Development of Surface Plasmon Resonance Imaging Apparatus for High-Throughput Study of Protein-Surface Interactions

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We report a parallel analysis of protein-surface interactions using an array presenting spots with different surface properties and a surface plasmon resonance (SPR) imaging apparatus. The array was fabricated by the photopatterning of self-assembled monolayers (SAMs) carrying different functional groups including methyl, hydroxyl, carboxylic acid, amine, and poly(ethylene glycol)-tethered amine. The SPR imaging apparatus was developed in-house. The adsorption behavior of bovine serum albumin (BSA) on SAMs was monitored by SPR imaging. The adsorption amounts obtained using the SPR imaging apparatus were well correlated with those obtained by the bicinchoninic acid (BCA) assay. The combination of the array and SPR imaging is suitable for high-throughput, real-time, label-free, and highly-sensitive studies of the interactions of proteins with surfaces with various properties.

Keywords: Biosensing and devices; Protein adsorption; Self-assembled monolayer; Surface plasmon resonance imaging

I. INTRODUCTION

Protein adsorption at the solid/liquid interface is of critical importance in a number of fundamental fields and applications, such as development of biomaterials, medical devices and biosensors, and thus has been extensively investigated [1]. However, many questions remain to be addressed to understand the phenomena of protein adsorption on artificial surfaces.

Various surface sensitive methods have been introduced to study protein adsorption on artificial surfaces including ellipsometry [2–4], Fourier transformed infrared reflection adsorption spectroscopy (FTIR-RAS) [5], atomic force microscopy (AFM) [6–8], quartz crystal microbalance (QCM) [9, 10], optical waveguide light mode spectroscopy (OWLS) [11], and surface plasmon resonance (SPR) [12–18]. Homogeneous and well-defined surfaces have also been developed. Alkanethiols with various terminal functional groups, HS(CH$_2$)$_n$X, that were commercially available, spontaneously form stable monolayers on metal surfaces, such as gold and silver [19, 20]. Self-assembled monolayers (SAMs) of alkanethiols can provide suitable model surfaces to study the interactions of proteins with artificial surfaces. Moreover, SAMs having a variety of surface properties can be easily generated by coadsorption of two or more kinds of alkanethiols with different terminal functional groups [2, 21, 22]. In addition, studies on protein adsorption onto SAMs have been reported [23–26].

SPR can offer real-time and label-free analysis of the interfacial events that occur on the surface of a metal (usually gold or silver) layer under physiological conditions [27]. Thus, it coordinates well with SAMs of alkanethiols formed on metal surfaces. Recently, the technique of SPR imaging (also denoted as SPR microscopy) has been developed and applied to monitor the adsorbing organic materials and biomolecules in a spatially resolved manner [28, 29]. The combination of SAMs of alkanethiols and a SPR imaging technique is expected to realize a high-throughput, real-time, label-free, and highly sensitive method to study protein-surface interactions. In this study, arrays of spots carrying SAMs of alkanethiols with different terminal functional groups, HS(CH$_2$)$_n$X, were made on a gold thin film. We examined the efficacy of the SPR imaging technique using a SPR imaging apparatus that we developed for quantitative analyses of protein adsorption on the functionalized SAM surfaces.

II. EXPERIMENTAL

A. Materials

Glass plates (S-LAL10, $n = 1.720, 25 \text{ mm } \times 25 \text{ mm } \times 1 \text{ mm}$) were purchased from Sigma Koki Co., Ltd., Tokyo. 11-mercaptop-1-undecanol (HS-OH, Sigma-Aldrich, Inc., St. Louis, MO, USA), bovine serum albumin (BSA, Sigma-Aldrich) and triethanolamine (TEA, Sigma-Aldrich), 11-mercaptoundecanoic acid (HS-COOH, Sigma-Aldrich), 1-hexadecanethiol (HS-C$_{16}$CH$_3$, Tokyo Kasei Kogyo Co., Ltd., Tokyo), 11-amino-1-undecanethiol, hydrochloride (HS-NH$_2$, Dojin Laboratories, Kumamoto), 1-dodecanethiol (HS-C$_{12}$CH$_3$, Wako Pure Chemical Industries, Ltd., Osaka), ethylene glycol (Wako Pure Chemical Industries), N-hydroxysuccinimidyl ester of methoxypoly(ethylene glycol) propionic acid (MW = 2,000) (PEG-NHS, Shearwater Co., Huntsville, AL, USA), bovine serum albumin (BSA, Sigma-Aldrich) and triethanolamine (TEA, Sigma-Aldrich) were used as received.

B. Formation of SAM

The glass plates were cleaned by oxygen plasma using a plasma reactor (PA300AT, O-kuma Engineering Co. Ltd., Fukuoka) for 1 min, rinsed with MilliQ water (18.2
MΩ) and 2-propanol three times, and then stored in 2-propanol. The glass plates were coated with 1 nm of a chromium underlayer and 44 or 199 nm of a gold layer by a thermal evaporation apparatus (V-KS200, Osaka Vacuum, Ltd., Osaka). The gold layers of 44 nm and 199 nm in thickness were used for X-ray photoelectron spectroscopy (XPS) and SPR imaging analyses, and for FTIR-RAS and contact angle measurements, respectively. The gold-coated substrates were immediately immersed in a 1 mM ethanol solution of HS-C₁₅CH₃ for at least 24 h at room temperature. The substrates were then rinsed with ethanol and water, followed by drying with a stream of nitrogen.

C. Preparation of SAM spot arrays

An array having SAM spots with different surface functional groups on a glass plate was prepared by the UV photopatterning method on a SAM film of HS-C₁₅CH₃ [30]. A quartz mask having 5×5 circular features of 1 mm in diameter was mounted on the glass plate containing the methyl-terminated SAM. The surface was irradiated with UV light through the photomask from a super-high-pressure mercury lamp (100 mW/cm², Ushio Inc., Tokyo) for 2 h. The glass plate was thoroughly washed with water and ethanol to remove oxidized alkanethiol from the UV light exposed areas. Spots with bare gold surface in the array were subsequently filled with droplets (300 nL) of 1 mM ethanol solutions of alkanethiols (HS-C₁₁CH₃, HS-OH, HS-COOH, or HS-NH₂). The glass plate was kept under the saturated ethanol atmosphere for 1 h to form SAMs on the spots, then thoroughly rinsed with ethanol and water three times, and finally dried with a stream of nitrogen gas. For preparation of PEG-tethered spots, spots with an amine-terminated SAM were applied with a droplet (400 nL) of 4 mM PEG-NHS in 100 mM TEA buffer (pH8) and left for 30 min at room temperature under the saturated condition of water, followed by the rinsing with water and ethanol three times.

D. Surface characterization

Surfaces of SAMs were characterized by FTIR-RAS, XPS and contact angle. Effective removal of an oxidized SAM of HS-C₁₅CH₃ and formation of SAMs with various functional groups on a gold surface were examined. The whole area of the HS-C₁₅CH₃ SAM on a gold layer was photo-oxidized by UV irradiation for 2 h without a photomask and the oxidized substance was removed by washing with water and ethanol. SAM layers of alkaniethiols (HS-C₁₁CH₃, HS-OH, HS-COOH, or HS-NH₂) were formed on the gold surface by immersing the photo-oxidized glass plates into ethanol solutions of each alkanethiol. These surfaces were analyzed by FTIR-RAS, XPS and water contact angle measurements.

Infrared absorption spectra of the SAMs were acquired by the reflection-adsorption technique using Spectrum One™ (PerkinElmer Inc., Boston, MA, USA) equipped with Reflection™ (Harrick Scientific Co., Ossining, NY, USA) and a liquid-nitrogen-cooled mercury-cadmiumtelluride (MCT) detector. The sample chamber was purged with dry nitrogen gas during the measurement, and a p-polarized beam with an incident angle of 75° was used. All spectra were recorded at 4 cm⁻¹ resolutions with 128 scans in the range of 750-4000 cm⁻¹. Next, the atomic compositions of the SAMs were determined by XPS analyses. XPS spectra were obtained using ESCA 850V (Shimadzu Co., Kyoto) equipped with a Mg Kα X-ray source. The take-off angle was 90° and the operating pressure was lower than 1×10⁻⁵ Pa. All spectra were referenced to Au(4f7/2) at 83.8 eV. Finally, static water contact angles on SAMs were determined by the sessile drop method using a contact angle meter (CA-X, Kyowa Interface Science Co. Ltd., Saitama) at room temperature. A droplet (10 µL) of water was placed on a SAM surface and the contact angle was measured three times. This procedure was repeated five times at different places on the same surface. The contact angle of a sample was expressed as the mean value of five contact angle measurements.
E. SPR imaging apparatus

The SPR imaging apparatus employed in this study was developed by referring to the report by Nelson, et al. [31] and is schematically depicted in Fig. 1. A glass plate with arrayed SAM spots was mounted on a S-LAL10 equilateral prism with index matching fluid \((n = 1.720, \text{Cargille Laboratories, Cedar Grove, NJ, USA})\). The flow cell was constructed using a washer made of silicone (diameter: 19 mm, thickness: 0.5 mm) and an acrylic lid with an inlet and outlet. The back side of the glass plate was illuminated by a \(p\)-polarized, collimated, and polychromatic white light through the prism in a Kretschmann configuration [31]. The reflected light was passed through an interference filter \((\lambda = 905 \text{ nm}, \text{half width: } 10 \text{ nm}, \text{Sigma Koki})\) and collected by a CCD camera (C3077-78, Hamamatsu Photonics K.K., Shizuoka). The data was acquired by internally designed software.

F. Calibration of SPR imaging apparatus

A series of ethylene glycol solutions with different concentrations ranging from 1 to 30 vol% in water were used to calibrate the SPR imaging apparatus. After the flow cell was filled with water, the ethylene glycol solutions were sequentially flowed for 5 min, and the intensities of the reflected light were determined for the calibration of the SPR apparatus. The refractive index of the solutions was calculated by the volume fraction of ethylene glycol using the refractive indices of ethylene glycol \((n = 1.4306)\) and water \((n = 1.3325)\) [32].

G. Protein adsorption measurements

Dulbecco’s phosphate buffered saline (DPBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4) was circulated into the flow cell using a peristaltic pump (Asahi Technoglass Co., Chiba) at 3 mL/min. The incident light was fixed at an angle slightly lower than the SPR angle. The intensity of reflected light was 20,000 (arbitrary unit) higher than that of the intensity at the SPR angle. For protein adsorption measurements, after 5 min of DPBS flow, a bovine serum albumin (BSA) solution of 1 mg/mL in DPBS was circulated for 2 h and then followed by DPBS for an additional 25 min to wash out the BSA solution, including weakly adsorbed BSA. The intensity of reflected light averaged for 7 \(\times\) 7 pixels in each spot was continuously monitored during the entire period of the flowing solutions. All measurement was carried out at 30°C.

H. Bicinchoninic acid (BCA) assay

Amounts of adsorbed BSA on the SAMs were directly determined by using the Micro BCA™ protein assay (Pierce Biotechnology, Inc., Rockford, IL, USA) [33]. To each of the SAMs that were formed on gold-coated glass plates, a silicone frame (inner area, 2 \(\times\) 2 cm) was mounted on the substrate plate to confine the protein-adsorbed area. The glass plate was placed in a polystyrene dish, and the inside of the silicone frame was filled with a BSA solution (1 mg/mL), and the plate was left for 2 h at room temperature to facilitate BSA adsorption. The BSA-adsorbed substrate was extensively washed with DPBS. After the DPBS was carefully aspirated, 250 \(\mu\)L of DPBS plus 250 \(\mu\)L of BCA working reagent was placed on the BSA-adsorbed substrate or non-adsorbed control substrate. On non-adsorbed HS-C\(_{11}\)CH\(_3\) SAM surfaces, 500 \(\mu\)L of DPBS plus 500 \(\mu\)L of BCA working reagent was placed to wet the whole surface area. To obtain a standard curve, 500 \(\mu\)L of a BSA standard solution (0.25-10 \(\mu\)g/mL) plus 500 \(\mu\)L of working solution was added to a polystyrene tube. After 1 h-incubation at 60°C, the BCA solution on the substrate was collected into a tube and cooled on ice. Absorbance at 562 nm of the solutions collected from the sample surfaces was determined by using a UV-VIS spectrophotometer (DU 640, Beckman Instruments Inc., Fullerton, CA, USA). The amount of adsorbed BSA was calculated from the absorbance difference between the non-adsorbed substrate from BSA-adsorbed substrates using a standard curve.

![FIG. 2: FTIR-RAS spectra of the SAMs in the (a) high and (b) low wavenumber regions of (I) HS-C\(_{15}\)CH\(_3\), (II) HS-C\(_{11}\)CH\(_3\), (III) HS-OH, (IV) HS-COOH, (V) HS-NH\(_2\), and (VI) HS-NHCO-PEG.](http://www.sssj.org/ejssnt)
III. RESULTS AND DISCUSSION

A. Surface characterization of SAMs

The SAM spot array was prepared by photo-oxidation of hydrophobic HS-C_{15}CH_{3} SAM surfaces by the following procedures: UV irradiation through a photomask carrying 5 × 5 circular open spots with 1 mm^2 area, removal of the oxidized HS-C_{15}CH_{3} SAM and then subsequent filling with alkanethiols carrying different functional groups to the oxidized spots. The spot area is too small to characterize the spot surface in an array in detail. Thus, surfaces were prepared by the same procedure as the fabrication of an array without the photomask. The progression of the multistep process was monitored using FTIR-RAS, XPS, and water contact angle.

Figure 2 shows the FTIR-RAS spectra of SAMs with different functionalities in the high and low wavenumber regions. In the high wavenumber region from 2800 cm\(^{-1}\) to 3000 cm\(^{-1}\) (Fig. 2a), for HS-C_{15}CH_{3} and HS-C_{11}CH_{3}, the peak at 2965 cm\(^{-1}\) is assigned to the CH\(_3\) asymmetric in-plane C-H stretching mode, \(\nu_{a} (\text{CH}_{3}, \text{ip})\), and those at 2879 cm\(^{-1}\) and 2937 cm\(^{-1}\) are assigned to the symmetric C-H stretching mode of CH\(_3\) group, \(\nu_{s} (\text{CH}_{3}, \text{FR})\) [34]. The peaks at 2850 cm\(^{-1}\) and 2917 cm\(^{-1}\) for all SAMs are the CH\(_3\) symmetric and asymmetric stretching mode, respectively. After photo-oxidation by exposure to UV and washing with ethanol, all peaks assigned to the C-H stretching modes were not observed (data not shown), suggesting that the preformed HS-C_{15}CH_{3} monolayer was effectively removed by photo-oxidation. In the low wavenumber region from 1000 cm\(^{-1}\) to 1800 cm\(^{-1}\) (Fig. 2b), SAM of HS-COOH shows two C=O stretching mode at 1744 cm\(^{-1}\) and 1720 cm\(^{-1}\), which arise from the free and hydrogen bonded carboxylic acids, respectively [35]. The PEG-tethered surface was prepared by the coupling PEG-NHS with the monolayer of HS-NH\(_2\). The peaks at 1660 cm\(^{-1}\) and 1576 cm\(^{-1}\) assigned to the amide I and II, respectively newly appeared. The band at 1148 cm\(^{-1}\) is due to the C-O-C stretch of the ethylene glycol units [36]. In addition, the characteristic peaks at 1070 cm\(^{-1}\) for HS-OH assigned to the C-O stretching mode [35] and at 1608 cm\(^{-1}\) and 1545 cm\(^{-1}\) for HS-NH\(_2\) assigned to the asymmetric and symmetric NH\(_3\)\(^+\) deformations [36] disappeared.

Table I summarizes the atomic concentrations of a set of SAMs determined from their XPS analyses. Most of values obtained are well in agreement with the compositions predicted from their molecular structures of SAMs. The high-resolution photoelectron spectra of the carbon 1s region for HS-OH and HS-COOH SAM showed the C(1s) peaks characteristic of methylene groups adjacent to the hydroxyl group (\(O\text{CH}_{2}\)) and a carbonyl carbon (\(C=\text{OOH}\)) (data not shown). Table I includes the water contact angles which are in agreement with those reported in the literature [25]. These analyses confirmed that the expected SAM surfaces were formed on the gold surface after oxidation and removal of the initial HS-C_{15}CH_{3} SAM.

| Table I: Properties of monolayers of thiols carrying different functional groups. | Atomic concentration from XPS (%) | Water contact angle (°) |
|---|---|---|
| Thiol | C(1s) | N(1s) | O(1s) | S(2p) | |
| HS-C_{15}CH_{3} | 95.7 | 0.7 | 0 | 3.6 | 108.5±0.5 |
| HS-C_{11}CH_{3} | 95.2 | 0 | 0 | 4.8 | 104.6±0.2 |
| HS-OH\(^*\) | 88.6 | 0.5 | 6.1 | 4.9 | 29.9±1.8 |
| HS-COOH\(^*\) | 86.2 | 0 | 9.6 | 4.2 | 20.8±2.5 |
| HS-NH\(_2^2\) | 84.6 | 6.0 | 5.0 | 4.4 | 64.4±1.1 |
| HS-NHCO-PEG\(^*\) | 76.1 | 5.4 | 16.2 | 2.3 | 38.4±0.8 |

B. SPR imaging apparatus and calibration of SPR signal

The SPR method is a sensitive technique to changes in the local refractive index near the surface of a thin metal film [37, 38]. Intensity of the reflected light around the SPR angle highly depends on the incident angle. We employed the near-infrared light for SPR imaging measurements, because the SPR reflectivity curve as a function of incident angle becomes sharper with increasing wavelength of the incidence light and thus, clear SPR images can be obtained due to an enhancement of the contrast in the SPR image [31].

In this study, one of the main goals was to determine the amount of adsorbed protein from the intensity changes of the reflected light from SPR imaging before and after protein adsorption. In the following, the relationship between changes of the reflected light intensity and amounts of protein adsorption will be discussed.

For calibration of our SPR imaging apparatus, changes of the refractive index near the surface of a thin metal film was introduced by a series of ethylene glycol/water solutions. The intensity of reflected light was monitored upon introduction of various ethylene glycol concentrations into a flow cell, which corresponded to refraction indices from 1.3325 to 1.3619. Figure 3 shows the plot of the intensity of the reflected light versus the refraction index of the ethylene glycol solution. The intensity of reflected light linearly increased for a change in bulk refractive index up to \(\Delta n = 0.008\). For the linear region, the relationship between the intensity change \(\Delta I\) and the refractive index change \(\Delta n\) is expressed as

\[
\Delta I = 2.29 \times 10^7 \cdot \Delta n.
\]
FIG. 3: Relationship between change in bulk refractive index and the intensity of reflected light. The intensity shows the averaged value at randomly selected 25 spots.

The shift in SPR angle for change in bulk refractive index can be obtained from the Fresnel relationship for multiple thin films using the program available on-line from Knoll’s research group [39]. Fresnel equation can be used to predict the reflectivity when incident light strikes an interface containing two or more phases. These calculations assume that each phase is homogenous and parallel and that they require the complex index of refraction of each phases, \( N(\omega) = n(\omega) + ik(\omega) \), which are dependent on the wavelength of the incident light. The relationship between the change in the refractive index \( \Delta n \) and the angle shift \( \Delta \theta \) in SPR angle for four layers (S-LAL10/Au/SAM/aq.solution) could be calculated using the Fresnel relationship with the optical constants listed in Table II, and is given by

\[
\Delta \theta = 1.077 \times 10^2 \cdot \Delta n. \tag{2}
\]

The shift in SPR angle due to protein adsorption can be also calculated using the Fresnel relationship for five layers (S-LAL10/Au/SAM/protein/water) with the assumption that the density of the protein layer is 1 g/cm\(^3\) [40]. The angle shift change, \( \Delta \theta \), is linearly proportional to the adsorbed amount of protein \( \Gamma \), as follows

\[
\Delta \theta = 5.161 \times 10^{-4} \cdot \Gamma. \tag{3}
\]

Therefore, combining Eqs. (1), (2), and (3) gives the relationship between changes of the reflected light intensity and the amount of adsorbed protein as

\[
\Gamma = 0.91 \times 10^{-2} \cdot \Delta I. \tag{4}
\]

We can evaluate amounts of adsorbed protein from brightness changes of spots of SPR imaging before and after the SAM was exposed to a protein solution using Eq. (4).

C. Protein adsorption measurement using SPR imaging apparatus

In a SPR imaging apparatus, an image collected by the CCD camera reflects the difference of resonance condition at each point on the surface due to changes in local index of refraction. Figure 4(a) shows the SPR image of an array in contact with DPBS. The spots carrying alkanethiols with different functional groups, methyl (CH\(_3\)), hydroxyl (OH), carboxylic acid (COOH), amine (NH\(_2\)) or PEG-coupled amine (PEG) groups, were formed on the array as depicted in Fig. 4(c). Spots appear as ellipses due to the oblique angle at which the surface is viewed. Spots except the PEG-tethered spots were darker than background. Supporting the notion that refractive indices of all alkanethiols are the same (i.e. 1.45) [41], the brightness in CCD image depends on the thickness of the monolayer. A number of repeating methylene units in an alkanethiol molecule on spots (\( n = 11 \)) are less than that on the surrounding area (\( n = 15 \)) and this difference in thickness is expected to be 0.5 nm. This caused darkness in the SPR images for spots with alkanethiols with methyl (CH\(_3\)), hydroxyl (OH), carboxylic acid (COOH), and amine (NH\(_2\)) groups. This result demonstrated the high vertical resolution of the SPR imaging apparatus. The PEG-tethered spots, where PEG-NHS (MW = 2,000) was reacted with NH\(_2\) spots, appear to be same brightness as the background. The brightness in the SPR image depends on the effective refractive index near the metal surface [29]. This might be due to hydration of the surface-bound PEG chains. The effective refractive index of hydrated chains is much lower than that of ethylene glycol \( (n = 1.4306) \) and dehydrated PEG film (high molecular weight poly(oxyethylene), \( n = 1.4563 \) [42].

We carried out parallel analysis of protein adsorption on the array surface by taking advantage of the SPR imaging apparatus. The intensity of reflected light averaged for 7 × 7 pixels in each spot (Fig. 4a) was continuously monitored during the entire period of the flowing solutions. Figure 5 shows the time course of BSA adsorption for multiple regions of the array. First, DPBS was introduced in a flow cell to see reflected light intensities of each spot before protein adsorption, and then a BSA solution in DPBS (1 mg/mL) was introduced. As shown in Fig. 5, the SPR signal sharply increased upon introduction of the BSA solution. Change in reflected light intensity is caused by the combination effects of the refractive index increase of DPBS by dissolving BSA and BSA adsorption on the surface. Net SPR signal increase by BSA adsorption can be demonstrated after replacement with DPBS. After a 2 h exposure, the BSA solution was replaced with DPBS. Figure 4(b) shows the difference between the two images collected before and after the exposure of the array to BSA. For protein adsorption studies, the change in the intensity of reflected light should be converted to the adsorbed amount of proteins. The changes of reflected light intensity for each spot with different functional groups were obtained by averaging five different spots. The adsorbed amounts of BSA on five kinds of surfaces with different functional groups, which were evaluated from the changes of reflected light intensity using Eq. (4), were listed in Table III.

The amount of adsorbed BSA on each SAM surface
FIG. 4: SPR image of the array in buffer (a) before and (b) after BSA adsorption. The surface functional groups of each spots are shown in (c). Image (b) was acquired by subtraction of the image from the image (a). Scale bar: 1 mm.

was also determined by the colorimetric micro BCA assay. The BCA assay involves the reduction of Cu$^{2+}$ to Cu$^{1+}$ by protein in an alkaline medium. The purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu$^{1+}$). This water-soluble complex exhibits a strong absorbance at 562 nm that is linear with increasing protein concentrations [33]. The adsorbed amounts of BSA on each SAM surface determined by the BCA method were also listed in Table III. These values correlate fairly well with those obtained from the SPR imaging apparatus except for the COOH-SAM.

The adsorbed amounts of BSA on the COOH surface obtained by the SPR and BCA methods did not correlate well with each other. One possible reason for this discrepancy is the conformational change of adsorbed proteins. Proteins undergo structural changes during adsorption [43, 44]. SPR is highly sensitive to the refractive index change near a metal film and the SPR response becomes larger if the optical change occurs closer to the surface because of exponential decay of the intensity of the evanescent field from the surface [45]. Conformational changes of proteins may increase the effective refractive index of a protein layer near a metal film. Nonetheless, more detailed analysis is needed to determine the exact cause of this difference.

The results shown in Figs. 4 and 5 demonstrated the usefulness of a SPR imaging apparatus for the real-time and parallel analysis of protein adsorption on many spots presenting different surface functional groups. Ostuni, et al. examined the relationship between characteristics of functional groups and the ability to resist the nonspecific adsorption of proteins on 58 kinds of surfaces on gold by SPR analysis in a one-spot format [17]. However, their method requires large numbers of measurements, one for each sample surface. In our system, protein adsorption on 25 different surfaces can be simultaneously examined. An array with smaller features can be fabricated by photopatterning with a photomask having smaller features which enables high-throughput studies of protein-surface interactions. However, the minimum size of the pattern is limited by the surface plasmon propagation length on the surface. The propagation length is calculated to be about 70 µm at a wavelength of 905 nm [37]. Nevertheless, parallel sensing using a SPR imaging apparatus has great advantages in its ability to allow label-free, high-sensitivity and real-time detection of interfacial events.

FIG. 5: Parallel monitoring of BSA adsorption onto an array carrying different functional groups. BSA concentration: 1 mg/mL.

TABLE III: Adsorbed BSA obtained by two methods (ng/cm$^2$). Data is shown as mean ± standard deviation.

| Functional group | SPR imaging | BCA assay |
|------------------|-------------|-----------|
| CH$_3$           | 115.6±6.4   | 157.4±58.6|
| OH               | 64.6±13.7   | 43.3±40.8 |
| COOH             | 192.0±20.0  | 63.2±23.0 |
| NH$_2$           | 131.0±10.9  | 137.0±20.7|
| PEG              | 38.2±3.6    | 7.8±17.5  |

http://www.sssj.org/ejssnt (J-Stage: http://ejssnt.jstage.jst.go.jp)
IV. CONCLUSIONS

We described a method for quantitative and parallel analysis of protein adsorption on a surface. This method uses an array and a SPR imaging apparatus. The array presenting spots with different surface characteristics (CH₃, OH, COOH, NH₂, and PEG) was fabricated by photopatterning of SAMs, which has the great advantage of stable and well-defined surfaces. The adsorption of BSA on five kinds of SAM surfaces was simultaneously monitored by the SPR imaging apparatus, and correlated well with a known protein measurement assay.

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