG or IgM antibody, indicating that the biomolecular interaction was limited by the reaction kinetics. As a result, the formation rate of the SARS-CoV-2 IgG/goat anti-SARS-CoV-2 IgG antibody complex or SARS-CoV-2 IgM/goat anti-SARS-CoV-2 IgM antibody complex at the biosensing surface, following pseudo-first-order kinetics, could be described as:

$$\frac{d[\mathrm{Ag:Ab}]}{\mathrm{dt}} = k_a[\mathrm{Ab}][\mathrm{Ag}]_{\max} - (k_a[\mathrm{Ab}] - k_d)[\mathrm{Ab:Ag}] \qquad (3)$$

where $[\mathrm{Ag}]_{\max}$ is the surface concentration of goat anti-SARS-CoV-2 IgG (or goat anti-SARS-CoV-2 IgM) antibody or all available binding sites on the biosensing surface prior to binding reaction; [Ab] and [Ab:Ag] represent the initial

concentration of SARS-CoV-2 IgG (or SARS-CoV-2 IgM) and the concentration of product complex formed on the biosensing surface, respectively. k_a and k_d are the affinity constant and dissociation constant, respectively. With the proceeding of the binding reaction, the amount of product complex formed on the biosensing surface can be recorded by the FRMB system, which is related with the change of Fresnel reflection light intensity I_t as following.

$$\frac{dI_t}{dt} = k_a C_0 (I_{\min} - I_t) - k_d I_t \tag{4}$$

where I_{min} is the minimum Fresnel reflection light intensity when all available binding sites of the biosensing surface are occupied by the SARS-CoV-2 IgG (or SARS-CoV-2 IgM), and C_0 is the concentration of SARS-CoV-2 IgG (or SARS-CoV-2 IgM). When the binding reaction reaches its quasi-steady-state, d[I_t]/dt equals to zero, Eq. 4 becomes:

$$I_{eq} = \frac{k_a C_0 I_{\min}}{k_a C_0 + k_d} \tag{5}$$

where I_{eq} is the Fresnel reflection light intensity at the quasi-steady-state. Eq. 5 can be rearranged as:

$$\frac{1}{I_{eq}} = \frac{1}{K_a I_{\min}} \cdot \frac{1}{C_0} + \frac{1}{I_{\min}} \tag{6}$$

where K_a is the equilibrium affinity constant at a certain concentration of antibody. Using the Eq. 6, the affinity constant K_a can be obtained after various concentrations of SARS-CoV-2 IgG or SARS-CoV-2 IgM are tested.

Analysis of SARS-CoV-2 IgG or IgM in real samples

To demonstrate the clinical application potential, the serum samples spiked by different concentrations of SARS-CoV-2 IgG or IgM are detected using the FRMB. After diluting 30 times, 30 μL serum sample was directly introduced into the microfluidic cell. The real-time Fresnel reflection light intensity was detected by the FRMB for 300 s (SARS-CoV-2 IgG) or 400 s (SARS-CoV-2 IgM) reaction. Finally, the bio-probe surface was regenerated using the SDS solution for 120 s. After washing by PBS solution for 90 s, the bio-probe was used for the next test.

Results and discussion

Biosensing mechanism of the FRMB and its characterization

To clarify the sensing mechanism, the geometric optics model was built for theoretical analysis because the diameter (Φ 600 μm) of the fiber bio-probe was far surpasses the incident light wavelength (635 nm). As we well known, the reflection coefficients r_s and r_p for s- and p- polarizations at the biosensing surface are calculated by Eqs. 7 and 8, respectively:

$$r_s = \frac{n_c\cos\theta - n_s\sqrt{1-\left(\frac{n_c}{n_s}\sin\theta\right)^2}}{n_c\cos\theta + n_s\sqrt{1-\left(\frac{n_c}{n_s}\sin\theta\right)^2}} \tag{7}$$

$$r_p = \frac{n_s\cos\theta - n_c\sqrt{1-\left(\frac{n_c}{n_s}\sin\theta\right)^2}}{n_s\cos\theta + n_c\sqrt{1-\left(\frac{n_c}{n_s}\sin\theta\right)^2}} \tag{8}$$

where n_c is the core RI of the fiber bio-probe, n_s is the local RI of the fiber bio-probe surface that changes with the formation or dissociation of antibody complex, and θ is the incident angle of the laser light. The total reflectivity is calculated by Eq. 9:

$$\overline{R} = \frac{|r_s|^2 + |r_p|^2}{2} \tag{9}$$

For simplicity, the incident light was assumed to be perpendicular to the biosensing surface. When the fiber bio-probe modified captured antibody (e.g., anti-SARS-CoV-2 IgG or anti-SARS-CoV-2 IgM antibody) was placed into the microfluidic cell, the Fresnel reflection light intensity I_t collected by PD-1000 was obtained by Fresnel formula:

$$I_t = k \cdot I_0 \cdot \left(\frac{n_c - n_s}{n_c + n_s}\right)^2 \tag{10}$$

where I_0 is the incident light intensity and k is a constant determined by the FRMB optical component transmittances. When the target biomolecules (e.g., SARS-CoV-2 IgG or SARS-CoV-2 IgM) were introduced over the biosensing surface, they specifically bound with the captured antibody. The biomolecules binding allowed the local RI (n_s) increase of the biosensing surface, thus resulting in the decrease of the Fresnel reflection light intensity (I_t) according to Eq. 10. As a result, the quantitative detection of target biomolecules could be achieved according to the relationship between the concentration of target biomolecules and the Fresnel reflection light intensity.

To verify this, the bio-probe functionalized goat anti-SARS-CoV-2 IgG antibody was vertically immersed into the microfluidic cell, and several control experiments were done (Fig. 3A). First, when 5 ng/mL SARS-CoV-2 IgG was introduced into the microfluidic cell, the Fresnel reflection light intensity decreased over the time (Fig. S3A). This contributed to that the SARS-CoV-2 IgG specifically bound with the goat anti-SARS-CoV-2 IgG antibody immobilized on the fiber bio-probe surface, which raised the local RI of

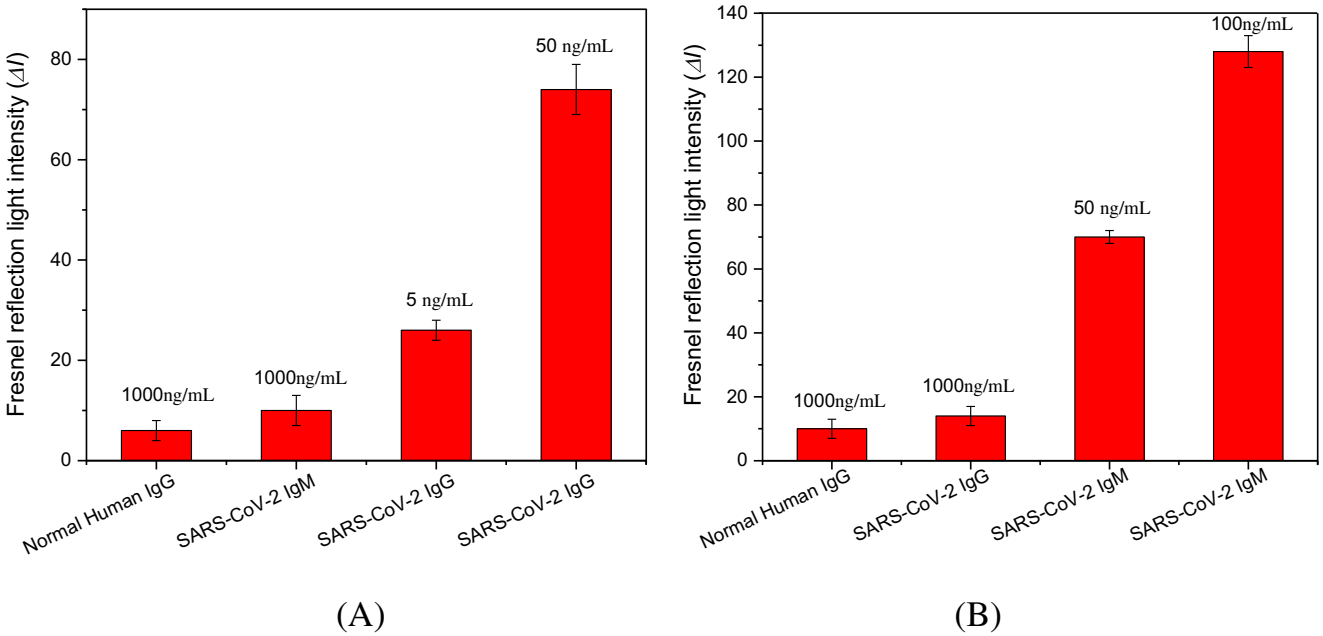


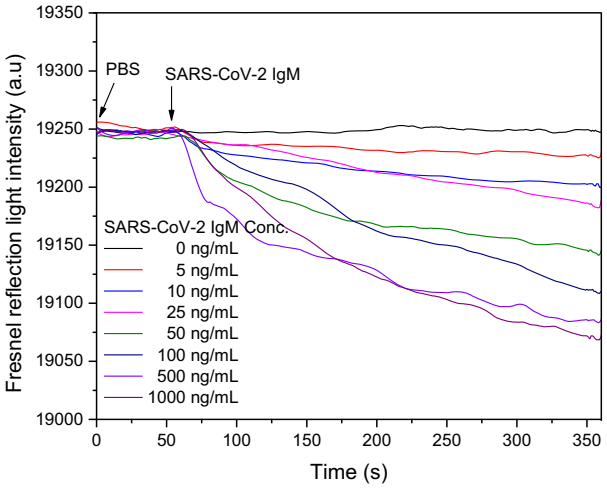
Fig. 3 Characteristics of the FRMB with functionalized fiber bio-probe. **A** Response of the bio-probe functionalized anti-SARS-CoV-2 IgG antibody for the normal human IgG, SARS-CoV-2 IgM, and SARS-CoV-2 IgG, respectively. Reaction time were 300 s. **B** Response of the bio-probe functionalized anti-SARS-CoV-2 IgM antibody for the normal human IgG, SARS-CoV-2 IgG, and SARS-CoV-2 IgM, respectively. Error bars corresponded to the standard deviation (SD) of data points in triple experiments

the biosensing surface and allowed the less lights to reflect according to Eq. 10. Second, when the higher concentration of the SARS-CoV-2 IgG (50 ng/mL) was added, the more SARS-CoV-2 IgG bound with the goat anti-SARS-CoV-2 IgG antibody, thus resulting in the lower light intensity. Third, when the normal human IgG or SARS-CoV-2 IgM antibody with 1000 ng/mL was pumped into the cell, no significant signal decrease was observed. The concentration of these samples was so low that their RIs was similar with that of PBS solution (Table S1). Therefore, the addition of them did not result in the obvious change of Fresnel reflection light intensity, indicating that the nonspecific adsorption of protein was negligible. Using the goat anti-SARS-CoV-2 IgM antibody modified bio-probe, the similar results were observed (Fig. 3B and Fig. S3B). Only when the SARS-CoV-2 IgM was introduced into the cell, the Fresnel reflection light intensity decreased. The higher concentration of the SARS-CoV-2 IgM was added, the lower light intensity was obtained. No significant signal decreased when normal human IgG or SARS-CoV-2 IgG was added. These results demonstrated that the goat anti-SARS-CoV-2 IgG antibody and goat anti-SARS-CoV-2 IgM antibody were successfully modified onto the bio-probe, and the observed signal decrease originated from the target antibody specifically bound with the captured antibody. The SARS-CoV-2 IgG and SARS-CoV-2 IgM had few cross-reactivity. Based on Fresnel reflection principle, the FRMB could be applied for the label-free detection of SARS-CoV-2 IgG and SARS-CoV-2 IgM.

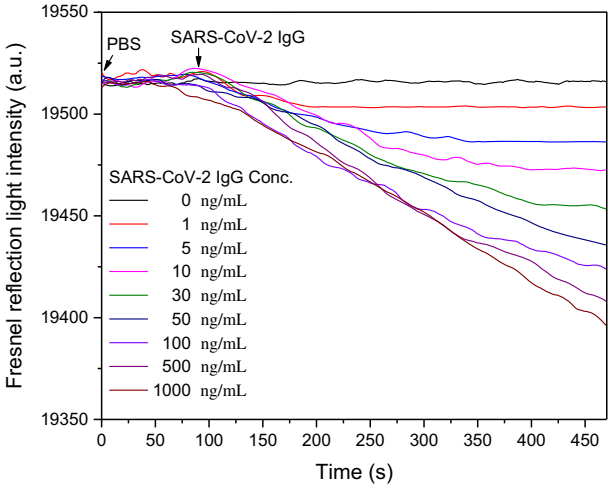
Detection of SARS-CoV-2 IgG or SARS-CoV-2 IgM in PBS

Figure 4A and B displays the exemplary signal traces for the detection of various concentrations of SARS-CoV-2 IgG and SARS-CoV-2 IgM, respectively. First, the PBS was introduced to the microfluidic cell, and the baseline was recorded. Then, various concentrations of the SARS-CoV-2 IgG or SARS-CoV-2 IgM was introduced to the cell, the Fresnel reflection light intensity initially rapidly decreased over time, and then gradually reached a plateau. Considering the detection time and signal value, the binding reaction time of between SARS-CoV-2 IgG or SARS-CoV-2 IgM with their respective antibodies-modified bio-probe was set as 300 s and 400 s, respectively. Finally, to reuse the bio-probe, the SARS-CoV-2 IgG (or SARS-CoV-2 IgM) bound with the goat anti-SARS-CoV-2 IgG (or goat anti-SARS-CoV-2 IgM) antibodies was removed by regeneration solution because they could

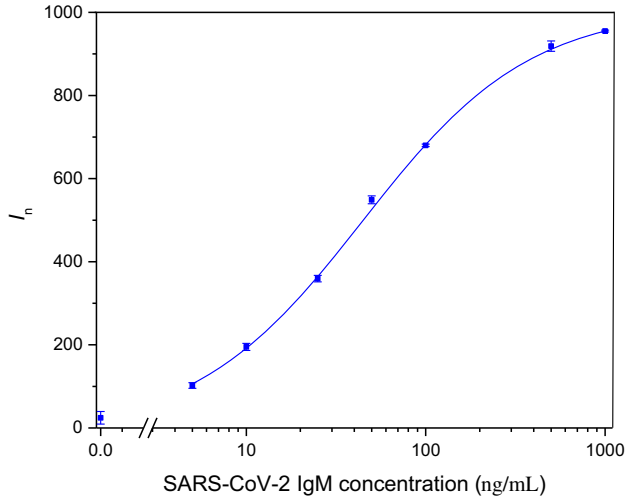
Fig. 4 Detection of SARS-CoV-2 IgG or SARS-CoV-2 IgM in PBS. **A** ▶ Representative signal curves of SARS-CoV-2 IgM in PBS. **B** Representative signal curves of SARS-CoV-2 IgG in PBS. **C** Dose-response curve of SARS-CoV-2 IgM in PBS (IgM concentrations ranged from 0 to 1000 ng/mL, reaction time was 300 s). **D** Dose-response curve of SARS-CoV-2 IgG in PBS (IgG concentrations ranged from 0 to 1000 ng/mL, reaction time was 400 s). **E** Dependence of $1/I_{eq}$ against $1/[C]_0$ for the association between SARS-CoV-2 IgM and goat anti-SARS-CoV-2 IgM antibody. **F** Dependence of $1/I_{eq}$ against $1/[C]_0$ for the association between SARS-CoV-2 IgG and goat anti-SARS-CoV-2 IgG antibody. Error bars corresponded to SD of the data points in triple experiments


PBS
SARS-CoV-2 IgM
SARS-CoV-2 IgM Conc.
0 ng/mL
5 ng/mL
10 ng/mL
25 ng/mL
50 ng/mL
100 ng/mL
500 ng/mL
1000 ng/mL
Fresnel reflection light intensity (a.u)
Time (s)

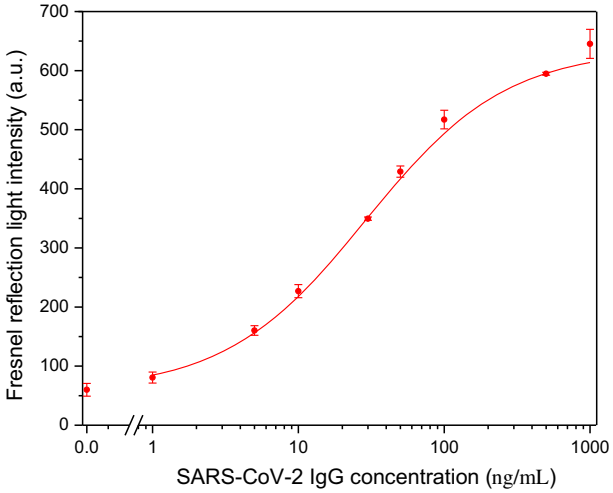
(A)


PBS
SARS-CoV-2 IgG
SARS-CoV-2 IgG Conc.
0 ng/mL
1 ng/mL
5 ng/mL
10 ng/mL
30 ng/mL
50 ng/mL
100 ng/mL
500 ng/mL
1000 ng/mL
Fresnel reflection light intensity (a.u.)
Time (s)

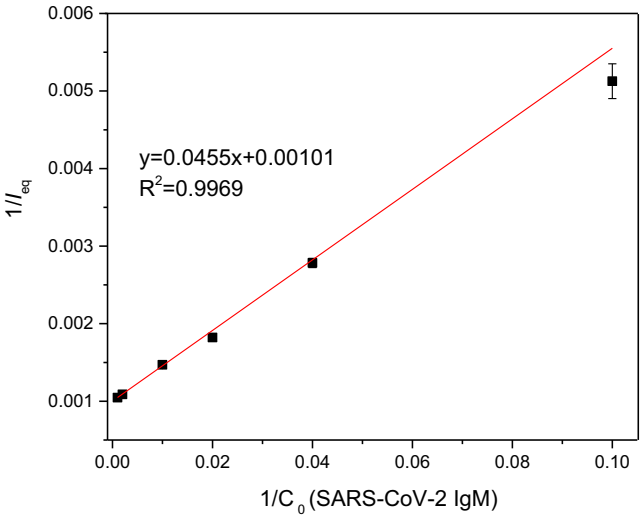
(B)


I_n
SARS-CoV-2 IgM concentration (ng/mL)

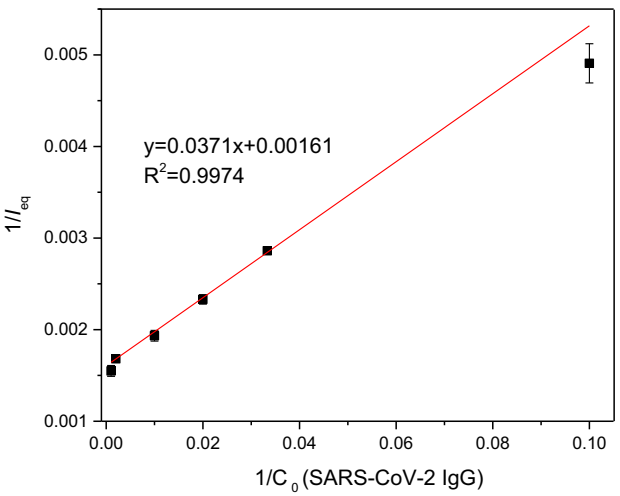
(C)


Fresnel reflection light intensity (a.u.)
SARS-CoV-2 IgG concentration (ng/mL)

(D)


y=0.0455x+0.00101
R^2=0.9969
$1/I_{eq}$
$1/C_0$ (SARS-CoV-2 IgM)

(E)


y=0.0371x+0.00161
R^2=0.9974
$1/I_{eq}$
$1/C_0$ (SARS-CoV-2 IgG)

(F)

destroy the binding of antibody complex. From Fig. 2B, one noted that the signal traces suddenly increased after the addition of SDS solution, which contributed to the lower RI of SDS than that of PBS. After regenerated, the PBS was pumped to wash the SDS solution and dissociated SARS-CoV-2 IgG or SARS-CoV-2 IgM. The signal traces came back to the baseline, indicating that the SARS-CoV-2 IgG (or SARS-CoV-2 IgM) bound to the goat anti-SARS-CoV-2 IgG (or goat anti-SARS-CoV-2 IgM) antibody could be completely removed. The biosensing surface could be reused for more than ten times without significant loss of bioactivity (Fig. S4), which was a desired feature for developing a good immunosensor to obtain accurate results. Meanwhile, the bio-probe retained more than 90% of its original response after 1 week of storage (Fig. S5).

As expected, the Fresnel reflection light intensity decreased with increasing the concentration of SARS-CoV-2 IgM or SARS-CoV-2 IgG in the PBS. The dose-response curves of SARS-CoV-2 IgG and SARS-CoV-2 IgM were demonstrated in Fig. 4C and D, which were plotted against the logarithm of the concentration of SARS-CoV-2 IgM or SARS-CoV-2 IgG based on the four-parameter logistic equation (Supporting information). The error bars in the Fig. 4C and D were less than 3.75%, indicating the good stability of FRMB. Using three times standard deviation of the mean blank values, the limits of detection (LODs) of SARS-CoV-2 IgM and SARS-CoV-2 IgG were 0.78 ± 0.031 ng/mL and 0.52 ± 0.028 ng/mL, respectively. The linear response of SARS-CoV-2 IgM and SARS-CoV-2 IgG ranged from 5.2 to 380.5 ng/mL and from 3.5 to 290.8 ng/mL, respectively. The high sensitivity of the FRMB for SARS-CoV-2 IgG or SARS-CoV-2 IgM detection was highly comparable to those of other immunoassay methods. An ELISA was developed for the detection of SARS-CoV-2 IgG with a LOD of 2.0 ng/mL [28], and the LOD of the opto-microfluidic sensing platform could reach 0.08 pg/mL through Au nanospikes enhancement [18]. However, these methods needed a long assay time (> 2 h). The high sensitivity of FRMB originates from the following features. First, compared with the single mode fiber, the Fresnel reflection light intensity is more sensitive for the local RI change on biosensing surface because the area of its end surface is four grades higher than that of the single mode fiber [23]. Second, the bigger area of multimode fiber bio-probe effectively enhances the interaction of biomolecules on the biosensing surface and increases the amount of SARS-CoV-2 IgG or SARS-CoV-2 IgM bound with anti-SARS-CoV-2 IgG or SARS-CoV-2 IgM antibody immobilized on the biosensing surface, respectively. In addition, the compact all-fiber optical structure improves the optical transmission and collection efficiency and reduces the light loss, which further raises the sensitivity of the FRMB.

The FRMB was also applied for the determination of affinity constants between SARS-CoV-2 IgG or SARS-CoV-2 IgM and their respective antibodies. Using Eq. 5, $1/I_{eq}$ was plotted against $1/C_0$ as shown in Fig. 5E and F, and the good linear relationships between $1/I_{eq}$ and $1/C_0$ for SARS-CoV-2 IgG or IgM and their respective antibodies were demonstrated. The affinity constants between SARS-CoV-2 IgM or IgG and their respective antibodies were determined as and 2.89×10^{11} M^{-1} and 2.23×10^{11} M^{-1}, respectively. These results displayed that the FRMB could serve as a simple label-free platform for the quantitative assay of binding kinetics between biomolecules (e.g., antibody proteins).

Detection of SARS-CoV-2 IgG or SARS-CoV-2 IgM in serum

Rapid and accurate detection of SARS-CoV-2 IgG or SARS-CoV-2 IgM in serum is very valuable for rapid diagnosis and timely treatment of COVID-19 patients [16, 18, 23]. However, the serum complexities are challenging for their immunodetection because the matrix can affect the binding reaction between antibody complexes [29, 30]. Meanwhile, the FRMB, as a label-free biosensor, is naturally sensitive to the RI change. As shown in Fig. S6, the Fresnel reflection light intensity suddenly decreased when the serum was directly introduced over the bio-probe, which mainly contributed to the high RI (1.3444) of the serum (Table S2) and unspecific adsorption. Diluting the serum was a simple and effective method to reduce matrix effect [29, 31]. With increasing the diluted times, their RI was gradually close to the PBS (Table S2), and the decrease value of Fresnel reflection light intensity also reduced (Fig. S6). When the serum was diluted to 30 times, the Fresnel reflection light intensity was similar with that of the PBS (Fig. S6B), indicating that the dilution serum could be directly used for the measurement of SARS-CoV-2 IgM or SARS-CoV-2 IgG. If not specific statement, the serum samples were diluted 30 times to test in the following experiments.

Different concentrations of SARS-CoV-2 IgG or SARS-CoV-2 IgM were added into human serum, respectively. They were sequentially introduced over the fiber bio-probe surface modified by goat anti-SARS-CoV-2 IgM antibody or goat anti-SARS-CoV-2 IgG antibody, respectively. The typical detection signal curves of SARS-CoV-2 IgM or SARS-CoV-2 IgG were demonstrated in Fig. 5A and B, respectively. The Fresnel reflection light intensity did not decrease when the serum without addition of SARS-CoV-2 IgM or SARS-CoV-2 IgG was pumped into the cell, indicating that the original serum did not contain SARS-CoV-2 IgM or SARS-CoV-2 IgG. With increasing the concentration of SARS-CoV-2 IgM or SARS-CoV-2 IgG, the Fresnel reflection light intensity decreased. Figure 5C and D displayed the dose-response curves of SARS-CoV-2 IgM or SARS-CoV-2 IgG, in which the error bars were less than 3.45%, indicating the good stability of FRMB for the detection of SARS-CoV-2

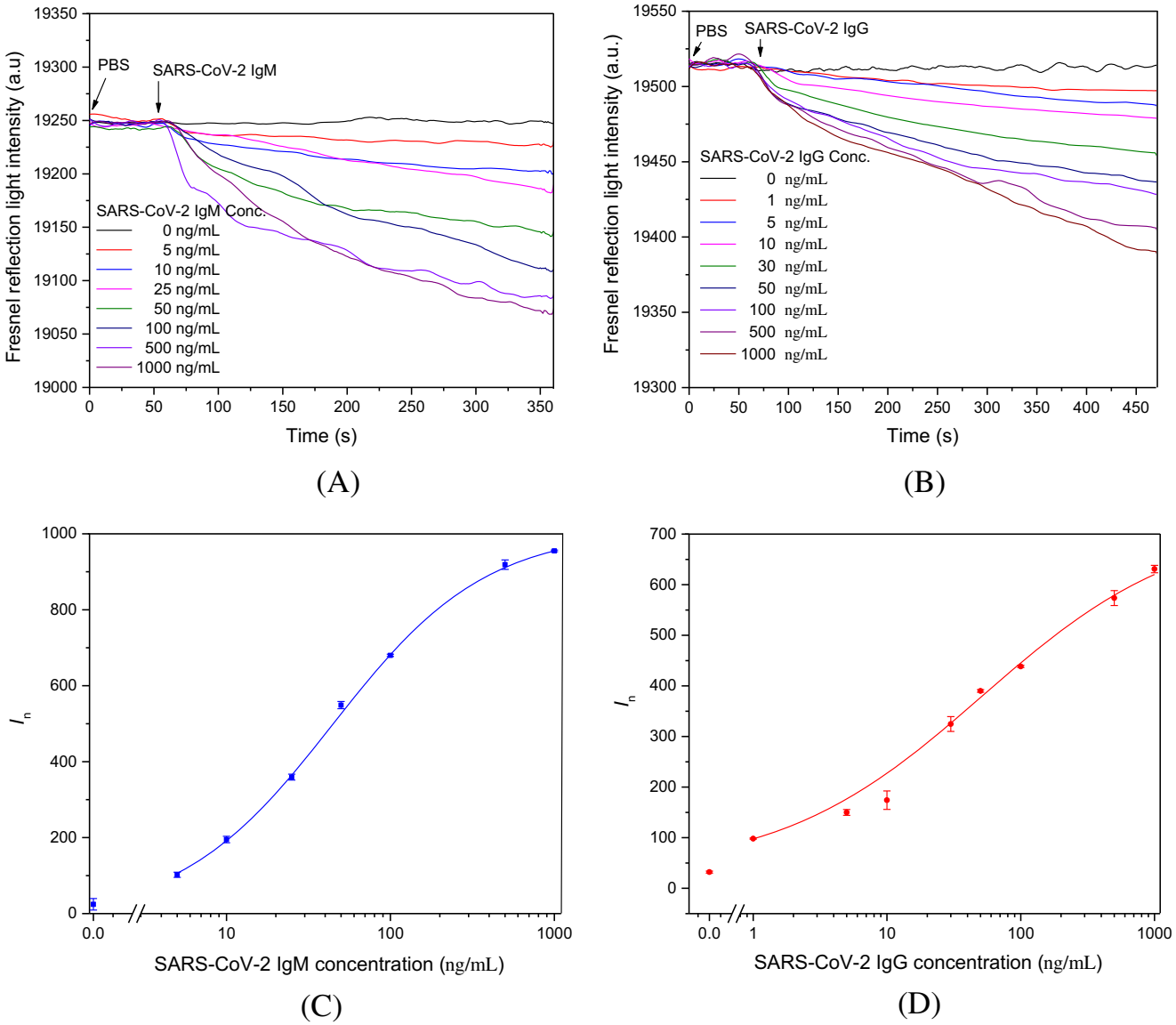


Fig. 5 Detection of SARS-CoV-2 IgG or SARS-CoV-2 IgM in serum. **A** Representative signal curves of SARS-CoV-2 IgM in diluted serum. **B** Representative signal curves of SARS-CoV-2 IgG in diluted serum. **C** Dose-response curve of SARS-CoV-2 IgM in diluted serum (reaction time was 300 s). **D** Dose-response curve of SARS-CoV-2 IgG in diluted serum (reaction time was 400 s). Error bars corresponded to SD of data points in triple experiments

IgM or SARS-CoV-2 IgG in the diluted serum. Using three times standard deviation of the mean blank values, the LODs of SARS-CoV-2 IgM and SARS-CoV-2 IgG were 0.82 ± 0.035 ng/mL and 0.45 ± 0.029 ng/mL, respectively. The linear response of SARS-CoV-2 IgM and SARS-CoV-2 IgG ranged from 5.2 to 266.3 ng/mL and from 3.2 to 350.8 ng/mL, respectively. These were similar with those in the PBS solution except for SARS-CoV-2 IgG having a wider detection range, which might contribute to the effect of serum matrix. Due to the serum was diluted 30 times, the actual LODs of SARS-CoV-2 IgM and SARS-CoV-2 IgG in real serum samples should be 24.6 ng/mL and 13.5 ng/mL, respectively. The studies demonstrated that the effective IgG concentration in COVID-19 patients' serum ranged between 1.4 and 4200 μg/mL, and the concentration of the neutralization antibodies should be higher than 1.0 μg/mL to ensure the effective protection [28, 32, 33]. Therefore, the FRMB is sensitive enough for serological testing for COVID-19 even for using 30 times dilution.

Analysis of real serum samples using the PRMB

To demonstrate the clinical application of the FRMB, 6 negative serum samples, spiked by various concentrations of SARS-CoV-2 IgM and SARS-CoV-2 IgG, were measured by the FRMB. Table S3 and S4 demonstrated that the SARS-CoV-2 IgM and SARS-CoV-2 IgG antibodies recoveries in the spiked serums were in the range of 80.6~119.7%

and 80.2~120.1%, and the relative standard deviations were less than 9.5% and 9.6%, respectively. These results indicated that the proposed method was capable of detecting SARS-CoV-2 IgG and SARS-CoV-2 IgM antibodies in serum samples, and the interference from real samples could be reduced by a simple dilution. Compared with ELISA or other biosensors (Table S5), the FRMB was simpler, easy to use, and time-saving (less than 7 min to obtain the results). Although lateral flow assays are well established, low cost, and easy to use for POCT, most of them only qualitatively detect IgG or IgM. The FRMB is label-free, and no nanomaterials (e.g., colloidal gold nanoparticle) is required. Using the FRMB, the SARS-CoV-2 IgG and SARS-CoV-2 IgM detection could be performed only directly immersing the bio-probe into the samples. Furthermore, in the present system, the bio-probe might be regenerated without comprising bioreactivity for multiple immunoassays, thus ensuring cost-effectiveness and the accurate results.

Conclusions

A novel FRMB system was successfully constructed through integrating all-fiber optical system, microfluidic chip, and multimode fiber bio-probe. In this system, the use of SMFC not only greatly simplified the optical structure of the FRMB and allowed it suitable for the label-free POCT of targets in nanoliter samples, but also greatly increased transmission efficiency of the incident light and reflection light, thus improving its sensitivity. Based on Fresnel reflection mechanism and immunoassay principle, both the SARS-CoV-2 IgG and IgM antibodies were rapidly and sensitively quantified in 7 min. The antibodies-modified biosensing surface allowed multiple immunoassays without comprising significant bioactivity. Using simple dilution method, the FRMB could perform one-step, accurate, label-free, and in situ/on-site detection of SARS-CoV-2 IgG or IgM antibodies in serum without matrix effect. Moreover, the proposed FRMB was capable of measuring the affinity constants of antibody interaction with high sensitivity and high specificity at low concentration in the ng/mL grade. We believe that this novel platform will pave the way for rapid screening of previous SARS-CoV-2 infections and identifying people recovered from COVID-19 to reengage with the society. The FRMB can be readily extended as a universal platform for label-free, sensitive, and in situ/on-site measurement of other biomarkers and the analysis of biomolecular interaction for which specific biorecognition molecules (e.g., antibody, aptamer) are available. However, further efforts need to be made to achieve high-through testing capacity and higher sensitivity through combining micro-/nanotechnologies.

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Declarations

Conflict of interest The author(s) declare that they have no competing interests.

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