Detection of influenza viruses with the waveguide mode sensor

— Development of a palmtop sized sensor—

Koichi Awazu*, Makoto Fujimaki, Subash C. B. Gopinath and Xiaomin Wang

We developed a highly sensitive sensor, based on optical waveguide modes, which was reported in the journal, *Synthesiology*.[1] The first part of the present paper reports the method for reducing sensor size. Applications include identification of influenza virus A H3N2 and other subtypes of influenza viruses. We also found that sialic acid based detection using the waveguide mode sensor system analysis was very useful in distinguishing between H3N2 and H5N1 viruses. Using these techniques, H3N2 and H5N1 strains of influenza viruses have been successfully identified with the waveguide-mode sensor. Sensitivity comparison was also conducted for waveguide-mode sensor, immuno-chromatography, enzyme-linked immunosorbent assay (ELISA), and surface plasmon resonance (SPR). Of these techniques, waveguide mode sensor showed the greatest sensitivity for the H3N2 Udorn strain. The palmtop sized, high sensitive sensor will be useful in border control against intrusion of infections, for example, in aircraft, at airports, and arenas.

Keywords : Bio sensor, waveguide mode, influenza virus, palmtop sensor

1 Introduction

Humankind faced three influenza pandemics in the 20th Century and has already experienced the first one in the 21st Century. The pandemics of the 20th Century include the Spanish flu of 1918, the Asian flu of 1957, and the Hong Kong flu of 1968. The 2009 flu pandemic is still fresh in our minds. Influenza is a communicable disease that must be reported to the authorities.[Note 1)] The Spanish flu developed into a pandemic in seven months, and although estimates of the number of those infected and those who died vary widely, of the affected world population of 1.8 billion people, 1 billion people were infected and 80 million died. In Japan, after the first patient was reported, the disease spread throughout the nation in three weeks, and 42 % of the Japanese citizens were infected and 450 thousand people died. The Spanish flu came to a close in 1920, and economists consider it as the remote cause of the Great Depression that started in 1929.[2] Regarding the 2009 flu that started in the spring of 2009, according to the Center for Disease Control and Prevention (CDC), about 270 thousand people were admitted to the hospital by October 2010 throughout the United States, and 12,000 people died.[3] The reason there were fewer deaths compared to the past pandemics was because the virus was hypovirulent.[Note 2)] It is reported that in total, 20.68 million people were infected in Japan.[4]

Why does such pandemic influenza appear periodically? First, there are three types of influenza: types A, B, and C. Type A has 144 subtypes according to the combinations of 16 types of hemagglutinin (HA) and nine types of neuraminidase (NA) with different antigenicity. In a new strains of influenza, mutation occurs in the HA and/or NA. Names such as type H1N1 influenza are given according to the type of HA and NA. In a new strain of influenza, new types of HA or NA appear among humans, and the antigenic drift and antigenic shift occur, or the influenza genes pass on from birds to humans. In past fact of research, it was found that the Spanish flu was caused by a hypovirulent H1N1 type derived from the avian flu.

There are three reasons why a new strain of influenza is serious. (1) Since a new strain never existed before, no one has immunity. (2) It is highly infectious. It is thought that only an influenza virus that developed the ability for airborne infection causes a pandemic. (3) Since it is an unknown virus, vaccines cannot be made beforehand. Also, as an outstanding characteristic of the Spanish flu, young people developed serious conditions such as systemic infection, multiple organ failure, or a cytokine storm, which is an excessive vital reaction to viral infection. For example, the peak deaths of the Spanish flu occurred in people of ages 24-29. In Japan, there were newspaper headlines that said, "Most productive people were first to fall victims." The patients spread amongst the most productive people that supported society, and they died and paralyzed the social functions. According to the estimates by the Ministry of Health, Labour and Welfare,[5] if a pandemic equivalent to the strength of the Spanish flu (2.0 % fatality) occurred, 640 thousand people may die. However, since a mutant virus of subtype H5N1 has 60 % fatality rate, there are estimates that greater damage

Electronics and Photonics Research Institute, AIST Tsukuba Central 5, 1-1-1 Higashi, Tsukuba 305-8565, Japan
∗ E-mail : k.awazu@aist.go.jp

Table 1. Number of deaths by avian flu virus

As of June 27, 2014[7][8]

	Number of deaths / Number of infected people
H5N1	393/667
H7N9	118/393

may occur.[6]-[8]

Influenza is a serious problem for animals as well as humans. When infection of poultry or cattle by avian flu virus is confirmed, the current measure is to conduct extensive culling and disposal of farm animals according to the Animal Infectious Diseases Control Law. For example, in 2010, a subtype H5N1 was detected in a poultry farm of Shimane Prefecture, and 21,549 birds were culled.[9] In addition to culling, inspection of the poultry farms in the 10-kilometer radius range, epidemic prevention work, and traffic blocks of the roads to poultry houses are needed, hence generating enormous economic damage. Therefore, if the source of the avian flu is found immediately, and the disease can be contained with minimum culling, wide-range measures do not have to be taken. It also becomes possible to prevent the development of a pandemic, and the economic effect is large. With this lesson in mind, when a highly pathogenic avian flu broke out in a poultry farm of Kumamoto Prefecture on April 13, 2014 (later this flu was determined to be H5N8), the spread of damage was kept to a minimum due to prompt early response.

The issues for types H5N1 and H7N9 are totally different even if they are similar avian flus that are currently at issue. Type H5N1 is hypervirulent and causes fatal infections to many birds including chickens, but the infection from birds to human is thought to be rare. In contrast, H7N9 is hypovirulent, and no symptoms occur in infected birds, and therefore, monitoring of birds' temperature is meaningless. However, although hypovirulent, infection from birds to humans does occur, and people develop serious conditions because humans have no immunity.

Type H5N1 and H7N9 avian flus are no longer problems of animals only. Both viruses can be transmitted from animals to humans. Moreover, as shown in Table 1, the fatality is extremely high. There is also concern for a complication of the problem. By frequent infection of humans, there is the possibility that the flu virus undergoes better adaptive mutations and the infection may spread from human to human efficiently. At this point, both viruses are thought to be transmitted from chickens to humans, and then the humans develop symptoms. However, no one can predict when the situation may change and an epidemic may occur among humans.

2 Benchmark

Normally, for the identification of pathogenic microorganisms, the identification of genes or antigenic proteins is necessary. When the polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR) are used for gene identification, there is a high possibility of producing false positive results due to the aerosol contamination that may be caused by a leakage of just one positive sample. Although dramatic progresses have been made in the last few years in the digital PCR and the real-time PCR such as qRT-PCR and qPCR that are gene amplification methods in sealed tubes, the possibility of aerosol contamination of the PCR products cannot be denied. Once this occurs, PCR false positive response becomes normal. The manufacturers' guidebooks spend several pages on the methods to avoid contamination, and recommend the preliminary and post treatments to be done in separate rooms, and to prepare exclusive reagent sets, pipettes, and disposable equipment for each room.[10] The PCR analysis method works well only when done by skilled personnel in a well-equipped research facility, but is not suitable for detection and identification in ordinary facilities such as general clinics, airport quarantine stations, meeting halls, or schools. In case of the avian flu virus, detection must be done in the field such as poultry houses, poultry markets, or slaughtering facilities, but PCR is not effective in such environments. If PCR is conducted in the field, even if the negative control is negative in the first test, the possibility of contamination by PCR products increases dramatically in the second measurement at the same place. Another disadvantage is that time is needed for detection and the procedure is complicated. In contrast, the detection by antibodies is a simple method with short detection time and does not require special procedures or gene amplification, although the detection sensitivity does not come close to the genetic test. There is almost no possibility of contamination of the antigenic proteins, because there is no process of amplification of the antigenic proteins.

The immuno-chromatography is the most common method for testing using antibodies. Because it has high National Health Insurance (NHI) points, the immuno-chromatography is widely used for diagnosing types A and B influenza. However, in a recent study in 2009, Hurt *et al.* conducted a sensitivity test using three types of immuno-chromatography and five types of influenza A H1N1 and H3N2.[11] As a result, it was found that the detection limits differed by test kits and target viruses, and the sensitivity was 10^3~10^5 TCID 50/mL. Yamaguchi *et al.* conducted sensitivity comparison using the virus positive control attached to the immuno-chromatography.[12] As a result, in the case of high infectivity titer of 0.7-1.4 × 10^3 TCID 50/mL, the hit rate was relatively high at 98.8 % for skilled clinical lab technicians and 85.4 % for lab personnel (assistant, nurse, or nursing school faculty). However, in the case of low infectivity titer 3.5-7.0 × 10^2 TCID 50/mL, the hit rate was extremely low at 60.7 % for

skilled clinical lab technicians and 43.8 % for lab personnel. Baccam *et al.* reported that the A/Hong Kong/123/77H1N1 strain sampled by nose smear reached the maximum value in two to three days after infection, and the value was about 5×10^2-1×10^3 TCID 50/mL nasal wash.[13] Considering the above three studies, it can be seen that the detection with good precision cannot be obtained by immuno-chromatography unless the virus count reached the maximum after infection.

According to the National Institute of Infectious Diseases, the false positive (true negative) by immuno-chromatography method is seen most frequently in the early stages of an outbreak when the influenza is not yet common in the community, as well as in the final stages.[14] Also, the false negative (true positive) by immuno-chromatography is seen commonly during an outbreak when the influenza is rampant in the community.

The situation is similar for avian flu, and the Ministry of Environment reported that the problem of immuno-chromatography was that infected birds might show negative.[15] For example, the manual presents a case where of the 60 birds that were positive in the definitive test, only 27 were tested positive in the simplified test. Conversely, there were cases where the birds were positive in the simplified test but negative in the definitive test. As a reason, it is explained that the virus in the sample became inactive due to drying or not being kept in low temperature during the period when the virus was taken to the lab and became subject to the definitive test of virus isolation. This means a technology for highly sensitive and quick detection at several digits higher than the current method is needed.

3 Developed content

3.1 Development of portable waveguide mode sensor

We have been developing our original waveguide mode sensors.[16][17] As reported in *Synthesiology*,[1] in the earliest waveguide mode sensor, the waveguide modes were formed in the reflective film layer and the waveguide layer at a certain angle of incidence. When the angle sweep is done using the He-Ne laser at a wavelength of 632.8 nm as the light source, the reflection intensity declines at a certain angle. When the surface condition changes such as by the adsorption of molecules on the surface, it is possible to measure the accompanying changes in the refraction index of the surface. However, in this case, it was necessary to conduct the sweep by synchronizing the two goniometers on the light source and detector sides as shown in the optical system in Fig. 1, and there was a problem that the optical system itself became large and complex. Therefore, we thought perhaps we could not satisfy the user demand for a potable sensor using the angle sweep type. Rather than sweeping the angle, we devised a method for sweeping the wavelength. If a spectrum is obtained by sweeping the wavelength, it would be possible to discard the two goniometers that were barriers to size reduction.[18] Figure 2 shows the simulation results of reflectivity changes before and after adsorption, assuming the adsorption of protein with 5 nm diameter (refractive index 1.45). The calculation was done with monocrystalline silicon layer thickness of 220 nm, silica waveguide layer thickness of 360 nm, prism material of silica, and at s-polarization. Several peaks were observed. For example, there was a peak in the range where the reflectivity change reached maximum at around 500 nm and 68°. From this calculation result, it was found that the target substance could be detected by fixing the angle of incidence and observing the reflection spectrum of the incoming light.
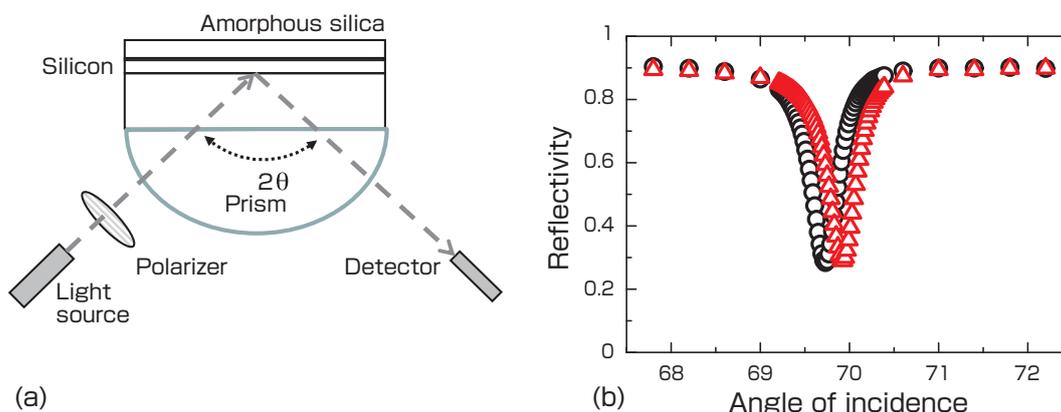

(a)

(b)

Fig. 1 (a) Optical arrangement of the angle sweeping waveguide mode sensor. Monocrystalline silicon (c-Si) film was laminated onto a SiO$_2$ glass substrate beneath the prism. The c-Si was thermally oxidized to form an amorphous SiO$_2$ (a-Si) film, and waveguide modes were formed inside this SiO$_2$ film. The reflection intensity was observed to change rapidly at a certain angle 2θ. When the molecule adhered to the amorphous silica surface, the value of 2θ changed. The angle 2θ synchronized the two goniometers.
(b) Conceptual image of the molecules adsorbing onto the amorphous silica surface in the optical system (a). The horizontal axis became 2θ. ○ is before the molecules were adsorbed and △ is after they were adsorbed.

Since it was possible to measure using the waveguide modes by sweeping the wavelength, we designed an optical system as shown in Fig. 3. The excitation light reached the back of the measuring site as white light, and the reflected light passed through the collimator lens and optical fiber and reached the spectrometer. The four-point measurement was possible at the measuring site using four light sources. The two goniometers for sweeping while synchronizing became unnecessary in this optical system, and this enabled size reduction.

Figure 4 is a detailed diagram of the measuring site of Fig. 3 during measurement. The detection plate that detected the surface reaction was a SiO_2 glass substrate on which monocrystalline silicon (c-Si) film was formed, and it was obtained by thermally oxidizing silicon to obtain amorphous silica (a-SiO_2). The mechanism was to sensitively detect the surface reaction by designing the thicknesses of c-Si film and a-SiO_2 film so it would be possible to obtain the maximum reflectivity change against the refraction index change on this

a-SiO_2 layer surface. Figure 5 is the calculation of electric field intensity distribution near the detection plate surface at a wavelength of 512 nm, silica waveguide layer thickness of 284 nm, and monocrystalline silicon layer thickness of 220 nm. It can be seen that a strong electric field was formed on the waveguide layer surface, and this enabled highly sensitive detection. Therefore, it was possible to capture the refraction index change of the surface upon which the antibodies were fixed and then were made to react with the virus. Also, it was possible to amplify the signal with gold nanoparticles, as explained later.

The process of size reduction is shown in Fig. 6. First, it was an angle-sweeping type where the optical system was set on a two stage plate at 1 m × 50 cm as shown in (a). Later, since it was possible to conduct measurement at waveguide mode by sweeping the wavelength as shown in Figs. 2 and 3, the optical system as shown in Fig. 3 was designed. The excitation light reached the back of the measuring site as white light, and the reflected light passed
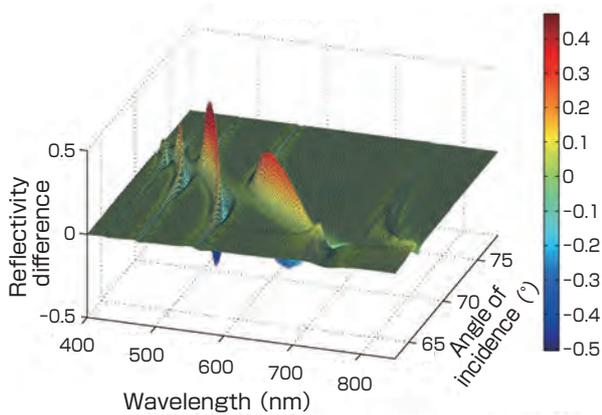

Fig. 2 Calculation results of the dependence of reflectivity change on angle and wavelength
The quantity of reflectivity change is shown in color. The peak of reflectivity change was observed when the wavelength was fixed and the angle was swept, but it can also be seen that the peak of reflectivity change was observed when the angle was fixed and the wavelength was swept.
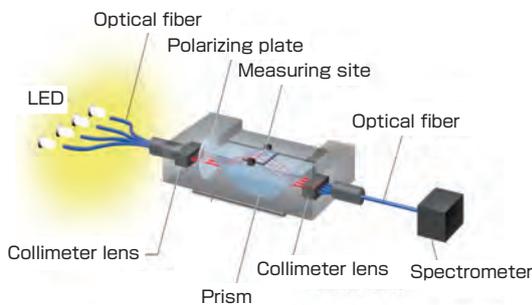

Fig. 4 Optical arrangement of the waveguide mode sensor
Configuration of the SiO_2 glass substrate on a prism and the detection plate was the same as Fig. 1. The outgoing light was dispersed by the spectrometer.

Fig. 3 Wavelength sweeping waveguide mode sensor
White LED was used as the light source. The measuring plate was installed in the measurement site, and the reaction on the measuring plate was detected by reflected light. The reflected light could be led to the spectrometer by installing the optical fiber optical axis.
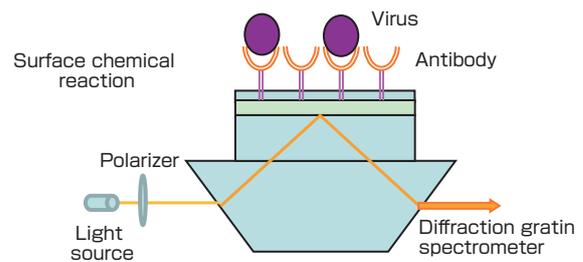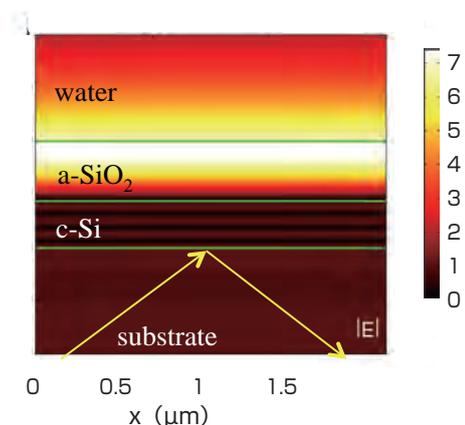

Fig. 5 Electric field intensity distribution of waveguide modes at 220 nm c-Si layer, 284 nm SiO_2 layer, and 512 nm excitation wavelength
Similarly to Fig. 1, the yellow arrow is the direction of incoming and outgoing light. The film thickness and others were designed so the electric field intensity reached the maximum at the SiO_2 layer surface. The scale bar on the side indicates electric field intensity.

through the collimator lens and optical fiber and reached the spectrometer. The two goniometers for sweeping while synchronizing became unnecessary in this optical system, and size reduction (30 cm × 15 cm × 20 cm) as shown in Fig. 6(b) was attained. This device was realized as a product by a company to which the technology was transferred. This device was equipped with a PC, Bluetooth communication, and a battery. We worked on further size reduction of the optical system, and achieved a 7 cm × 5 cm × 15 cm device as shown in (c).

3.2 Identification of influenza subtypes

The identification of influenza subtypes was attempted using the waveguide mode sensor.[20] The polyclonal antibodies were obtained by immunizing healthy rabbits with H3N2 A/Udorn/307/197. These polyclonal antibodies were fixed onto the detection plate surface. After decomposing the four

types of H3N2 influenza virus with surfactant Triton X-100, these were reacted with the polyclonal antibodies fixed on the detection plate surface. Then, it was highly sensitized using a label in which the polyclonal antibodies were fixed around the gold nanoparticles. The virus used in measurement was 1 μg. Figure 7 shows the results, and the dashed line shows the reaction with the virus alone, while the solid line shows the reaction of antibodies highly sensitized with gold nanoparticles. The virus types were: (a) A/Shandong/9/1993, (b) A/Brisbane/10/2007, (c) A/Panama/2007/1999, and (d) A/Wisconsin/67/2005. Changes in spectrum reflectivity were confirmed in all types, and although there were differences in reactivity, it was found that H3N2 bonded with the antibodies of the same H3N2. The error was within 20 % for reflectivity changes.

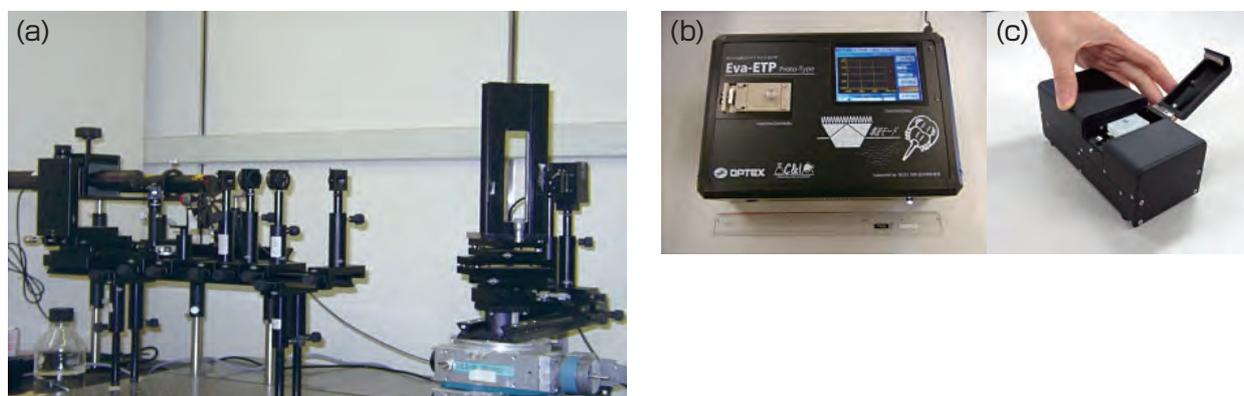Next, the anti-H3 antibodies were fixed on the surface and

Fig. 6 Process of size reduction of the wavelength mode sensor
(a) Device number one. The optical system was set on a two-stage plate of 1 m × 50 cm. (b) A small device (30 cm × 15 cm × 20 cm) was commercialized as a product from a company to which the technology was transferred. This device was equipped with a PC, Bluetooth communication, and a battery. Further size reduction was conducted, and as shown in (c), device size of 7 cm × 5 cm × 15 cm was achieved.[19]
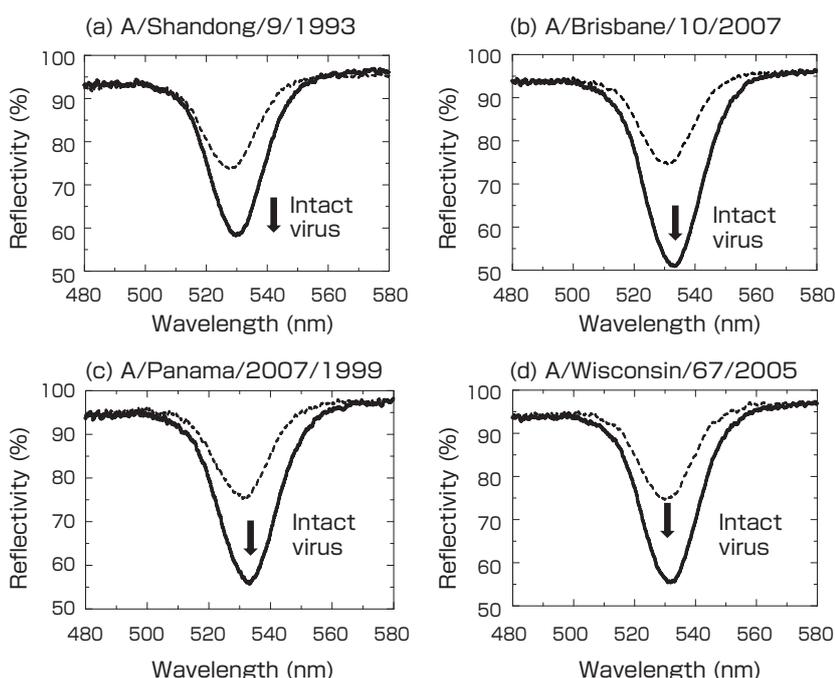

Fig. 7 Results of detection for various types of H3N2 virus using the detection plate to which the H3N2 antibodies were fixed
The subtypes were (a) A/Shandong/9/1993, (b) A/Brisbane/10/2007, (c) A/Panama/2007/1999, and (d) A/Wisconsin/67/2005.

the virus subtypes other than H3N2 were used as detection targets. Since the handling of the H5N1 virus was difficult in Japan, HA proteins were used for all samples. The results are shown in Fig. 8. The dashed line shows the reaction with the virus alone, while the solid line shows the reaction of antibodies highly sensitized with gold nanoparticles. The virus used in the measurement was 1 μg. Figures 8 (a), (b), (c), and (d) show the results of the HA detection test for A/Wisconsin/67/2005(H3N2), A/chicken/India/ NIV33487/2006(H5N1), A/California/07/2009(H1N1), and A/Japan/305/1957, respectively. For H3N2, as shown in (a), spectrum change was seen just for the HA. However, no spectrum change was detected in the HAs of other sub-species. This means that the HAs of different sub-species did not bond with the fixed antibodies. Therefore, when the detection plate onto which the H3N2 antibodies were fixed was used, the H3N2 virus could be detected while other sub-species could not be detected, and it was possible to identify the sub-species using the waveguide mode sensor.

We shall introduce another identification method for the influenza virus subtypes.[21] The schematic diagram of the various reactions and the experiment results are shown in Figs. 9 and 10. The detection targets were the virus particles of human influenza type A H3N2, and the HA of avian influenza H5N1. To identify each virus, two types of gold nanoparticles coated with 2,6-sialic acid and 2,3-sialic acid were used as labels. High sensitivity was achieved by using such gold nanoparticles as labels. The HA protein of the human influenza H3N2 virus reacted with the 2,6-sialic acid on the surface of gold nanoparticles, and the spectrum changed [Fig. 9(a) and (c)], while there was no spectrum change since it did not react with the 2,3-sialic acid on the

Table 2. Comparison of the detection limit concentration of the waveguide mode sensor, immuno-chromatography, and ELISA, using the H3N2 Udorn strain

Method	H3N2 pfu/ml
Waveguide mode sensor	800
Immuno-chromatography	8×10^4
ELISA	2×10^3

gold nanoparticle surface [Fig. 9(b) and (d)]. In contrast, the HA protein of avian influenza H5N1 did not react with the 2,6-sialic acid on the gold nanoparticle surface and the spectrum did not change [Fig. 10(a) and (c)], while it reacted with the 2,3-sialic acid on the gold nanoparticle surface and the spectrum changed [Fig. 10(b) and (d)]. Hence, simple identification of the HAs of human influenza H3N2 and avian influenza H5N1 viruses became possible.

Next, type A influenza virus H3N2 Udorn was used for the detection sensitivity test using viruses. Here, comparison was conducted using the plaque forming unit (pfu), which is the index of infectivity, and the results are summarized in Table 2. In the detection test using the waveguide mode sensor, the virus and the antibodies labeled with gold nanoparticles were mixed, and left for 10 min. The mixture was dropped onto the sensor, and signals 30 min later were measured. The data indicated that the detection limit of the waveguide mode sensor would be 8×10^2 pfu/ml. For immuno-chromatography, the detection limit was 8×10^4 pfu/ml using the same sample. The H3N2 virus detection limit using the Sandwich ELISA method was 2×10^3 pfu/ml.
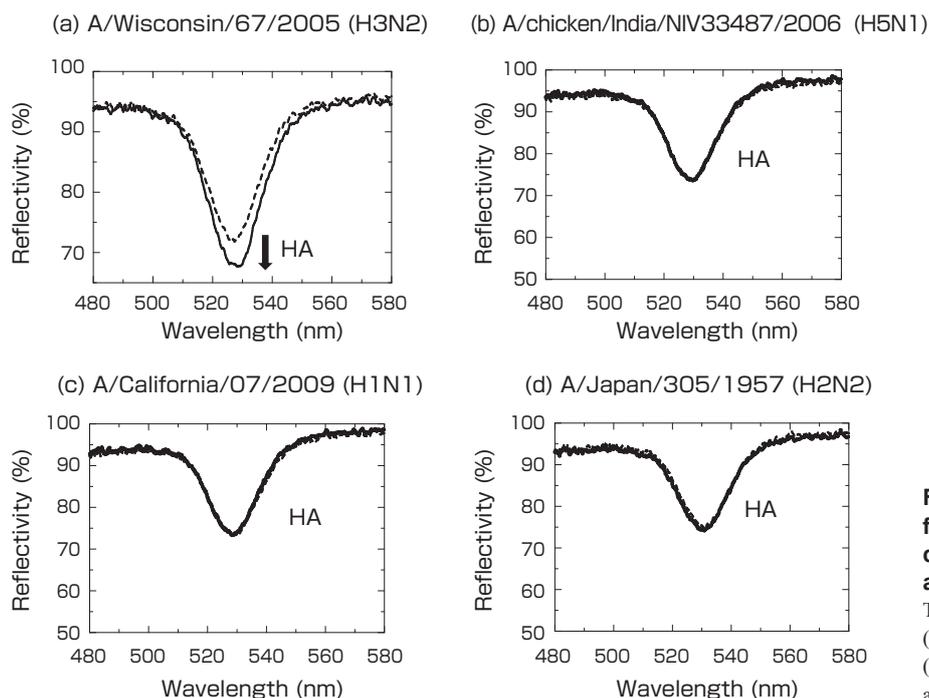

Fig. 8 Results of the HA detection for various subtypes using the detection plate to which the H3N2 antibodies were fixed
The subtypes were (a) A/Wisconsin/67/2005 (H3N2), (b) A/chicken/India/NIV33487/2006 (H5N1), (c) A/California/07/2009 (H1N1), and (d) A/Japan/305/1957 (H2N2).

From the results of the above quantitative tests, it was found that the waveguide mode sensor was one to two digits higher in sensitivity compared to the immuno-chromatography and direct adsorption ELISA methods. The Sandwich ELISA method was troublesome to conduct since the preliminary treatment was complicated and the procedure took several hours to a day until detection, and also it depended on the skill of the testing personnel. Therefore, the waveguide mode sensor technology is thought to have overwhelming superiority over other methods in terms of simplicity of preliminary treatment and time required.

3.3 Nonspecific adsorption inhibiting surface
The important point in the detection by antigen-antibody reaction is to prevent nonspecific adsorption. Surface formation as shown in Fig. 11 was created in a joint research with the Biomedical Research Institute, AIST.[22] Methoxyoligoethyleneglycol-triethoxysilane was used as the modifying material for the silane surface to inhibit nonspecific adsorption of protein. This is an oligoethyleneglycol group where the terminal has been methylated, and it is capable

of almost completely inhibiting nonspecific adsorption. Succinimide ester-triethoxysilane was used as the silane surface modifying material to fix the antibodies. The succinimide group was present on the surface terminal to which the antibodies were fixed. By soaking the detection plates with these silica surfaces in two types of hybrid solutions, a high level blocking property and an antibody fixing property were achieved. Currently, it has been confirmed that nonspecific adsorption could be efficiently inhibited in the detection of viruses in serum and plasma. It is expected to specifically detect influenza viruses in nose smear samples.

3.4 Synthesiological discussion
Figure 12 shows the synthesiological description of the above process. The "realization of a simple and highly sensitive influenza virus detector" is the demand and the goal. On the other hand, we already have significant accumulation of elemental technologies of silicon technology, optics, and electromagnetics. To achieve an integrated technology, the fusion of the four fields of elemental technologies, where there were hardly any overlaps, was necessary. By conducting
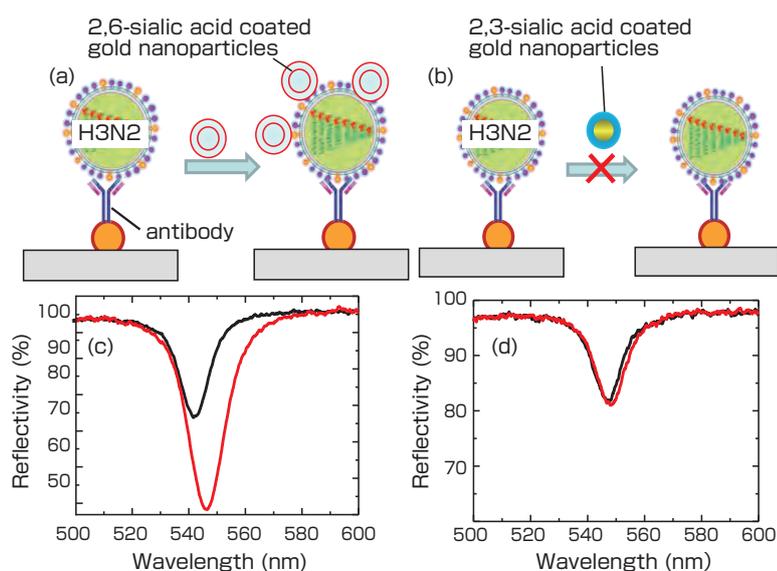

Fig. 9 The H3N2 virus was fixed on the waveguide mode detection plate using antibodies. The gold nanoparticles with diameter of 5 nm were coated with 2,6-sialic acid and 2,3-sialic acid, and were used as labels. High sensitivity was achieved by using such gold nanoparticles. (a) The 2,6-sialic acid on gold nanoparticle surface bonded with the HA protein of H3N2. (b) The 2,3-sialic acid on gold nanoparticle surface did not bond with the HA protein of H3N2. When the reactions of (a) and (b) were actually observed using the waveguide mode sensor, (c) the 2,6-sialic acid on gold nanoparticle surface bonded with the HA protein of H3N2 and the spectrum changed (from black line to red line). (d) The 2,3-sialic acid on gold nanoparticle surface did not bond with the HA protein of H3N2 and the spectrum did not change.
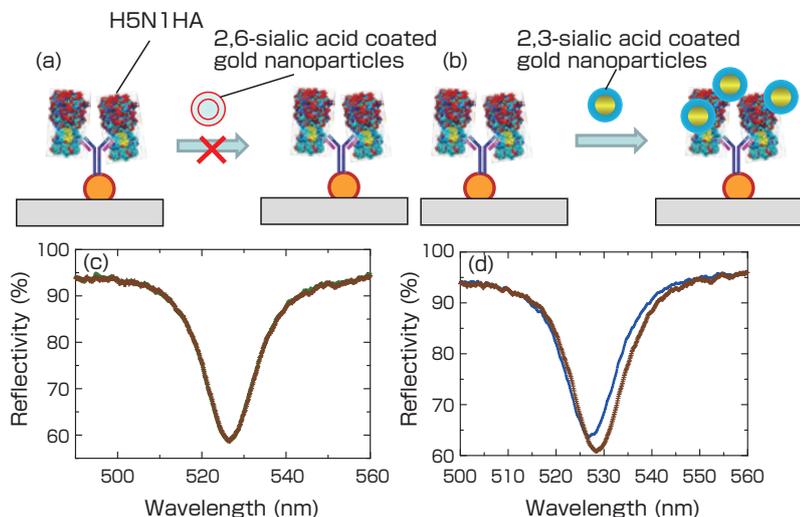

Fig. 10 The H5N1 HA was fixed using antibodies. The gold nanoparticles with diameter of 5 nm were coated with 2,6-sialic acid and 2,3-sialic acid, and were used as labels. (a) The 2,6-sialic acid on gold nanoparticle surface did not bond with the H5N1. (b) The 2,3-sialic acid on gold nanoparticle surface bonded with the H5N1. When the reactions of (a) and (b) were actually observed using the waveguide mode sensor, (c) the 2,6-sialic acid on gold nanoparticle surface did not bond with the H5N1 and the spectrum did not change. (d) The 2,3-sialic acid on gold nanoparticle surface bonded with the H5N1 and the spectrum changed (from red line to brown line).

joint research with the experts of surface chemistry and virology, we succeeded in developing an intermediate integrated technology. Toward our goal, we returned to the elemental technologies several times, optimized the intermediate integrated technologies, and constructed the integrated technology.

4 Process of the fusion of different fields

In this research, the cooperation of the researchers of optical experiment and calculation was necessary to design and fabricate the palmtop sensor. Moreover, we needed cooperation of the companies to realize the design. As the social issue for which the sensor that we fabricated could be used, we received suggestions from an internist for a quick testing method for new strains of influenza. In 2008, the internist commented that if a pandemic of a new strain of influenza occurred, they would be helpless as physicians because the PCR technology at the time took too long and the immuno-chromatography could not determine whether a virus was a new strain. This was one year before the spring of 2009 when a new strain of influenza broke out in Mexico and developed into a pandemic. We believe we can respond

to the urgent social demand for quick, on-site identification of new strains of influenza using this sensor. The most important point in observing the surface reaction was the inhibition of nonspecific adsorption. Although there were no descriptions about inhibiting nonspecific adsorption in most papers, it was an important issue in measuring viruses in samples. This was solved through joint research with the Biomedical Research Institute within AIST. This research was achieved through the collaboration of information and technical elements of the researchers in different fields.

5 Conclusion

To achieve a safe and secure society in the future, we think it is necessary to be able to detect various viruses, not just of influenza. Since there is no boundary for viral infection, it is necessary for each country to regularly and carefully monitor the viruses and stop the virus spread at the border. Although it is said that virus infection and temperature, humidity, and bacteria are related, the whole picture is not yet clear. By managing all data with geographical information, it can be developed into a new business such as IT medicine or IT agriculture. In such a case, rather than selling a sensor as a product, there is a possibility for business of packaging the product with service, information, and maintenance.

In this paper, the content was specifically on the detection of influenza viruses. Currently, we are developing a simplified blood testing device using waveguide mode sensors, and we aim to determine the presence of infectious diseases such as B or C type hepatitis as well as blood types. If this can be achieved, it may be possible to provide blood transfusion at refugee shelters in disaster areas or conduct blood tests in ambulances. Also, since it is a system to observe surface reaction, it can be used in the management of production lines such as in the continuous monitoring for deterioration of plating solutions. Several companies that operate plating plants have already shown interest in this application.
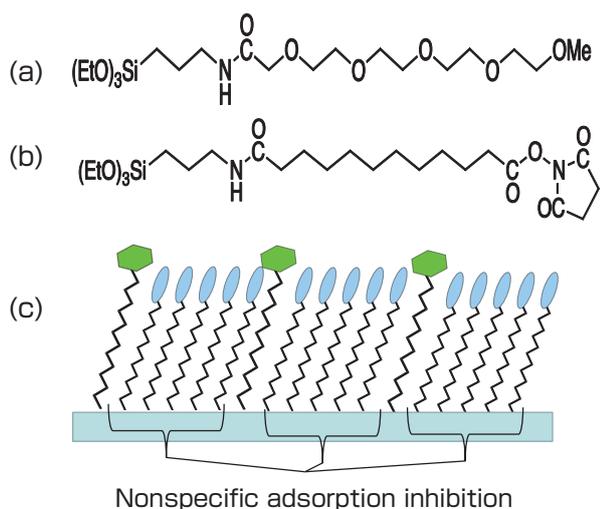
Acknowledgement

Fig. 11 Nonspecific adsorption inhibiting surface
Triethoxysilane was bonded with (a) methoxy oligoethyleneglycol and (b) succinimide ester. The (a) inhibited nonspecific adsorption, and (b) of the succinimide group bonded with the carbonyl group of antibodies.
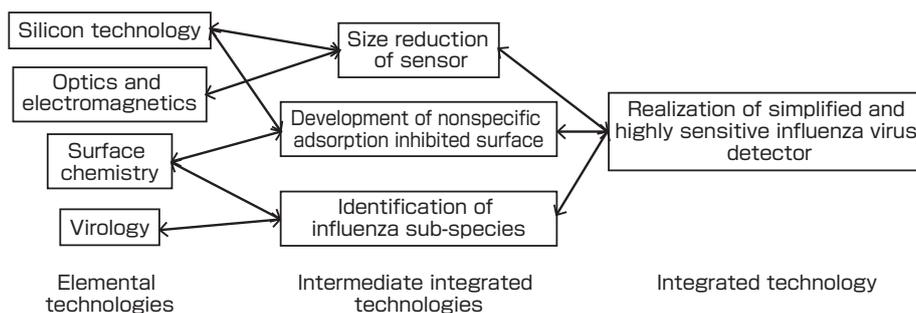

Fig. 12 Synthesiological description

University helped us with the experiments. We received advice on biochemistry and virology from: Associate Professor Kazumichi Kuroda, School of Medicine, Nihon University; and Special Appointment Professor Kazufumi Shimizu, Center for Infectious Disease, School of Medicine, Kobe University.

Notes

Note 1) In Japanese, words for cold and influenza are used interchangeably, but the two are totally different. Influenza is a communicable disease that must be reported to the authority. There are some infectious diseases that must be reported and others that must be reported only at certain designated hospitals. The seasonal influenza falls in the latter category.

Note 2) A clear definition of hypervirulent influenza is difficult. Normally, it is used for avian flu, and one of the conditions is that the protease cleavage site of HA is hypervirulent. In that sense, the Spanish flu is a hypovirulent type.

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Authors

Koichi AWAZU
Completed the doctorate course at Tokyo Institute of Technology in 1991. Doctor (Engineering). Joined the Electrotechnical Laboratory in 1991, and engaged in research of accelerator application engineering. Visiting Researcher, Université de Montréal, Quebéc, Canada in 1996-1998; Senior Researcher, New Energy and Industrial Technology Development Organization (NEDO) in 2001-2002; Visiting Professor, Institute for Molecular Science in 2002-2004; Team Leader, Center for Applied Near-Field Optics Research, AIST in 2003-2010; Visiting Professor, Faculty of Engineering, The University of Tokyo in 2005-2007; Director, Research Planning Office of Information Technology and Electronics, AIST in 2009-2012; and Deputy Director, Electronics and Photonics Research Institute, AIST from 2012. Engages in research for nanophotonics and fused discipline with medicine. In this paper, investigated the observation of interface and surface states.

Makoto FUJIMAKI
Completed the doctorate course at Waseda University in 1998. Doctor (Engineering). Engaged in the research for optical communication devices at Waseda University and Université de Montréal on Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists. Engaged in the development of power electronics devices and optical communication elements at the Electrotechnical Laboratory as Domestic Research Fellow, Japan Science and Technology Corporation. After taking position as Associate Professor of Waseda University, joined AIST in 2004. Chief Planner, Research Planning Office of Information Technology and Electronics, AIST in 2012-2013; and Group Leader, Optical Sensing Group, Electronics and Photonics Research Institute in 2013 to present. Works on the development of biosensing technology using near-field optics. Appointed director of the AIST Technology Transfer Venture, and works on the practical application of technologies developed at AIST. In this paper, engaged mainly on the optical design.

Subash C. B. GOPINATH
Completed the doctorate course at University of Madras, India in 1999. Joined the Academia Sinica, Taiwan in 1999; post-doctoral researcher and technical staff, AIST in 2003-2013; and Associate Professor, Institute of Nano Electronic Engineering, University Malaysia Perlis in 2013 to present. Engages in research of fused disciplines of bioscience and nanotechnology. In this paper, was in charge of overall virus detection.

Xiaomin WANG
Withdrew from the doctorate course at the Department of Electronic Engineering, Graduate School of Engineering, The University of Tokyo in 1999. After working as Visiting Researcher, Telecommunications Advancement Organization of Japan, participated in Femtosecond Technology Research Association in 2000 and engaged in the research for ultra high-speed optical transmission. Engaged in the development of new optical device at the Center for Applied Near-Field Optics Research, AIST in 2003-2010. Transferred to the Nanoelectronics Research Institute, AIST and engaged in the research of new phase-change functional devices. Doctor of Engineering, Waseda University in 2011. In this paper, conducted the numerical calculation of magnetic wave and the optical design for achieving high sensitivity.

Discussions with Reviewers

1 Overall
Comment (Naoto Kobayashi, Center for Research Strategy, Waseda University)

This paper is a report on the R&D of a device that enables simple and quick identification of the influenza virus by detecting the changes in light reflection spectrum using the waveguide mode sensor. It is a detailed description of a process by which an extremely useful method for solving an urgent health and medical issue of society was created. It is an excellent case of innovative creation, and the content is appropriate for *Synthesiology*.

However, since the first draft was a review of the authors' published papers shown in the references, and I suggested restructuring the paper as a description of innovation methodology. As a result, the objective, scenario, elemental technologies, and their syntheses were described in an organized manner, and the paper became appropriate for *Synthesiology*.

Comment (Yasushi Mitsuishi, AIST)

This paper is about the size reduction of a virus detector using the waveguide mode sensor that can sensitively detect the adsorption of nanoparticles, and the company to which the technology was transferred has developed a palmtop device for practical use. This is a paper befitting *Synthesiology*. However, the first draft of the paper spent much space on the presentation of detection data based on the antigen-antibody reaction, and there was very little description about what kind of work was done to reduce the device size or how it was made usable for on-site use. I felt that the goals of the paper were not sufficiently explained.

2 Synthesiological discussion
Comment (Naoto Kobayashi)

In *Synthesiology*, you are required to describe the goal of research, the scenario to achieve the goal, the selection and integration of elements (elemental technologies) to achieve the goal, and the evaluation of the results. Please present a figure that enables ready understanding of the relationship of the above, and describe the relationships of the elemental technologies, intermediate integrated technologies, and the integrated technology (goal) of this research. Also, please describe the general evaluation and future prospects for the R&D method for simplified and quick influenza virus detection.

Answer (Koichi Awazu)

I added Fig. 12 as a synthesiological diagram. The "realization of simple and highly sensitive influenza virus detector" is the demand and the goal. On the other hand, we already have significant accumulation of elemental technologies of silicon technology, optics, and electromagnetics. To achieve an integrated technology, the fusion of the four fields of elemental

technologies, where there were hardly any overlaps, was necessary. By conducting joint research with the experts of surface chemistry and virology, we succeeded in developing the intermediate integrated technologies. Toward our goal, we returned to the elemental technologies several times, optimized the intermediate integrated technologies, and constructed the integrated technology. I added such a description to subchapter 3.4 "Synthesiological discussion." I also added chapter 4 "Process for the fusion of different fields" for the general evaluation and future prospects, to match the objective of *Synthesiology*.

3 Superiority of the performance
Comment (Yasushi Mitsuishi)

Since it is already widely known that the specificity of antigen-antibody reaction is extremely high, in this paper, I think the objective will be achieved if you thoroughly describe the work on sensor design to introduce the wavelength sweeping property without sacrificing the sensitivity of the angle sweeping detector, as well as the implementations done to the palmtop device that will make it appropriate for on-site use. Also, please organize the descriptions so you can clearly convey the superiority of the device that can detect influenza virus subtypes precisely, quickly, with high sensitivity compared to the detection methods using the conventional antigen-antibody reaction.

Answer (Koichi Awazu)

Since the concept of shifting from angle sweep to wavelength sweep is difficult to communicate to the readers, I added Fig. 2 that shows the results of the new calculation. I think this figure will help three-dimensional understanding of waveguide modes that appear in the respective sweeping method. The superiority of this method against the conventional ones is shown in Table 2, with the description of sensitivity and measurement time.

4 Response to the result of this research
Comment (Naoto Kobayashi)

In this research, you have developed an innovative method from the perspective of simplification and quickness, compared to the conventional methods that require a long period of time for preliminary treatment and measurement and yet have low sensitivity. I believe the research results have attracted much attention of persons concerned, but how are their responses? If you have any comments or evaluations from such parties, please mention them.

Answer (Koichi Awazu)

As the social issue for which the sensor that we fabricated would be used, we received suggestions from an internist for a quick testing device for new strains of influenza. In 2008, the internist commented that if a pandemic of a new strain of influenza occurred, they would be helpless as physicians because the PCR technology at the time took too long and the immuno-chromatography could not determine whether the virus was a new strain. I think we can respond to the urgent social demands for quick, on-site identification of new strains of influenza using this sensor. I added this point in chapter 4 "Process of the fusion of different fields."