Chapter 3

Array-Based Spectral SPR Biosensor: Analysis of Mumps Virus Infection

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Summary

Spectral SPR biosensor is a useful system for a rapid analysis of protein arrays, as the biosensor with a fiber optic spectrometer can be easily aligned with the reflected light from protein arrays. The spectral SPR biosensor was constructed by Kretschmann geometry, based on the wavelength interrogation with various modules such as protein arrays, optical unit, programs for data acquisition and processing, and a motorized *x–y* stage for scanning. Protein arrays consist of glass/gold film/linker layer/protein/air. The surface of gold arrays was modified with poly(diallyldimethylammonium chloride) and 11-mercaptoundecanoic acid to immobilize mumps virus. The virus arrays were used to analyze anti-mumps virus in a buffer or human serum by the line-scanning mode of the spectral SPR biosensor. The array-based spectral SPR biosensor has a strong potential for a high-throughput serodiagnosis of infectious diseases.

Key words: Spectral SPR biosensor, Line scanning mode, Protein array, Mumps virus, Serodiagnosis, Resonance wavelength, PDDA.

1. Introduction

Surface plasmon resonance (SPR)-based biosensors have been widely used for biomolecular interactions, as the optical method allows real-time measurement of biomolecular interactions without labeling and the device is simple *(1–3)*. The SPR method is an optical technique that uses the evanescent wave to measure changes in a refractive index on the sensor surface *(4, 5)*. Biomolecular interactions have been intensively investigated by three types of SPR-based biosensors such as angular (angular interrogation-based) SPR biosensors, spectral (wavelength

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interrogation-based) SPR biosensors and surface plasmon microscopy biosensors *(2, 6, 7)*.

Spectral SPR biosensors are based on wavelength interrogation and scan wavelengths at a constant incidence angle to analyze biomolecular interactions *(8)*. Recently, we have demonstrated the possible use of the spectral SPR biosensor in a high-throughput analysis of protein interactions and serodiagnosis in an array format *(9, 10)*. In this chapter, system integration of the spectral SPR biosensor and analysis of protein arrays are introduced. Protein arrays were prepared by immobilizing mumps virus onto the modified surface of gold arrays and analyzed by the line-scanning mode of the spectral SPR biosensor.

2. Materials

2.1. Optical Components

1. 10–100W Series Q quartz tungsten halogen (QTH) Source (#66892) (ORIEL, Stratford, CT) (*see* **Note 1**).
2. Achromatic lenses (#42540, #42570, #42650) (ORIEL, Stratford, CT).
3. Maximum reflection flat mirrors (#44152) (ORIEL, Stratford, CT).
4. Iris diaphragms (#62030, #71400) (ORIEL, Stratford, CT)).
5. Glan-Taylor polarizing prism (#03 PTA 401) (Melles Griot, Carlsbad, CA).
6. Precision beamsteering assembly and StableRod™ horizontal mounting platform with fine height adjustment (#07 BSF 503, #07 BSB 003, #07 DSS 511) (Melles Griot, Carlsbad, CA).
7. Rotation stage (#07 TRT 507) (Melles Griot, Carlsbad, CA).
8. Precision flexure mirror mount (#07 MFM 513) (Melles Griot, Carlsbad, CA).
9. Right angle UV fused silica prism (#46161) (ORIEL, Stratford, CT).
10. Optical table (#10770) (ORIEL, Stratford, CT).

2.2. Fiber Optic Spectrometer

1. Fiber optic spectrometer (Ocean Optics, Dunedin, FL) (*see* **Note 2**).
2. Fiber optic cable with SMA termination for the fiber optic spectrometer (Ocean Optics, Dunedin, FL).
3. ADC2000-PCI+ A/D Converter for PCI-bus (Ocean Optics, Dunedin, FL).

2.3. Motorized Translational Stage

1. Motorized translation stages (40 mm traveling range with a resolution of 20 μm) (#MTS-40) (Cheung Won Mechatronics, Korea) (*see* **Note 3**).
2. PCI-GPIB, NI-488.2 for Windows Me/9x (#778032-01) (National Instruments, Austin, TX).

2.4 .Gold Arrays

1. Two types (metallic and Cr) of masks for preparing gold arrays with 1 or 1.5 mm spots (e.g. 5 × 10, 7 × 16 and etc). Metallic (stainless still) and Cr masks are prepared by a drill machine and the traditional semiconductor technology, respectively.
2. Gold arrays are prepared by depositing Ti/Au (5/45 nm) onto pyrex glasses with the masks by an RF-magnetron sputtering apparatus at a vacuum of 3×10^{-6} Torr (*see* **Note 4**).
3. Cleaning solution of gold arrays: H_2O_2/NH_4OH/ milliQ water (1:1:5, v/v).

2.5. Antigen Arrays

1. Mumps virus (strain 98-10) (the Kangwon National University College of Medicine, Korea): was prepared in VeroE6 cells (American Type Culture Collection, CRL 1585).
2. Poly(diallyldimethylammonium chloride) (PDDA) (Sigma, St. Louis, MO), M_w ~100,000: 1 mg/mL solution in 0.5 M NaCl.
3. poly L-lysine: 1 mg/mL solution in milliQ water.
4. 11-mercaptoundecanoic acid (MUA) (Sigma, St. Louis, MO): 1 mM solution in ethanol.
5. Phosphate-buffered saline (PBS): 8.1 mM Na_2HPO_4, 1.2 mM KH_2PO_4, 2.7 mM KCl and 138 mM NaCl, pH 7.4.
6. 0.1% Tween 20 in PBS.
7. 3.7% formaldehyde in PBS.
8. 0.2% Triton X-100 in PBS.

2.6. Antibodies

1. The hybridoma cell line producing a monoclonal antibody against mumps virus was established by fusion of Sp2/0-Ag14 mouse myeloma cells (ATCC, CRL-1581) with spleen cells of Balb/c mice immunized with mumps virus strain 98–40 *(11)* (*see* **Note 5**).
2. Monoclonal anti-GST (Boditech, Korea).

3. Methods

The spectral SPR biosensor is very useful for a high-throughput analysis of biomolecular interactions and serodiagnosis in

an array format, as the method is simple and label-free. The array-based spectral SPR biosensor consists of various modules such as protein arrays, signal acquisition, motion control, and data processing. The schematic diagram and optical components of the SPR system are shown in **Figs. 1** and **2**, respectively. Gold arrays are modified with octadecyltrichlorosilane to prevent reaction solutions from being mixed between spots during incubation, and protein arrays are prepared by immobilizing mumps virus onto the modified surface of gold arrays *(10)*. To obtain reproducible results, it is essential to perform beam alignment to obtain a parallel and p-polarized light, to determine an incidence angle above the critical angle for the total internal reflection, considering the sensitivity of the system and the dynamic ranges of a spectrometer, and to calculate the resonance wavelength with a polynomial curve fitting technique *(5, 8)*. Protein arrays are analyzed in the line-scanning mode of the spectral SPR biosensor and the obtained results are displayed by a color spectra *(10)*.

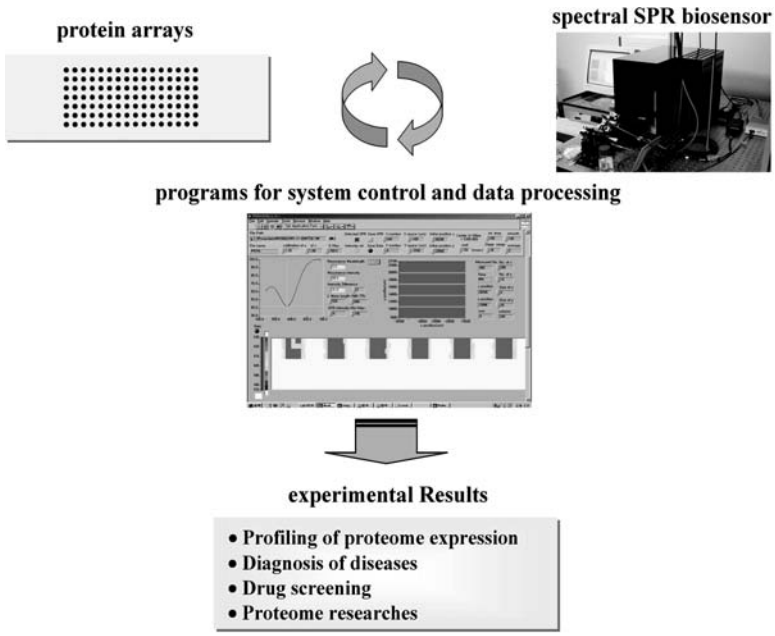

Fig. 1. Schematic diagram of array-based spectral SPR biosensor. Protein arrays consist of various proteins on the gold surface. Collimated white light enters the polarizer and passes through the right angle prism contacted with a thin Au metal (45 nm thick) of protein arrays at the fixed angle of θ_{sp}. The reflected light is collected into a fiber optic spectrometer. Scanning, data acquisition and data processing are performed by self-developed LabVIEW™ software. The array-based spectral SPR biosensor can be used for various biological applications such as profiling of protein expression, diagnosis of diseases and proteome researches.

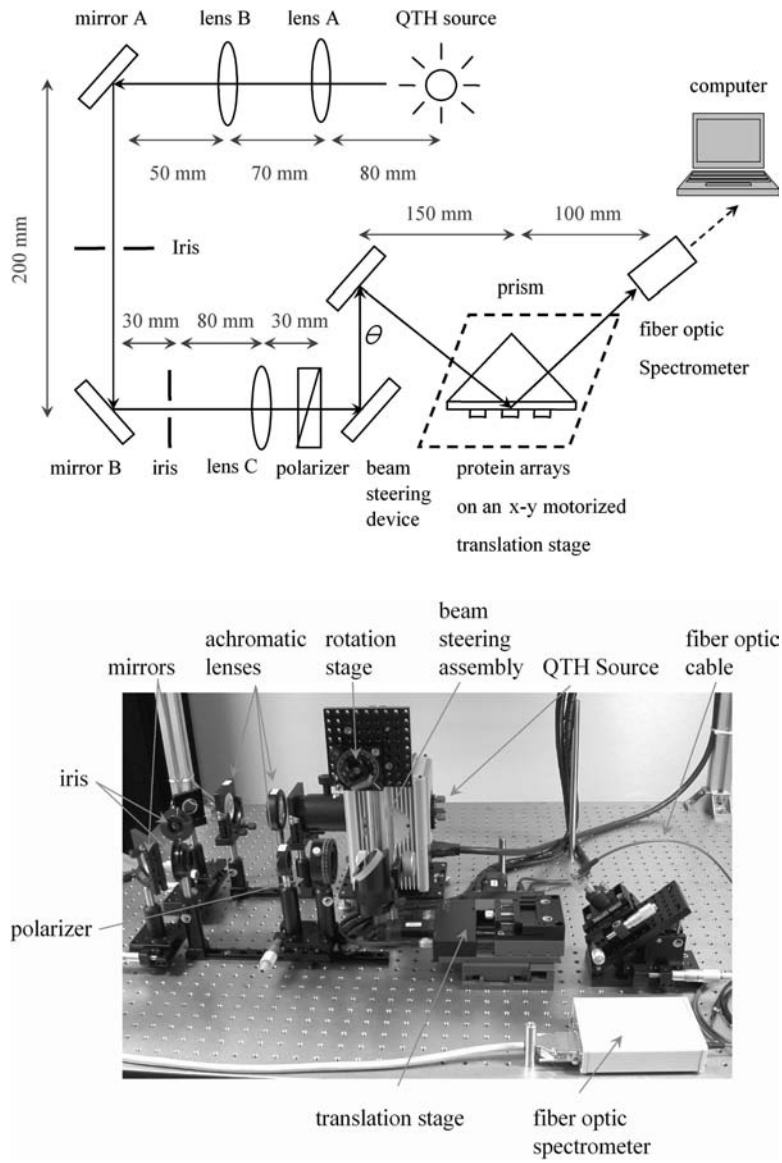

Fig. 2. Optical components of the spectral SPR biosensor. Array-based spectral SPR biosensor is configured by the Kretschmann geometry of the attenuated total reflection method. A polarizer is positioned at the input light path to obtain transverse magnetic polarized light. Reflected light from the protein arrays is collected into an optical fiber and analyzed by the fiber optic spectrometer. (**a**) A schematic diagram. (**b**) A photograph.

3.1. Integration of Spectral SPR Bio sensor; Optical Components, a Fiber Optic Spectrometer and Motorized Translation Stages

1. These instructions assume the use of a white light lamp, a fiber optic spectrometer and motorized translation stages. A 20 W quartz halogen lamp is installed in a lamp housing (*see* **Note 6**). **Figure 2** shows the components of the spectral SPR biosensor.
2. Iris diaphragms are installed in front of the light source to obtain parallel light.

3. Achromatic convex lenses are located in front of iris diaphragms by monitoring the status of collimated light on the screen (*see* **Note 7**).
4. To obtain p-polarized light, a Glan-Taylor polarizing prism is positioned in front of a beam steering device. The beam steering device consists of a horizontal mounting platform and a rotation stage combined with a precision flexure mirror mount. The rotation stage is used to change an incidence angle.
5. Incidence angle is set at 48° using the beam steering device.
6. A PCI-GPIB card is installed in the PC slot to communicate with a motion controller.
7. Motorized translation stages are installed on the optical table and connected with the GPIB cable located between the PCI-GPIB card and the motion controller. The motorized translation stage is used to move the sample holder.
8. A gold array is put onto the translation stage (*see* **Note 8**).
9. A fiber optic spectrometer is installed to collect the reflected light from the gold arrays with the same incidence angle (*see* **Note 9**).

3.2. Calculation of a Resonance Wavelength; Polynomial Curve Fitting

1. A wavelength range of the experimental SPR spectrum is selected to calculate a resonance wavelength. Generally, the wavelength range below 30% of maximal intensity is selected.
2. The fourth polynomial curve fit technique such as $y = \sum_{j=0}^{4} c_j x^j$, which is a matrix type of $Ac = y$, is selected. x is a wavelength and y is the intensity of experimental SPR spectrum.
3. The polynomial fit coefficient (c) is calculated with the linear algebra $c = A^{-1} \cdot y$.
4. The fourth polynomial curve fit is evaluated with the calculated coefficients and the index of a minimal value is calculated with a polynomial curve fitted SPR spectrum.
5. The index of a minimal value of the polynomial curve fitting data is converted into a resonance wavelength.
6. The best polynomial fitting data is compared with an experimental SPR spectrum.

3.3. Interface and Data Analysis

1. When the hardware is installed into the computer to interface between the devices and the software, it is essential to prevent electrostatic discharge from damaging the device and the components by using a grounding strap or by holding a conduction material such as the computer chassis.
2. GPIB driver software for LabVIEW™ is installed in the computer.
3. Power is turned off and the computer is unplugged.

4. Any static electricity, that might be on one's clothes or body, is discharged by touching the metal part of the power supply case inside the computer.
5. The GPIB card-edge connector is lined up with the expansion slot receptacle.
6. The computer is plugged in and the installation is verified.
7. The Pprocedure *(1–6)* is repeated until an SPR spectrum is acquired with the fiber optic spectrometer with ADC2000-PCI+ A/D Converter for PCI-bus (*see* **Note 9**).
8. Data acquisition is performed by the fiber optic spectrometer when reflected light is collimated into the optical fiber entrance.
9. Resonance wavelength is calculated by the fourth-order polynomial curve fitting method (*see* **Subheading 3.2**).
10. Statistical analysis of protein array is performed by the line scanning mode (*see* **Subheading 3.7**). Each protein array is scanned every 100 μm along the central line by moving the *x*–*y* translation stage, which is systemically controlled by the motion controller. Average resonance wavelengths and standard deviations of each spot are calculated by the collected SPR data.

3.4. Calibration of SPR Spectrum

1. The SPR spectrum is very sensitive to the changes of the refractive index on the metal surface and so the spectrum is easily affected by various experimental environments. It is important to find an exact resonance wavelength from the experimental SPR spectrum to monitor biomolecular interactions. To obtain an exact resonance wavelength, the experimental SPR spectrum is calibrated with a theoretical SPR spectrum when the QTH lamp is replaced.
2. A resonance wavelength of 577 nm is calculated with 3-phase Fresnel's equations (fused silica prim/Au (45 nm) film/air) at the incidence angle of 48° *(12)*.
3. An exact incidence angle is adjusted to find the resonance wavelength of 577 nm for the 45 nm-thick Au coated chip with the beam steering device at the incidence of angle 48° (*see* **Note 10**).

3.5. Surface Modification of Gold Arrays

1. The Au arrays are washed with the cleaning solution at 70°C for 10 min (*see* **Note 11**).
2. The arrays are incubated with a mixture of cyclohexane/carbon tetrachloride/octadecyltrichlorosilane (40:10:0.08, v/v) to generate a hydrophobic glass surface (hydrophobic wells) at 45°C for 30 min.
3. The arrays are then washed with a mixture of cyclohexane and carbon tetrachloride (4:1, v/v), carbon tetrachloride and ethanol in order.

4. The arrays are again cleaned with the cleaning solution at 70°C for 10 min.
5. Then, the Au arrays are incubated with 1 mM MUA in ethanol for 16 h and washed with ethanol to remove the excess MUA.
6. The gold arrays are incubated with 50 mM NaOH in milliQ water for 10 min to make the MUA surface negative.
7. The modified arrays are incubated with 1 mg/mL PDDA in 0.5 M NaCl or 1 mg/mL poly L-lysine in milliQ water for 30 min and then washed with milliQ water.

3.6. Preparation of Protein Arrays

1. Mumps virus is immobilized on the PDDA surface of gold arrays at 37°C for 2 h.
2. The virus arrays are incubated with 3.7% formaldehyde and 0.2% Triton X-100 each for 30 min (*see* **Note 12**).
3. The virus arrays are washed with PBS and incubated with 10 mg/mL of bovine serum albumin in PBS for 30 min to reduce nonspecific interactions.
4. The virus arrays are washed with 0.1% Tween 20 in PBS.
5. Then, the virus arrays are incubated with various concentrations of anti-mump virus or anti-GST antibody in PBS or human serum for 2 h at 37°C. For analysis of mumps virus infection, human sera are diluted ten times with PBS and applied to the virus arrays for 2 h at 37°C.
6. The virus arrays are washed with 0.1% Tween 20 in PBS and milliQ water, and dried under N_2 gas.
7. The arrays are analyzed by the line-scanning mode of the spectral SPR biosensor to determine the amount of anti-mumps virus in the samples that has interacted with the virus arrays (*see* **Note 13**).

3.7. Analysis of Protein Arrays by the Line Scanning Mode of the Spectral SPR Biosensor; Display by Color Spectra

1. Protein arrays are analyzed by the line-scanning mode of the spectral SPR biosensor and the resulting resonance wavelengths are displayed by the color spectra. Protein arrays are horizontally divided into a plurality of pixels. The pixels are determined by the resolution of the stage and the diameter of the optical fiber.
2. Protein arrays are sequentially scanned by moving an *x*–*y* motorized translation stage.
3. Reflected spectra are sequentially acquired and SPR spectra are selected by considering the spectral characteristics of dip shape.
4. Resonance wavelengths are calculated by the fourth polynomial curve fitting method.
5. The net shift of each resonance wavelength is sequentially encoded into a one-dimensional array pixel, which is assigned

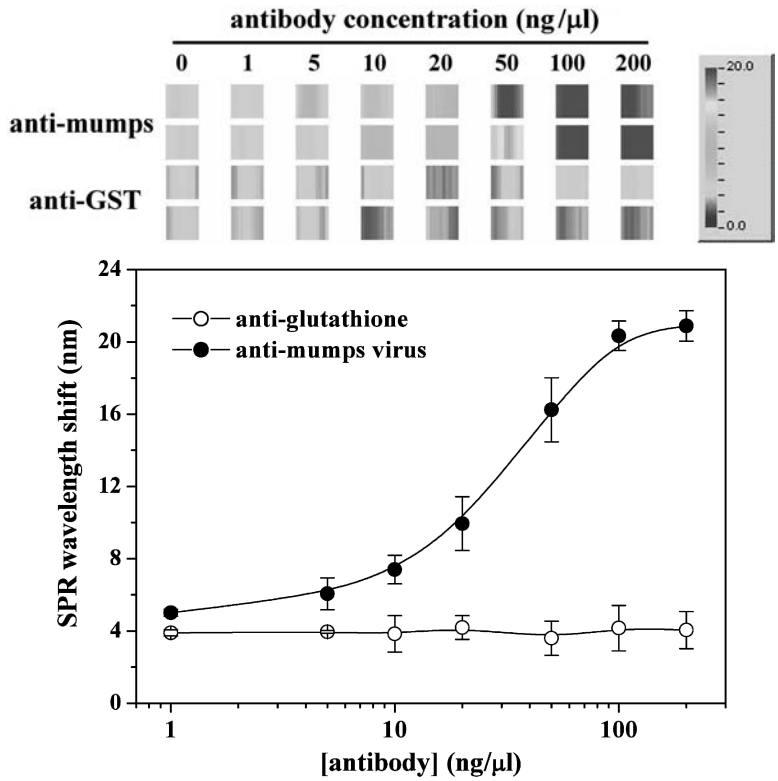

Fig. 3. High-throughput analysis of anti-mumps virus antibody in human serum on the viral arrays by the spectral SPR biosensor. Mumps virus arrays were prepared by immobilizing mumps virus on the PDDA surface of arrays. The virus arrays were incubated with the indicated concentrations of monoclonal antibodies against mumps virus and glutathione in human serum for 2 h at 37°C. Following washing, the arrays were analyzed by the line-scanning mode of the spectral SPR biosensor and the shift of resonance wavelengths was were expressed by color spectra (**a**). The results are expressed as means ± S.D. from three independent experiments (**b**).

to an appropriate color from the GRB color tool bar in proportion to the wavelength.

6. This procedure is repeated until the end of a plurality of pixels *(7)* (*see* **Note 14**). **Figure 3** shows analysis of anti-mumps virus in human serum by the line-scanning mode of the spectral SPR biosensor.

4. Notes

1. A 20 W QTH lamp was used since as it provides a stable spectral irradiance curve.
2. The spectrometer used was an S2000 series with a spectral range of 500–700 nm, 25 μm slit and 1,200 lines/mm.

3. A two-axis controller of the stage is connected with a GPIB card and controlled by IEEE-488.2 command.
4. The surface of the pyrex glass is required to cleaned before depositing Ti/Au films.
5. The specificity of the monoclonal antibody is established by immunofluorescence staining and immunoblotting.
6. When the lamp is installed, it is required to handled the lamp with poly-vinyl gloves (powder-free).
7. Generally, several achromatic convex lenses are required to obtain collimated parallel light. If the filament shadow of the quartz tungsten halogen lamp appears during beam collimation, beam focusing is inappropriate for obtaining collimated light. This process is called "alignment of the optics".
8. A sample holder is designed to install protein arrays contacted with a prism.
9. To obtain an SPR spectrum with LabVIEW™ software, the OOILVD LabVIEW driver (32-bit) (Ocean Optics, Dunedin, FL) is used.
10. If the shape of the SPR spectrum is not similar to that of inverse Gaussian distribution, it is necessary to align the optical lens system again. If the intensity of the SPR spectrum is over 60%, it is necessary to check the thickness of gold arrays.
11. The quality of gold arrays is controlled by checking the gold surface after cleaning the arrays.
12. To determine the titer of mumps virus in solution, virus-immobilized arrays are analyzed without incubating with 3.7% formaldehyde or 0.2% Triton X-100.
13. The protein arrays can be recycled several times after cleaning with the cleaning solution.
14. The scanning speed of the spectral SPR biosensor is mainly dependent on the performance of the hardware motorized stages.

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