**TOF-SIMS Imaging of Protein Adsorption on Dialysis Membrane by means of Information Entropy**

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Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is expected to contribute to the evaluation of biomaterial surfaces interacting with proteins, cells and microbes because it requires no sample pre-treatments, such as labeling with probes and coating with metallic thin films for insulated samples. TOF-SIMS measurement provides chemical distribution of biomaterials with high spatial resolution. Since it is difficult for TOF-SIMS to generate enough characteristic secondary ions for imaging from large molecules such as proteins, multivariate analysis techniques such as principal component analysis (PCA) have been employed to characterize the spectra with fragment ions considered to be related to proteins. However, some of those fragment ions are also related to substrates especially polymers. Information theory was employed for the first time to analyze TOF-SIMS spectra of hollow-fiber dialysis membranes treated with a protein solution. TOF-SIMS spectra and images of protein adsorbed membranes and native membranes were compared based on mutual entropy in order to discriminate secondary ions related to protein from membranes. TOF-SIMS images with the fragment ion groups related to proteins and membrane were obtained respectively, and they show clearly the distribution of adsorbed protein on the dialysis membranes. [DOI: 10.1380/ejssnt.2003.67]

Keywords: TOF-SIMS; Information entropy; Bovine serum albumin; Hollow fiber; Dialysis membrane

I. INTRODUCTION

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is one of the most useful techniques for the evaluation of biomaterial surfaces, which proteins adsorb on, and cells and microbes adhere to. The advantages of TOF-SIMS measurement are 1) requiring no pre-treatment, such as labeling with probes and coating with metallic thin films for insulated samples and 2) providing chemical distribution of biomaterials with high spatial resolution. TOF-SIMS is expected to provide submicron-scale distribution of proteins on biomaterials in principle. However, TOF-SIMS is not suitable for ionization of large molecules such as proteins and polymers. Therefore some analysis techniques have often been employed for characterization of TOF-SIMS spectra with fragment ions from large molecules.

Since every protein consists of the same 20 amino acids, it is difficult to discriminate among proteins based on the simple comparison of the fragment ions. Multivariate analysis techniques, such as principal component analysis (PCA) and linear discriminant analysis (LDA), have been employed to interpret TOF-SIMS spectra using fragment ions related to proteins [1, 2]. PCA is a very useful technique, especially in terms of characterization and determination, and is expected to be helpful to obtain chemical imaging [3]. However, some of those fragment ions are also related to substrates such as polymers. Though secondary ions related to substrates themselves should be also analyzed with an appropriate analysis, some of the multivariate analysis techniques, PCA and LDA, have strict limitation of the number of peaks of secondary ions for analysis through matrix calculations. Therefore selection of appropriate peaks is needed for TOF-SIMS measurement of proteins on biomaterials such as polymers. In this study, information theory [4, 5, 6] was employed to select peaks from numerous candidate peaks in TOF-SIMS spectra of protein adsorbed on dialysis membranes.

The dialysis membranes with nano-size pores [7], having the shape of hollow-fiber, have been used for a treatment of nephric patients. Albumin loss [8] and permeability change caused by protein adsorption during treatments should be reduced in order to improve quality of life for the patients. Several surface analysis techniques, such as scanning electron microscopy (SEM) [9], atomic force microscopy (AFM) [9, 10] and confocal laser-scanning fluorescence microscopy [11], have been used to estimate protein adsorption onto the membranes. However, SEM and AFM only provide rough outline change of membrane surfaces. Though confocal laser-scanning fluorescence microscopy is able to observe the cross-section of the hollow-fiber membrane, it requires complicated pre-treatment of samples, which may change the sample condition. TOF-SIMS is expected to be capable of chemical imaging of the cross-section, inside and outside of protein adsorbed hollow-fiber dialysis membranes without any pre-treatment.

In this paper, the commercially available hollow-fiber dialysis membrane and bovine serum albumin (BSA) were used as the model sample. Mutual information theory was employed to analyze TOF-SIMS spectra of BSA ad-

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FIG. 1: Mutual entropy of each peak in TOF-SIMS spectra. White square: peaks of the native APS-E membrane, red square: peaks of the BSA adsorbed APS-E membrane.

sorbed dialysis membranes in order to select appropriate peaks related to the protein and the membrane, respectively. Chemical images of protein adsorbed on the hollow-fiber dialysis membranes, obtained by the selected peaks based on mutual information theory, contribute to understand protein permeability of the membrane and adsorption mechanisms.

II. EXPERIMENTAL

A. Protein Adsorption

The commercially available hollow-fiber membrane, APS-E (Asahi Medical, Tokyo, Japan), made of polysulfone, was used. Bovine serum albumin (Sigma Chemicals Co, St Louis, MO) in pH 7.4 of 0.1 M-phosphate buffer saline (PBS), BSA concentration of 3.75 g/dl, were fed into the hollow of dialysis membranes at 1.2 ml/hr per one fiber for 7 hr and then rinsed with PBS at the same rate for 55 min and finally rinsed with distilled water. Before TOF-SIMS measurement the membranes were dried in a desiccator. Dried native APS-E membrane was prepared as a reference sample.

B. TOF-SIMS Analysis

Positive ion spectra by TOF-SIMS, TFS-2100 (Physical electronics, Eden Prairie, MN) and TRIFT III (Physical electronics) using 12 keV Ga+ primary ion source, were acquired up to 1000 m/z while maintaining the primary ion dose less than $10^{12}$ ions/cm$^2$ to ensure static conditions [12]. Both TOF-SIMS machines performed measurements in the same conditions. A pulsed low-energy electron flood gun was used for charge neutralization for all samples.

TOF-SIMS images of BSA adsorption on the cross-section of the membranes and those of native membranes were obtained with the selected secondary ions related to BSA and membranes respectively by means of mutual information.

C. Mutual Information

Suppose the number of TOF-SIMS spectra is $N$ and they are classified in two categories, the sample and the reference sample. The number of spectra belonging to the sample is $n(a1)$ and that belonging to the reference is $n(a2)$. In terms of sample categories information entropy $S(A)$ is defined by Eq. (1) [4, 5].

$$S(A) = -\sum_{i} p(ai) \log_2 p(ai).$$  

The probability is

$$p(ai) = n(ai)/N, \quad (i = 1, 2).$$

$S(A)$ is the amount of information needed to determine the a priori category of a spectrum. With a certain peak threshold $V$, the set of spectra are split into two subsets B1 and B2. The peak intensity greater than $V$ is classified to B1 and the number of the spectra containing these peaks is $n(b1)$, and that less than $V$ is classified to B2 and the number of the spectra containing these peaks is $n(b2)$. Therefore the information entropy of splitting induced by $V$, $S(B)$ is defined by Eq. (2).

$$S(B) = -\sum_{j} p(bj) \log_2 p(bj).$$
FIG. 2: Typical secondary ion images of native membranes based on the selected peaks by mutual entropy. (a) Image of secondary ions related to the membrane, (b) image of secondary ions related to BSA, (c) total ion image. The maximum intensity of secondary ions is shown in white (the color bar is intensity scale).

FIG. 3: Typical secondary ion images of membranes treated with BSA based on the selected peaks by mutual entropy. (a) Image of secondary ions related to the membrane, (b) image of secondary ions related to BSA, (c) total ion image. The maximum intensity of secondary ions is shown in white (the color bar is intensity scale).

The probability is

\[ p(b_j) = \frac{n(b_j)}{N}, \quad (j = 1, 2). \]

Mutual entropy \( I(A; B) \) is defined by Eq. (3) [4, 5, 6].

\[
I(A; B) = S(A) - S(A | B),
\]

\[
S(A | B) = - \sum_i \sum_j p(b_j) p(a_i | b_j) \log_2 p(a_i | b_j).
\]

The probability is

\[ p(a_i | b_j) = \frac{n(a_i | b_j)}{n(b_j)}. \]

\( S(A) \) means the \textit{a priori} uncertainty and \( S(A | B) \) is the \textit{a posteriori} uncertainty [5]. \( S(A | B) \) is the entropy in terms of the category when it is shown the result about \( V \). The \( n(a_i | b_j) \) is the number of spectra belonging to sample category \( I \) out of the spectra containing peaks greater than \( V \). The best value of \( V \) is chosen to provide the largest \( I(A; B) \). When \( I(A; B) = S(A) \), peak intensity of each spectra is completely classified to the right category. Number of TOF-SIMS spectra of native membranes and BSA-adsorbed membranes, used for the calculation of mutual information, were seven and ten, respectively. Before the calculation, peaks were normalized to their total intensity.

In this way, mutual information of each peak in every spectrum is calculated. When sample categories are more than three, mutual entropy of each datum is calculated based on the same process with the classification and recognition tree [13].

### III. RESULTS AND DISCUSSION

The mutual entropy value of each peak in the TOF-SIMS spectra was calculated comparing the spectra of BSA adsorbed APS-E membranes and the reference samples. Based on mutual entropy, peaks of secondary ions were classified into those related to BSA and the membrane, though some secondary ions from APS-E membranes, mainly made of polysulfone (PS) with various reagents such as polyvinylpyrrolidone, are similar to those from proteins. Figure 1 shows the values of mutual entropy, in terms of the category, of each peak in the TOF-
FIG. 4: Relative secondary ion images of membranes treated with BSA based on the selected peaks by mutual entropy. (a) Relative image of secondary ions related to the membrane (the native APS-E membrane), (b) relative image of secondary ions related to BSA (the native APS-E membrane), (c) relative image of secondary ions related to the membrane (the BSA adsorbed APS-E membrane), (d) relative image of secondary ions related to BSA (the BSA adsorbed APS-E membrane). The maximum intensity of secondary ions is shown in white (the color bar is intensity scale).

SIMS spectra. Since the a priori information entropy is 0.98, peaks with value 0.98 are complete peaks for the discrimination. In terms of peaks related to BSA, the CHS peak at $m/z = 45$ is 0.98 and three peaks of $C_2H_6NO$, $C_4H_{10}N$, $C_3H_6NO_2$, at $m/z = 60, 72$ and 88, are 0.67. On the other hand, the entropy values of these four peaks in the reference spectra are very low. Therefore these peaks are considered to be mainly from BSA. Even though a peak shows the same value of the a priori entropy, it is not an appropriate peak when that of the reference also shows high value, because it is greatly related to both the sample and the reference. In addition, comparing intensities between the samples and the reference samples are also important, because at this mass resolution around 600 (imaging mode), each peak may contain more than one chemical. In this way, the $C_4H_2$, $C_5H_6N$, and $C_5H_6O$, $C_6H_{10}NO$ peaks at $m/z = 50, 82, 86, 112$ are found to be from APS-E membrane.

Figures 2 and 3 show secondary ion images from native APS-E membranes and BSA-adsorbed APS-E membranes, respectively. These secondary ions, related to BSA, were all selected by means of mutual information, and shown in Table 1. It is shown in TOF-SIMS images of secondary ions related to BSA of the APS membranes treated with BSA that there is BSA adsorption both at the inside and the outside of the membranes, comparing with those of the native membranes, but no BSA adsorption was observed on the cross-section of the membranes. Figure 4 shows the images of the secondary ions normalized with the intensity of the total ion images, influenced mainly by the sample topography. It is also shown clearly in Fig. 4 that BSA adsorption both on the inside and the outside of the membranes, but none on the cross-section. Intensity of the secondary ions related to the APS mem-
brane is less in the images of the membrane with BSA than those of the native membranes, because adsorbed BSA covers the membrane surfaces.

The APS membrane has two skin layers, consisting of nano-size pores, at the surface of the inside and the outside and the average pore size at the inside is smaller than the outside, the average pore diameter of the inside is 10.5 nm, and that of the outside is 17.9 nm [7]. Since the APS membrane is hydrophilic, it is expected that proteins do not adsorb much on APS-E membrane. As the results, BSA adsorption onto the APS membrane occurs only at the skin layers near the inside and the outside surfaces and does not onto the middle area of the cross-section of the membrane.

TOF-SIMS images obtained by means of mutual entropy show clearly the distribution of adsorbed BSA on the dialysis membranes and indicate that BSA permeability and interaction between the membranes and BSA. Therefore TOF-SIMS is capable of evaluating protein adsorption on the cross-section of dialysis membranes. Further study is needed to obtain the cross-section, inside and outside distribution of various proteins in human blood for evaluating the dialysis medical treatment with membranes. Comparison of BSA adsorption on various dialysis membranes will be the subject of future report [14].

IV. CONCLUSIONS

TOF-SIMS is a promising technique for the study of protein adsorption on biomaterials, especially with an appropriate data analysis techniques such as information theory TOF-SIMS provides useful information on protein adsorption and permeability on membranes with nano-scale pores. TOF-SIMS images, obtained with information theory, show distribution of adsorbed BSA on the cross-section, inside and outside of the hollow-fiber dialysis membranes and indicate that BSA adsorption and permeability on the APS-E membrane. Mutual entropy is also a powerful tool in order to characterize and select appropriate secondary ion peaks from numerous candidates in all of the TOF-SIMS spectra of samples and reference samples. With information theory, required peaks are selected for other analysis such as PCA, imaging, and determination without toil. TOF-SIMS data analysis with information theory is expected to contribute to accurate characterization of unknown samples in future.

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**TABLE I:** Peaks of secondary ions from BSA and the APS-E membrane, respectively, selected for TOF-SIMS imaging.

| Peak of secondary ion (m/z) from BSA | Peak of secondary ion (m/z) from APS-E membrane |
|--------------------------------------|-----------------------------------------------|
| 45 (CHS+)                           | 50 (C_{6}H_{3}^{+})                           |
| 60 (C_{2}H_{8}NO^{+})               | 82 (C_{2}H_{6}N^{+})                          |
| 72 (C_{4}H_{10}N^{+})               | 86 (C_{5}H_{10}O^{+})                         |
| 88 (C_{4}H_{10}NO^{+})              | 112 (C_{6}H_{10}NO^{+})                       |

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