DETECTION OF THE UNDERWATER MUCUS BY USING LASER RAMAN SPECTROSCOPY

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ABSTRACT

Laser Raman Spectroscopy measure contactlessly and non-destructively Raman scattering shift conveying the information of molecular vibration. The study describes technique to measure the thickness of individual transparent multiple layer with micrometer-scale resolution by using Laser Raman Spectroscopy. We applied edge detection method to the Raman scattering spectra obtained from a single parylene layer between glasses, and succeeded to measure the thickness with error range less than 1.0 µm. We also applied the technique to the measurement of underwater mucus on the scale. Thus, the technique is adaptable to measure various biomaterials under the wet condition, such as mucus, and the inside of transparent micro device.

KEYWORDS

Laser Raman Spectroscopy, edge detection method, mucus

INTRODUCTION

The cell is most sophisticated micro device, which consists various highly polymerized biomaterials, such as proteins and lipid layer. For example, mucus is a multifunctional biomaterial, such as keeping moist, pathogens trapping and protection from the physical damages, and is the main component of interface between external environment and the surface of cells [1]. Mucus also consists the interface between the skin of aquatic animals and aqueous phase. The chemical main component of mucus is biologically produced glycoproteins and water. Because the visible light transmittance of these biomaterials is near to 100 %, various optical microscopes and fluorescence imaging techniques were developed to observe the microstructure of these biomaterials. Laser Raman Spectroscopy (LRS) is possible to measure contactlessly and non-destructively Raman scattering shift conveying the information of molecular vibration. Thus, it allows the imaging of distribution of biomaterial under the wet condition [2]. Here, we describe the technique to identify the interface of multiple transparent materials by using LRS and zero-crossing based edge detection method. We applied the technique to measure the mucus layer on the surface of salmon scale in water, and succeeded to estimate its thickness.

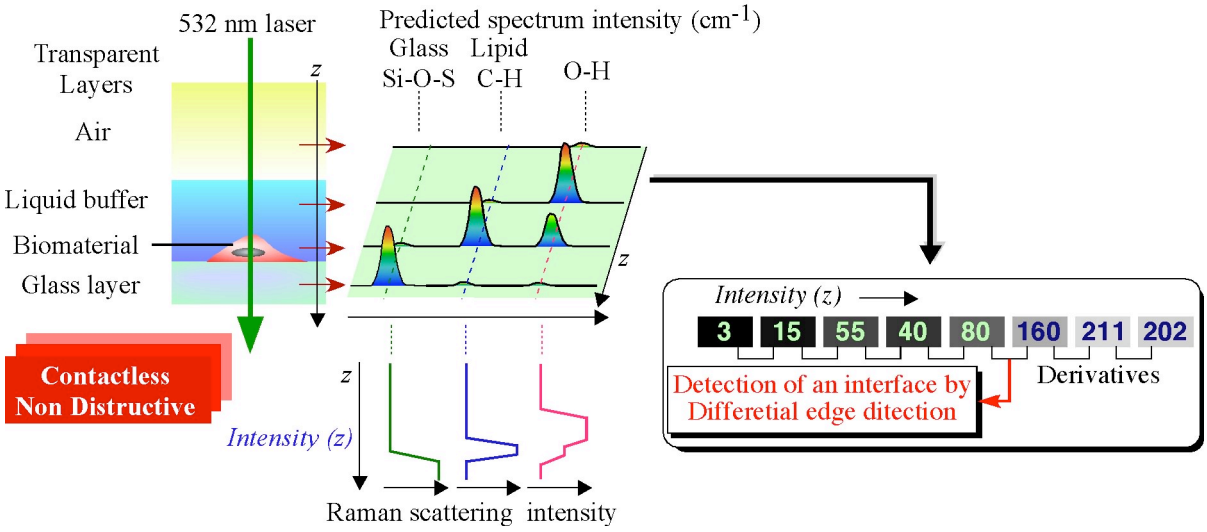

Figure 1. Concept of the study. Transparent biomaterials are scaned by laser beam to obtain the information about chemical bond distribution. Data were processed to detect interfaces of materials by using edge detection method.

EXPERIMENTAL PROCEDURE

Figure 1 shows the concept of the study. We used water, glass and parylene as the model of multilayered transparent material. Theoretically, Raman spectra image gives the information of transparent material interface between the different chemical compositions. However, the pseudocolor image of non-processed Raman spectra was insufficient to identify the interface between glass substance and liquid buffer. We found that the resolution of pseudocolor image obtained from glass substance in liquid solution was dramatically lower than that of dry glass sample, suggesting the difficulty of high resolution detection of the surface of wet samples. To overcome the

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problem, we applied the zero-crossing based edge detection method [3] to Raman scattering intensity of the target chemical bond (figure 1). The method searches for zero crossing in a second-order derivative expression in order to find the interface of materials. For the offline-data analysis, spectra data were processed by a hand-made macro-program running on IgorPro. The program consists of the data interface to load the three-dimensional Raman scattering data, data processing and visualization of results.

The fresh and non-frozen chum salmon (*Oncorhynchus keta*, 80 cm SL.) was purchased from a local fish market. Scales were removed from the skin and immersed into the milliQ water for the LRS measurement to detect the mucus layer.

RESULTS and DISCUSSION

We applied the technique to Raman Spectra acquired from layers of glass substrates and parylane (figure 2). To estimate the parylene thickness, we focused on the data of Raman scattering intensity of C-H bond in parylene (2930 cm^{-1}). We first processed the Raman scattering intensity by using the smoothing function to leave out quantization noise. Then, second-order derivatives were calculated along *z*-axis. Although multiple zero crossing points were observed in second order derivatives, the side zero crossing points of peak Raman scattering intensity corresponded to the interface between transparent materials (figure 2B). The validity was checked by the comparison of measured and real parylene thickness (figure 2C). The method allows measurement of >3 μm thickness with error range less than 1.0 μm. Decreasing of confocal *z*-axis stepping slightly improved the estimation resolution, and detection threshold. We were not able to detect the Raman scattering of 50 nm thickness parylene. Although we found the slight gap in interface between glass substrate and parylene (figure 2B), the thickness of gap was less than the error range.

Finally, We measured the thickness of mucus on the surface of salmon scale in water, smilar with the natural condition (figure 3). Based on the spectra of collagen and hydroxy apatite consisting scales and organic compounds in mucus, our estimation of mucus layer was 38±1 μm (figure 3B, C; n=3 scales, mean±S.D.). Mucus layer on the scale under the natural condition was ticker than that on the human respiratory epithelial cells (0.5 ~ 5 μm) [1]. The results suggested the importance of mucus of aquatic animals under the wet condition. Proteomics studies showed that mucus contain a variety of metabolic enzymes [4]. Further chemical identification will shed the light on the unknown mucus function.

Under the wet condition, the estimation of thickness of scale was 6.2±2 μm (n=4). The estimation was greatly different from that of dried and fixed scale samples by using the scanning electron microscopes (~1μm). The result suggested the importance of characteristics test of biomaterials under the wet condition.

We believe that our simple image processing technique is applicable to not only analyzing the wet biomaterials, but also universally usable Non Destructive Inspection for transparent micro devices.

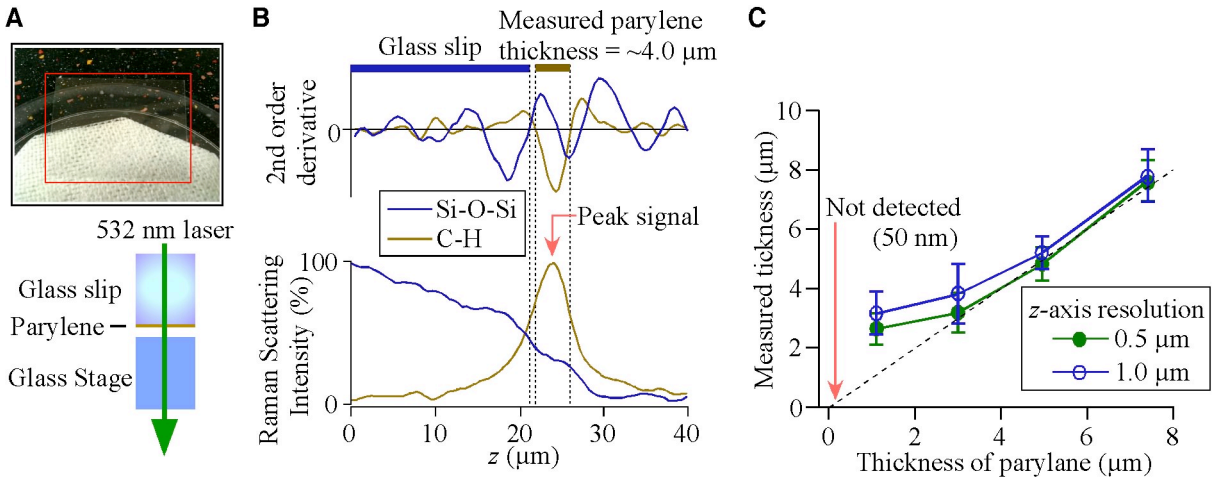

Figure 2. Application of LRS and edge detection method to the measurement of parylene layer tickness between glass materials (A). The spectra intensity and its second order derivative was plotted (B).C, Comparison of real parylene thickness and measured parylene layer between coated glass and glass stage by using LRS and edge detetction method. Averages of 6 trials and S. D. at different confocal z-axis stepping mode were plotted (n=6 at each data point).

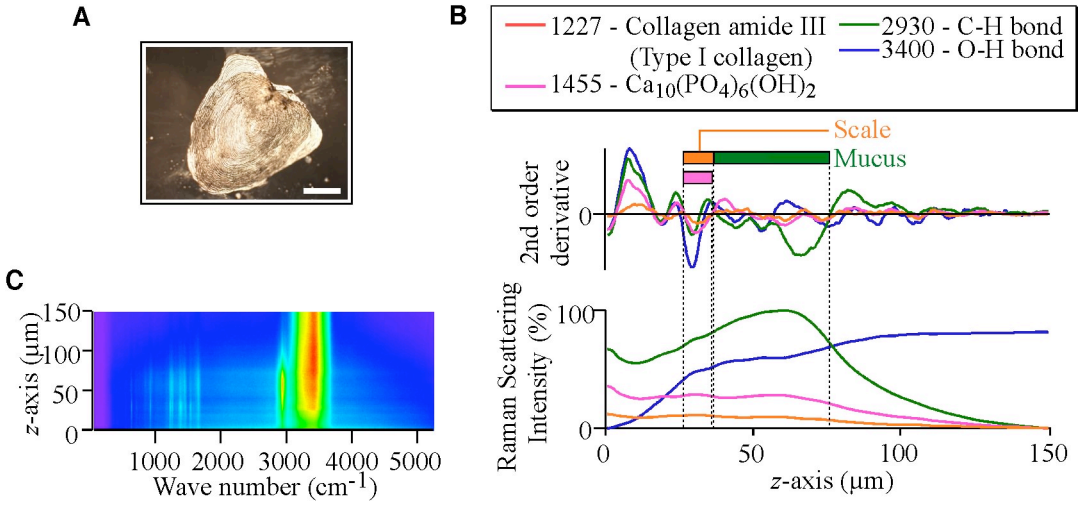

Figure 3. Detection of mucus layer on a salmon scale (A) in water by using LRS and edge detection method. Second order derivative (B) was calculated from the raw spectra data (C). Measured thickness of mucus was ~38 μm. Scale bar = 1.0 mm.

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