Rapid detection of SARS-CoV-2 nucleocapsid protein using dual-comb biosensing

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Testing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is essential along with vaccination and inactivation to fight against the coronavirus disease 2019 (COVID-19) pandemic. Reverse-transcription polymerase chain reaction (RT-PCR), based on reverse transcription of RNA into DNA and amplification of specific DNA targets, is the current standard for COVID-19 testing; however, it hampers from laborious and time-consuming multiple steps. If the testing is largely simplified and shortened, it will be a powerful deterrent to the spread of COVID-19. Here we demonstrate the optical biosensing based on optical frequency comb (OFC), enabling the rapid detection of SARS-CoV-2 nucleocapsid protein. The virus-concentration-dependent optical spectral shift caused by antigen-antibody interaction and multimode-interference fiber sensor is transformed into a photonic radio-frequency (RF) shift by coherent frequency link between optical and RF regions in OFC, benefiting from high precision, rapid, simple, and low cost in electric frequency measurements. Furthermore, the active-dummy compensation of temperature drift with dual-comb configuration extracts the imperceptible change of virus-concentration-dependent signal from the large background signal that changes moment by moment. Such the dual-comb biosensing has a potential to reduce the testing time down of COVID-19 to a few tens minute, which is one order of magnitude shorter than that of RT-PCR (typically, 5 hours). Furthermore, it will be applied for sensitive sensing of not
only virus of emerging and re-emerging infectious diseases but also RNA, bio-marker, and endocrine disruptor by selecting the surface modification of biomolecule interaction.
Introduction

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread in no time and is still going around all over the world. One reason not to suppress the rapid spread is in time-consuming testing of COVID-19. The current standard for COVID-19 testing is reverse transcription polymerase chain reaction (RT-PCR) [1-3], benefiting from sensitive detection of SARS-CoV-2 by selective amplification of nucleic acid sequence of target gene. However, it hampers from time-consuming multiple steps which involve purification, nucleic acid amplification, and fluorescence detection. To avoid overwhelming hospitals, there is a considerable need for rapid detection of SARS-CoV-2.

Optical biosensor [4,5] is a potential method for rapid detection of SARS-CoV-2 due to their sensitivity, cost-effectiveness, versatility, ease of testing, point-of-care (POC) prospects, and more importantly no need for nucleic acid amplification. For example, surface plasmon resonance (SPR) [6,7] has been widely used for analyzing bio-molecules and virus, in which the spectral shift of SPR dip is measured in wavelength or angular spectrum. Due to high quality kinetics, real-time data acquisition, label-free analysis, faster automated experiments, and lower sample consumption, SPR has been applied for the detection of human immunodeficiency virus [8], Ebola virus [9], norovirus [10], influenza virus [11], and even SARS-CoV-2 [12-14]. However, the relatively broad SPR dip and/or the instrumentational resolution
often spoil the precision of virus detection, hampering from the further enhancement of sensing performance.

If the virus-concentration-dependent optical spectral shift is transformed into a photonic radio-frequency (RF) signal in combination with a sharpened spectrum, the optical biosensing benefits from high precision, high functionality, convenience, and low cost by making use of frequency standards and precise measurement apparatuses in the electric RF region. Recently, an optical frequency comb (OFC) \([15-18]\) has attracted attention for a photonic RF sensor via coherent frequency link between optical and RF regions \([19,20]\). For example, a refractive-index-dependent optical spectrum shift was converted into a change of an OFC mode spacing \(f_{rep}\) around several tens MHz by placing a multimode-interference (MMI) refractive-index (RI) fiber sensor \([21,22]\) inside a fiber OFC cavity \([23-25]\). Due to the ultra-narrow linewidth of the \(f_{rep}\) signal, this RI-sensing OFC enables us to precisely measure the sample RI with a resolution of \(4.9 \times 10^{-6}\) refractive index units (RIU). The RI-sensing OFC has a potential to be further extended for optical biosensing, namely biosensing OFC, by surface modification of biomolecule interaction in the MMI fiber sensor similar to SPR. However, the residual temperature drift of \(f_{rep}\) signal hampers from high precision in the RI-sensing OFC and its extension to the biosensing OFC. In this article, we first suppress the temperature drift of \(f_{rep}\) signal by using dual-comb configuration of an active-sensing OFC and a dummy-sensing OFC in the same manner as the active-dummy temperature compensation of strain sensors \([26]\). Then, for proof of
concept, we apply the active-dummy dual RI-sensing OFCs for RI sensing of a glycerol solution. Finally, we demonstrate the rapid detection of SARS-CoV-2 nucleocapsid protein (N protein) antigen by combining the active-dummy dual RI-sensing OFCs with the surface modification of SARS-CoV-2 N protein antibody, namely dual-comb biosensing.

Results

Principle of operation

Figure 1a shows the principle of operation for the biosensing OFC. Function of the biosensing OFC is implemented by three steps: (1) antigen-antibody interaction in the antibody-modified sensor surface, (2) RI-dependent tunable bandpass filtering in MMI fiber sensor [21,22,27], and (3) photonic-to-RF conversion via wavelength dispersion of a cavity fiber [23-25]. In (1), the selective combination of a target antigen with the antibody changes the effective RI on the sensor surface depending on the antigen concentration. In (2), since the MMI fiber sensor functions as an RI-dependent tunable optical bandpass filter (bandpass center wavelength = $\lambda_{\text{MMI}}$) via the multimode interference and the Goos-Hänchen shift, the OFC shows RI-dependent and hence antigen-concentration-dependent shift of optical spectrum. In (3), the antigen-concentration-dependent shift of optical spectrum is converted into that of optical cavity length $nL$, where $n$ is RI of the cavity fiber and $L$ is the physical length of OFC cavity, via the wavelength dispersion of the cavity fiber. Finally, a change in the antigen
concentration can be read out as \( f_{\text{rep}} \) shift via \( f_{\text{rep}} = c/nL \), where \( c \) is a velocity of light in vacuum.

Figure 1b shows a schematic drawing of the dual-comb biosensing. Mechanical sharing dual-comb configuration [28] of an active biosensing OFC (center optical wavelength = 1550 nm, repetition frequency = \( f_{\text{rep}} = 29.38 \) MHz) and a dummy biosensing OFC (center optical wavelength = 1550 nm, repetition frequency = \( f_{\text{rep}} = 29.47 \) MHz) was adopted for the active-dummy compensation of the temperature drift. In this configuration, although \( f_{\text{rep}} \) and \( f_{\text{rep}} \) fluctuate depending on the cavity temperature via thermal expansion or shrink of \( nL \), their fluctuations were common to each other due to the same thermal disturbance. Therefore, frequency difference between \( f_{\text{rep}} \) and \( f_{\text{rep}} \) (\( = \Delta f_{\text{rep}} = f_{\text{rep}} - f_{\text{rep}} = -85.2 \) kHz or -88.6 kHz) remains steady regardless of temperature drift in \( f_{\text{rep}} \) and \( f_{\text{rep}} \). If the active biosensing OFC measures a target antigen solution under a certain temperature condition while the dummy biosensing OFC measures a reference material under the same temperature condition, \( \Delta f_{\text{rep}} \) reflects the antigen concentration without the influence of temperature drift. In other words, one-to-one correspondence is established between \( \Delta f_{\text{rep}} \) and the antigen concentration independent of temperature drift. A photonic RF signal of the dual-comb biosensing was detected by a pair of photodetectors (PDs) and was measured by a RF frequency counter synchronized to a rubidium frequency standard. Details of the dual-comb biosensing are given in the Methods section together with details on the experimental and analytical methodology employed for the following measurements.
Active-dummy compensation of temperature drift in RI-sensing OFC

We first evaluated the dependence of $f_{rep1}$ on the temperature in the active RI-sensing OFC. To this end, we measured the temporal fluctuation of $f_{rep1}$ when the cavity temperature of the active RI-sensing OFC was not controlled. A pure water was used for a sample and was put into a glass sample cell together with the MMI fiber sensor. Figure 2a shows the $f_{rep1}$ shift ($\Delta f_{rep1}$) in the active RI-sensing OFC when the cavity temperature changed within a range of 1 ºC. $\Delta f_{rep1}$ was calculated as the frequency deviation from the initial value of the measurement. The temporal behavior of $\Delta f_{rep1}$ indicated a temperature sensitivity of about 400 Hz/ºC. To suppress the $\Delta f_{rep1}$ fluctuation below 1 Hz, it is required to stabilize the cavity temperature within 0.0025 ºC. Although the cavity temperature was actively controlled in the following experiments (see the Methods section), such the temperature stability is technically challenging. For an alternative method, we applied the dual-comb configuration for the active-dummy compensation of temperature drift. Green and blue lines in Fig. 2b shows a shift of $f_{rep1}$ and $f_{rep2}$, namely $\Delta f_{rep1}$ and $\Delta f_{rep2}$, with respect to the elapsed time when a pure water was used for a sample for both active and dummy RI-sensing OFCs. The $\Delta f_{rep1}$ and $\Delta f_{rep2}$ suffers from the temperature drift over -38.2 Hz due to the decreased cavity temperature; however, their behaviors were almost common to each other. The resulting $\Delta f_{rep}$ shift ($\Delta \Delta f_{rep}$) was stable within a range of 1.18 Hz as shown by a red line in Fig. 2b, being equivalent to the temperature stability within a range of 0.0030 ºC.
We next adopted the active-dummy temperature compensation for the RI sensing of a liquid sample. We used glycerol solution with different mixture ratios between glycerin and pure water (= 0 vol%, 1 vol%, 2 vol%, 3 vol%, 4 vol%, and 5 vol%, corresponding to 1.3334 RIU, 1.3350 RIU, 1.3366 RIU, 1.3382 RIU, 1.3398 RIU, and 1.3414 RIU) for a target sample in the active RI-sensing OFC. We exchanged the sample by using a peristaltic pump. On the other hand, a pure water (0-vol% glycerol solution, corresponding to 1.3334 RIU) was used for a reference sample in the dummy RI-sensing OFC. To avoid the temperature increase of the pure water in the sample cell during the measurement, the pure water was exchanged into another pure water by another peristaltic pump when the target sample was exchanged into the different RI glycerol solution. Blue and green lines in Fig.3a show sensorgrams of $\delta f_{rep1}$ and $\delta f_{rep2}$ when the concentration of the glycerol solution was increased from 0 vol% to 5 vol% while the pure water was exchanged into another pure water at the same timing. $\delta f_{rep2}$ in the dummy OFC indicated a slow drift with some jagged fluctuations, which are caused by disturbance of the water flow at the timing of the sample exchange. On the other hand, $\delta f_{rep1}$ in the active OFC indicated the combined behavior of the step-like change of the sample RI and the slow drift. Such the combined behavior in $\delta f_{rep1}$ spoils the RI sensing performance of the single RI-sensing OFC in the previous studies [23-25]. Figure 3b shows a sensorgram of $\delta f_{rep}$, which is calculated by subtracting the green line ($\delta f_{rep2}$) from the blue line ($\delta f_{rep1}$) in Fig. 3a. The temperature drift almost disappeared, and only the step-like change of the sample RI was confirmed in $\delta f_{rep}$. 
We calculated the mean and the standard deviation of $\delta f_{\text{rep}}$ at each concentration: 0.76±0.19 Hz at 0 vol%, -7.58±0.24 Hz at 1 vol%, -16.48±0.52 Hz at 2 vol%, -25.64±0.53 Hz at 3 vol%, -34.59±0.31 Hz at 4 vol%, and -43.12±0.34 Hz at 5 vol%.

Red plots of Fig. 3c shows a relation between the sample RI and $\delta f_{\text{rep}}$. A negative linear relationship was confirmed between them with a slope coefficient of -5470 Hz/RIU, corresponding to RI sensitivity. For comparison, we also investigated a relation between the sample RI and $\delta f_{\text{rep1}}$ as shown by the blue plot of Fig. 3c. A linear relationship was confirmed between them with a RI sensitivity of -9906 Hz/RIU; however, it was significantly shifted from that in $\delta f_{\text{rep}}$ due to the temperature drift of $f_{\text{rep1}}$. Importantly, the RI sensitivity of $\delta f_{\text{rep1}}$ fluctuates from moment to moment depending on the temperature fluctuation because the behavior of temperature drift also fluctuates from moment to moment (see and compare blue lines between Figs. 2a and 2b), spoiling the precision of RI-sensing OFC. In contrast, one-to-one correspondence between the sample RI and $\delta f_{\text{rep}}$ is always maintained independently of the temperature fluctuation. The resulting enhanced RI precision enables us to apply this dual RI-sensing OFCs for rapid detection of SARS-CoV-2.

**Rapid detection of SARS-CoV-2**

The antigen-antibody interaction of virus protein was adopted for the detection of SARS-CoV-2 with the dual-comb biosensing. We selected N protein for the antigen-antibody interaction because of abundant existence, low mutation-introducing probability, high specificity, high sensitivity, and high stability. We formed a
self-assembled monolayer (SAM) on the surface of the MMI fiber sensor and then immobilized the monoclonal antibody of N protein on the SAM for the active biosensing OFC. On the other hand, the SAM without immobilizing the antibody was made on the surface of the MMI fiber sensor in the dummy biosensing OFC. Those MMI fiber sensors were placed together into the same sample cell (see Fig. 1b), and solution samples of N protein antigen and phosphate-buffered saline (PBS) with different molar concentrations were poured consecutively into the sample cell by the peristaltic pump. The antigen-antibody interaction of N protein occurs on only the sensor surface of the active biosensing OFC.

Figure 4a shows sensorgrams of $\Delta f_{\text{rep}1}$ and $\Delta f_{\text{rep}2}$ when the molar concentration of the antigen/PBS solution was increased from pure PBS (purple zone) to 1 aM (blue zone), 1 fM (green zone), 1 pM (yellow zone), and 1 nM (red zone). In gray zones, we performed the following three steps: (1) the introduction of the antigen/PBS solution into the sample cell by the peristaltic pump (1.5 min), (2) the waiting time for the antigen-antibody interaction (5 min), and (3) the PBS rinse of the sensor surface to flush the N protein antigen without the antigen-antibody interaction. In the behavior of $\Delta f_{\text{rep}1}$, the temperature drift completely spoils the step-like change caused by the change of antigen concentration. On the other hand, as the temperature behavior of $\Delta f_{\text{rep}2}$ indicated the temperature drift similar to $\Delta f_{\text{rep}1}$, we calculated the frequency difference ($\Delta \Delta f_{\text{rep}}$) between them. Figure 4b shows a sensorgram of $\Delta \Delta f_{\text{rep}}$. The step-like change of $\Delta \Delta f_{\text{rep}}$ was observed depending on the molar concentration.
although a little drift of $\Delta f_{\text{rep}}$ was still remained within a range of a few Hz. From this sensorgram, we calculated a relation between the molar concentration and $\Delta f_{\text{rep}}$ as shown by red plots in Fig. 4c. Roughly speaking, the negative slope is valid considering the RI dependence of $\Delta f_{\text{rep}}$ (see red plots of Fig. 3c) because the progression of antigen-antibody reaction increases the effective RI in the MMI fiber sensor. Strictly speaking, since the antigen-antibody reaction in biosensors is represented by the sigmoidal behavior, the sigmoidal fit to the experimental data is a good indicator to evaluate a potential of the dual-comb biosensing for sensing of SARS-CoV-2 antigen. The purple line in Fig. 4c represents the sigmoidal fit of the experimental data using the Logistic function. The correlation coefficient ($R^2$) of 0.9988 was obtained within a range of 1 aM to 1nM in the curve fitting analysis, thus testifying the validity of the proposed method. Importantly, the sensitivity close to that of RT-PCR has been achieved in the rapid measurement.

**Discussion**

The residual drift of $\Delta f_{\text{rep}}$ in Fig. 4b hampers the more sensitive measurement of SARS-CoV-2 N protein antigen. We first discuss a possibility to further suppress the residual drift of $\Delta f_{\text{rep}}$. Although the mechanical sharing dual-comb configuration enables the effective active-dummy temperature compensation, temporal behaviors of $f_{\text{rep}1}$ and $f_{\text{rep}2}$ in Fig. 2b is not exactly common to each other due to use of two different fiber cavities, leading to the residual drift of $\Delta f_{\text{rep}}$. Recently,
single-cavity dual-comb fiber lasers based on multiplexing mode-locked oscillation in wavelength [29,30], polarization [31,32], or propagation direction [33,34] have attracted attentions for a light source of dual-comb spectroscopy. Since these fiber lasers achieve dual-comb oscillation in the same single cavity, the resulting fluctuation of $\Delta f_{\text{rep}}$ can be reduced below 1 Hz. Although installation of the MMI fiber sensor in the single-cavity dual-comb fiber laser is technically challenging, the combination of them will enhance the sensing performance of dual-comb biosensing for SARS-CoV-2 N protein antigen.

Assuming that the residual drift of $\Delta f_{\text{rep}}$ is well suppressed, we next discuss a possibility of further enhancement in the biosensing sensitivity while securing the sufficient compensation of temperature drift. Since the dual-comb biosensing is based on the frequency measurement of $\Delta f_{\text{rep}}$ signal, one potential method to increase the sensitivity is a frequency multiplication of $\Delta f_{\text{rep}}$ signal. For example, when a $m$-order harmonic component of $f_{\text{rep}1}$ and $f_{\text{rep}2}$ (freq. = $mf_{\text{rep}1}$ and $mf_{\text{rep}2}$) is measured by using a faster photodetector, a frequency difference between them ($= mf_{\text{rep}1} - mf_{\text{rep}2} = m\Delta f_{\text{rep}}$) is corresponding to a $m$-fold frequency-multiplied $\Delta f_{\text{rep}}$ signal while securing the active-dummy temperature compensation. Unfortunately, as a frequency response of fast photodetectors is typically within a range of several GHz, a frequency multiplication factor $m$ is remained around 100. To further increase $m$, a combination of dual-comb biosensing with a dual-THz-comb spectroscopy [35,36] will be used (see Figs. 5a and 5b). When an active biosensing OFC (mode spacing = $f_{\text{rep}1}$) is incident onto a
photoconductive antenna (PCA1) for generation of THz comb, a m-order harmonic component of $f_{rep1}$ radiates from PCA1 and propagates in free space as an m-order frequency mode (freq. = $mf_{rep1}$) of an electromagnetic-wave THz comb (EM-THz comb). On the other hand, when a dummy biosensing OFC (mode spacing = $f_{rep2}$) is incident onto another photoconductive antenna (PCA2) for detection of THz comb, a m-order harmonic component of $f_{rep2}$ is generated in PCA2 as an m-order frequency mode (freq. = $mf_{rep2}$) of a photocarrier THz comb (PC-THz comb) [37]. Multifrequency-heterodyning photoconductive mixing between EM-THz and PC-THz combs in PCA2 results in generation of a secondary comb in RF region, namely RF comb (mode spacing = $\Delta f_{rep}$). A beat signal between $mf_{rep1}$ and $mf_{rep2}$ (freq. = $mf_{rep1} - mf_{rep2} = m\Delta f_{rep}$) has a frequency multiplication factor $m$ over 10,000 under the active-dummy temperature compensation. In other words, dual THz combs function as a higher-order frequency multiplier of $f_{rep1}$, $f_{rep2}$, and $\Delta f_{rep}$. Work is in progress to implement the frequency multiplication function in dual-comb biosensing.

In summary, we have demonstrated the rapid detection of SARS-CoV-2 N protein antigen by using dual-comb biosensing. The mechanically sharing dual-comb configuration was successfully installed in the RI-sensing OFC to suppress the temperature drift of $f_{rep}$ signal based on the active-dummy temperature compensation. Use of $\Delta f_{rep}$, in place of $f_{rep}$, in RI-sensing OFC enables us to reduce the temperature drift down to 1/33 and opens the door to the biosensing application by combining with the antigen-antibody interaction. The N protein antigen of SARS-CoV-2 was detected
at a molar concentration down to aM level by immobilizing the monoclonal antibody of N protein in the active biosensing OFC. The required measurement time was significantly shorter than that in RT-PCR. The proposed dual-comb biosensing will greatly enhance the applicability of optical biosensors for virus, bacteria, protein, biomarker, environmental hormone, and so on.
Methods

Dual-comb configuration of active and dummy biosensing OFCs

We used a pair of linear fiber cavities mode-locked by saturable absorption for the active biosensing OFC and the dummy biosensing OFC in dual-comb configuration (see Fig. 1b). Each linear cavity (total dispersion = -0.10 pm/s²) includes a 2.6 m length of single-mode fiber (SMF, SMF-28, Corning, dispersion at 1550 nm = 17 ps•km⁻¹•nm⁻¹), a 0.6 m length of erbium-doped fiber (EDF, ER30-4/125, LIEKKI, dispersion at 1550 nm = -15 ps•km⁻¹•nm⁻¹), a saturable absorbed mirror (SAM-1550-55-2ps-1.3b-0, BATOP, high reflection band = 1480-1640 nm, absorbance = 55 %, modulation depth = 2.4%, relaxation time constant ~2 ps, size = 1.3-mm width, 1.3-mm height, 0.4-mm thickness), a wavelength-division-multiplexing coupler (WDM, WDM-1-9855-N-B-1-F, AFR), a pumping laser diode (pumping LD, BL976-PAG900, Thorlabs, wavelength = 976 nm, power = 900 mW), a 90:10 fiber coupler (OC, PMOFM-55-2-B-Q-F-90, AFR), and an intracavity MMI fiber sensor (MMI). Sharing of the same pumping LD for those two cavities cancels the influence of power fluctuation in the pumping LD. The fiber cavity was enclosed in an aluminum box, and its temperature was controlled to 25.0 °C by a combination of a Peltier heater (TEC1-12708, Kaito Denshi, power = 76 W), a thermistor (PB7-42H-K1, Yamaki), and a temperature controller (TED200C, Thorlabs, PID control) (not shown in Fig. 1b). We set frequency spacing of the active and dummy sensing OFCs around 29.4~29.5 MHz by adjusting their cavity length; the resulting $\Delta f_{\text{rep}}$ was -85.2 kHz in RI sensing and -
88.6 kHz in biosensing. We adopted mechanically sharing of those two fiber cavities to implement equivalent environmental temperature disturbance to them [28]. The output light from dual OFCs was detected by a pair of photodetectors (PDs, PDA05CF2, Thorlabs, wavelength = 800~1700 nm, frequency bandwidth = 150 MHz), and the resulting frequency signals of \( f_{\text{rep}1} \) and \( f_{\text{rep}2} \) were measured by an RF frequency counter (53230A, Keysight Technologies, frequency resolution = 12 digit\( \cdot \)s\(^{-1}\)), which was synchronized to a rubidium frequency standard (FS725, Stanford Research Systems, accuracy = 5 \times 10^{-11} \) and instability = 2 \times 10^{-11} at 1s).

**MMI fiber sensor**

Supplementary Figure S1 shows a schematic diagram of the intracavity MMI fiber sensor with the surface modification of antibody on its surface. The MMI fiber sensor was composed of a clad-less MMF (FG125LA, Thorlabs, core diameter= 125 \( \mu \)m, fiber length = 58.94 mm) with a pair of SMFs at both ends (core diameter=6\( \mu \)m, clad diameter=125\( \mu \)m, fiber length = 150 mm) [23-25]. The OFC light passing through the input SMF is diffracted at the entrance face of the clad-less MMF, and then repeats total internal reflection at the boundary between the clad-less MMF surface and the sample solution. Only the OFC modes satisfying the multi-mode interference wavelength \( \lambda_{\text{MMI}} \) can exit through the clad-less MMF and then goes toward the output SMF. \( \lambda_{\text{MMI}} \) is given by

\[
\lambda_{\text{MMI}} = \frac{n_{\text{MMF}}^m}{L_{\text{MMF}}} \left\{ D(n_{\text{sam}}) \right\}^2,
\]

(1)
where $L_{MMF}$ and $n_{MMF}$ is the geometrical length and the refractive index of the clad-less MMF, $m$ is the order of MMI, $n_{sam}$ is the sample RI, and $D(n_{sam})$ is the effective diameter of the clad-less MMF. Since $D(n_{sam})$ is influenced by the Goos-Hänchen shift on the side of the clad-less MMF, $\lambda_{MMI}$ is a function of sample RI. The intracavity MMI fiber sensor in this study functions as a RI-dependent optical bandpass filter tunable around $\lambda_{MMI}$ ($= 1556.6$ nm) by setting the constructive interference with $m = 4$. Such RI-dependent $\lambda_{MMI}$ shift of the OFC is converted into the RI-dependent $f_{rep}$ shift via the wavelength dispersion of the cavity fiber (see Fig. 1a) [23].

**Antibody modification on MMI fiber sensor**

The SARS-CoV-2 antibody modification on the MMI fiber sensor enables the optical biosensor for the specific detection of SARS-CoV-2 associated with antibody-antigen reactions because the RI-dependent $f_{rep}$ shift is converted into the virus-concentration-dependent $f_{rep}$ shift (see Fig. 1a). We briefly describe a chemical modification method of N protein antibody of SARS-CoV-2 on the sensor surface. First, the surface of the clad-less MMF was cleaned and organics on it was removed by UV ozone. Second, amino-terminated group was modified on the surface of the clad-less MMF by silane coupling reaction using 1%(v/v) of 3-aminopropyltriethoxysilane (APTS) in ethanol for 1 hour, following washed by ultra-pure water and dried at 110 °C for 10 min. Third, the monoclonal antibody of N protein was immobilized on amino-group-coated MMF by dehydration-condensation reaction using 10 mM of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) in PBS buffer
(pH 7.4).

**Antibody and antigen of SARS-CoV-2 N protein**

N protein is a promising candidate for the antigen-antibody interaction of SARS-CoV-2 because of abundant existence, low mutation-introducing probability, high specificity, high sensitivity, and high stability. To select suitable N protein antibody from commercialized products, we evaluate the affinity of commercialized antibodies (HM1064, EastCoast Bio; HM1054, EastCoast Bio; FPZ0553, Fapon; FPZ0548, Fapon; NB100-56576, Novus Biologicals) to commercialized antigens (LA600, EastCoast Bio; FPZ0513, Fapon; FPZ0514, Fapon; FPZ0517, Fapon) for SARS-CoV-2 N protein by enzyme-linked immunosorbent assay (ELISA). We selected the combination of the N protein antibody FPZ0553 and the N protein recombinant antigen FPZ0513 because this combination indicated high affinity in ELISA as shown by Supplementary Fig. S2, indicating a relation between concentration of FPZ0513 and optical density at 450 nm in ELISA. We made the surface modification of FPZ0553 on the MMI fiber sensor whereas we prepared FPZ0513/PBS solutions with different molar concentrations as the samples.

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**Contributions**

Tak.Yas. conceived the project. S.M., R.O., and T. N. developed the dual-comb biosensing system, performed the experiments and analyzed the data. T.K., S.T., Y.T. and S.O. made the MMI fiber biosensor. K.O. and A.S performed the ELISA experiment. K.Y., T.S., T.M., T.K., S.T., and Tak.Yan. discussed the results and commented on the manuscript. S.M. and Tak.Yas. wrote the manuscript. All authors reviewed the manuscript.

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**Competing interests**

The authors declare no competing interests.
Figure 1. Dual-comb biosensing. a, Principle of operation for biosensing OFC. Concentration of target antigen is read out as a mode spacing $f_{rep}$ of OFC by antigen-antibody interaction in the antibody-modified surface of MMI fiber sensor, RI-dependent tunable bandpass filtering in MMI fiber sensor, and photonic-to-RF conversion via wavelength dispersion of a cavity fiber. b, Experimental setup for dual-comb biosensing. Mechanically sharing of an active biosensing OFC cavity and a dummy biosensing OFC cavity implements equivalent environmental temperature disturbance to them, enabling the active-dummy compensation of the temperature drift with $\Delta f_{rep}$. LD, laser diode; SMF, single-mode fiber; OFC, optical frequency comb; sensor, intracavity multimode-interference fiber sensor; WDM, wavelength-division-multiplexing coupler; OCs, fiber output coupler; PDs, photodiodes.
Figure 2. Basic performance of dual RI-sensing OFCs. a, Dependence of $f_{\text{rep}1}$ on the temperature in the active RI-sensing OFC. Pure water was used as a sample. Temperature sensitivity of the active RI-sensing OFC was about 400 Hz/ºC. b, Temporal fluctuation of $\delta f_{\text{rep}1}$, $\delta f_{\text{rep}2}$, and $\delta \Delta f_{\text{rep}}$ when the pure water was used as a sample of the active and the dummy RI-sensing OFCs. $\delta \Delta f_{\text{rep}}$ was passively stable because fluctuations of $\delta f_{\text{rep}1}$ and $\delta f_{\text{rep}2}$ were almost common to each other. $\delta f_{\text{rep}1}$, $\delta f_{\text{rep}2}$, and $\delta \Delta f_{\text{rep}}$ were respectively calculated as the frequency deviation from the initial value of $f_{\text{rep}1}$, $f_{\text{rep}2}$, and $\Delta f_{\text{rep}}$. 
Figure 3. RI-sensing of dual RI sensing of glycerol solution. a, Sensorgrams of $\delta f_{rep1}$ in the active RI-sensing OFC and $\delta f_{rep2}$ in the dummy one. Glycerol solution with different mixture ratios between glycerin and pure water was used for a target sample in the active RI-sensing OFC; a pure water was used for a reference sample in the dummy RI-sensing OFC. $\delta f_{rep1}$ indicated the combined behavior of the sample RI change and the slow drift while $\delta f_{rep2}$ indicated a slow drift. b, Sensorgram of $\delta f_{rep}$ with respect to different mixture ratios between glycerin and pure water. The step-like change of the sample RI was confirmed without influence of temperature drift. c, Relation between the sample RI and $\delta f_{rep}$ or $\delta f_{rep1}$. 
Figure 4. Biosensing of SARS-CoV-2 N protein antigen. a, Sensorgrams of $\Delta f_{rep1}$ and $\Delta f_{rep2}$ with respect to different molar concentrations of SARS-CoV-2 N protein antigen. $f_{rep1}$ was measured by the active biosensing OFC equipping SAM with immobilizing the antibody whereas $f_{rep2}$ was measured by the dummy one equipping SAM without immobilizing the antibody. The step-like change of antigen concentration was completely spoiled by the background temperature drift in $\Delta f_{rep1}$. b, Sensorgram of $\Delta f_{rep}$ with respect to different molar concentrations of SARS-CoV-2 N protein antigen. The step-like change of the antigen concentration was confirmed without influence of temperature drift. c, Relation between the antigen molar concentration and $\Delta f_{rep}$. Experimental plots matches well with the curve fitting analysis of sigmoidal curve.
Figure 5. Sensitivity enhancement based on frequency multiplication function in dual THz combs. a, Experimental setup for $\Delta f_{\text{rep}}$ multiplier using dual-THz-comb. OFC, optical frequency comb; PCA1, photoconductive antenna for THz generation; PCA2, photoconductive antenna for THz detection; PC-THz comb, photocarrier THz comb; EM-THz comb, electromagnetic-wave THz comb; AMP, current preamplifier. b, Spectral behavior of frequency comb in optical, THz, and RF region.
Supplementary Figure S1. Schematic diagram of biosensing MMI fiber sensor.

The antigen-antibody interaction in the surface of MMI fiber sensor converts the antigen concentration change into the effective RI change around the sensor surface while the MMI fiber sensor functions as an RI-dependent tunable bandpass filter via the MMI process. The combination of the antigen-antibody interaction with the MMI fiber sensor causes the antigen-concentration-dependent shift of optical spectrum.
Supplementary Figure S2. Affinity test between a commercialized antibody (FPZ0553, Fapon) and a commercialized antigen (FPZ0513, Fapon) for SARS-CoV-2 N protein by ELISA.