Calculation of Reflection Spectrum with Actual Layer Thickness Profile in Nacre of Akoya Pearl Oyster

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Abstract. Pearls have beautiful color and luster which are caused by the interference of light within a multilayer in nacre. The nacre is composed of submicron aragonite layers and nanoscale organic sheets of chitin. The value of a pearl is determined by its size, color, and luster. To improve the quality of cultured pearls, we investigate reflection properties and layer thickness profiles in pieces of nacre of Akoya pearl oyster. SEM images show us how large the thickness variation of the nacre of Akoya oyster is. We discuss the reflection properties of the pieces of nacre to connect the relation between the reflection spectra and the thickness profiles obtained by SEM observation.

1. Introduction
People have been fascinated by the beautiful colors of pearl and opal jewelries since a long time ago. The colors of the pearls and opals are the structural colors caused by multiple reflections from a nanostructure [1-3]. The study of the structural color is intimately associated with photonic crystals which have been widely studied to develop new optical applications such as low threshold lasers [4-10] and nanoscale optical waveguides [11,12]. The optical properties of nanostructures in nature can also be analyzed by calculation techniques for the photonic crystals. Not only pearls and opals, but also morpho butterflies and gold bugs have attracted much attention from a scientific point of view [13].

We have investigated the layer structure of Akoya pearl oysters and its structural colors to improve the quality of the pearls harvested from pearl farming [14]. In general, the price of a pearl is determined by its size, color, and luster. For example, pink Akoya pearls are particularly popular with women. However, the color of a pearl cannot be completely controlled in pearl farming. Therefore, a screening method to obtain desired color in pearl farming is very important [14,15]. In addition, the development of grading of a pearl’s quality based on optical calculations is also a goal.

A cultured Akoya pearl consists of a polished sphere nucleus made from freshwater mussel shell and a thin coating of nacre. The nacre has a layered structure of aragonite crystals and conchiolins. The aragonite is one of the two common naturally occurring crystal forms of calcium carbonate (CaCO₃), and the conchiolin is a complex protein. In general, the thickness range of the aragonite layer is from 300 nm to 700 nm, and that of the conchiolin is from 10 nm to 20 nm. In the point of quality of pearls, it is needless to say that the uniformity of thickness is a key factor. However, the relationship
of the layer profile and optical properties of nacre are not investigated in detail yet. In this paper, we observe the layer profiles of several samples to know how much the aragonite layer varies in the nacre and measure their reflection spectra to investigate optical properties. Furthermore, we also try to reproduce reflection spectra of the samples by transfer matrix method using the measured layer profiles.

2. Reflection spectra of pieces of nacre

At the start of pearl farming, a small piece of mantle tissue is cut from an Akoya pearl oyster (piece oyster), and then nucleus with the piece of mantle tissue is inserted into another Akoya oyster (mother oyster), as shown in figure 1. The small piece of mantle tissue from the piece oyster grows up in the mother oyster, and then the piece gradually covers the nucleus. During the process of holding mother oysters in a farming point, the mother oyster provides nourishment to the piece in sea. Therefore, the genetic information of the piece oyster is important because the small piece becomes the nacre of cultured pearl. Here, we focus on the aragonite layer thickness of the piece oyster on the assumption that the color of a cultured pearl is reflected by the layer thickness.

Figures 2(a)-2(d) show the samples of pieces of nacre measured in this study. We prepared the two red pieces of nacre A and B and two pale green pieces of nacre C and D whose sizes were about 1 cm². These pieces were cut from red and green Akoya pearl oysters, as shown in figure 2(e). To investigate the optical properties of these pieces of nacre, we measured reflection spectra of the samples. Figure 3 shows the reflection spectra of the pieces of nacre. The reflection spectra of the pieces of nacre were obtained by a charge-coupled device (CCD) multichannel spectrometer (BlueWave-VIS, StellarNet) attached to a microscope (Eclipse 50i POL, Nikon). The reflection spectra of nacre A and B have two peaks at 480 nm and 700 nm. The colors of reflection peaks of 480 and 700 nm correspond to blue-green and deep red, respectively. The peaks are caused by Bragg reflection from the periodic structure.

**Figure 1.** Schematic illustration of process of pearl farming.

**Figure 2.** Samples used in this study: (a) nacre A, (b) nacre B, (c) nacre C, (d) nacre D, and (e) green and red Akoya pearl oysters.
of the aragonite and conchiolin layers. The lowest point of reflectance is about 40% because light traveling in the nacre is strongly scattered by defects in the nacre. Due to the strong scattering, the color of the piece of nacre becomes whitish. Therefore, the colors of the pieces of nacre A and B would be slightly pink. Figure 3(c) shows the reflection spectrum of the piece of nacre C. The spectrum has a small peak at 540 nm and a large peak at 820 nm. The piece of nacre C also strongly scattered light, therefore, the base line of the reflection spectrum was about 40%. The wavelengths of 540 nm and 820 nm correspond to green and near infrared. The small green reflection and light scattering from the piece of nacre C show us the color of pale green, as shown in figure 2(c). Figure 3(d) shows the reflection spectrum of the piece of nacre D. The spectrum does not show a clear large peak, and the reflection at near 520 nm is slightly high. The reflection peak and color of the piece of nacre D is the most unclear in the four samples.

![Reflection spectrum of nacre A](image1)

![Reflection spectrum of nacre B](image2)

![Reflection spectrum of nacre C](image3)

![Reflection spectrum of nacre D](image4)

**Figure 3.** Reflection spectra of the pieces of nacre of Akoya oysters.

3. **SEM observation of nacre in Akoya oyster**

We observed periodic structures in the nacre of the samples by using scanning electron microscope (SEM) (S-3100H, Hitachi). To investigate the thickness profile of aragonite crystal layers, we observed the four samples from the bottom part to the top part of the pieces. Figure 4 shows the SEM images of the piece of nacre B from the top to the bottom. We arranged 42 SEM images in line to show the thickness profile of the piece. The upper left corner is the top part of the piece of nacre and the lower right corner is the bottom. As mentioned above, the thickness range of the aragonite layer is generally from 300 nm to 700 nm, and that of the conchiolin is from 10 nm to 20 nm. We could not distinguish between thin conchiolin layers and aragonite crystal layers from the SEM images because the SEM did not have enough resolution for observation of a thin conchiolin layer. However, the periodicity of the nacre B could be measured from these images. To obtain the thickness profile in the piece, we counted the number of aragonite crystal layers in all SEM images, and then we estimated the average layer thickness in each SEM image. The estimated layer thicknesses for all pieces are plotted.
Figure 4. SEM images of the piece of nacre B.

Figure 5. Layer thickness profiles of the pieces of nacre.

in figure 5. Since we just counted the number of layers, this estimated layer thickness corresponds to the total thickness of the aragonite crystal and conchiolin layers. Figures 5(a) and 5(b) show the thickness profiles of the pieces of nacre A and B, respectively. The layer thicknesses for nacre A and B are distributed at around 420 nm, and the thickness profile does not show a large variation through the top to the bottom. In contrast, the thickness profiles of nacre C and D had large variations, as shown in figures 5(c) and 5(d). In particular, the layer thickness of nacre D shows a large variation from 450 nm to 690 nm. Figure 6(a) shows the SEM images of the piece of nacre D, and figures 6(b)
and 6(c) are magnified views in two parts of figure 6(a). The numbers in figures 6(b) and 6(c) are the number of aragonite layers. As is evident from these numbers in figures 6(b) and 6(c), we can easily confirm that the variation of the layer thickness is about 1.5 times. These SEM images show us how large the thickness variation of the nacre of Akoya oyster as a natural structure is.

![SEM images](image)

**Figure 6.** (a) SEM images of the piece of nacre D. (b) and (c) are magnified views.

### 4. Calculations of reflection spectra from Akoya pearl oyster

In this section, we again discuss the reflection properties of the pieces of nacre to connect the relation between the measured reflection spectra and the thickness profiles obtained by SEM observation. From the SEM images, we confirmed that the layer thicknesses of the nacre A and B were thinner than those of the nacre C and D. However, the reflection color of nacre A and B was approximately red, and that of nacre C and D was pale green. The pieces of nacre A and B having thinner layers showed us the longer wavelength reflection in the visible region. The reason is that the colors of the pieces of nacre A and B are caused by the second order reflection and that from the pieces of nacre C and D are caused by the third order reflection. The peak wavelength of the $m$-th order reflection $\lambda_m$ is given by

$$\lambda_m = \frac{2(n_1d_1 + n_2d_2)}{m} \quad (1)$$

where $n_1$ and $d_1$ are refractive index and the thickness of the aragonite crystal layer, and $n_2$ and $d_2$ are refractive index and the thickness of the conchiolin layer. When $d_1 = 400$ nm and $d_2 = 20$ nm, $\lambda_2$ and $\lambda_3$ are 691 and 461 nm, respectively. These wavelengths approximately match to the reflection spectra in figures 3(a) and 3(b) and the thickness profiles in figures 5(a) and 5(b). Here, we assumed $n_1$ and $n_2$ are 1.65 and 1.55. When $d_1 = 500$ nm and $d_2 = 20$ nm, $\lambda_2$ and $\lambda_3$ are 856, and 571 nm, respectively. These wavelengths also approximately match to the reflection spectrum in figure 3(c) and the thickness profile in figure 5(c).
The reflection spectra of the four pieces were calculated by transfer matrix method using thickness profiles obtained by SEM observation. The transfer matrix method is widely used for analysis of a one-dimensional layered structure. The data of layer thickness was obtained by spline interpolation. Figure 7 shows the measured and calculated reflection spectra for the four pieces of nacre. Red curves are calculated spectra which were filtered by Gaussian filter to reduce high order interference effect. In figure 7(a), although the calculated peak positions were slightly mismatched with the measured positions, the shape of the calculated curve showed a good agreement with that of experiment. Since the thicknesses of layers in the nacre are not uniform, depending on the xy position, we consider that the cause of the mismatch in peak position is the difference in measured area between optical and electron microscopes. On the other hand, the peak positions of the calculated reflection spectra in figures 7(b) and 7(c) showed a good agreement with those of experiment. The calculated curve in figure 7(d) did not show a clear large peak. This characteristic is similar to the measured spectrum. We consider that the reflection spectra calculated by transfer matrix using measured thickness profiles qualitatively agree with the experimental results. If light scattering could be simulated, the color of nacre of Akoya oyster would be reproduced in calculation. We believe such simulation is useful for pearl farming and grading of pearls.

![Figure 7. Measured and calculated reflection spectra of the pieces of nacre of Akoya oysters.](image)

5. Conclusions
The reflection properties of the pieces of nacre of Akoya oysters were measured and the layer thickness profiles of the pieces were also observed by SEM. The layer thicknesses for red nacre were distributed at around 420 nm, and the thicknesses were approximately constant through the top to the bottom. In contrast, the thickness profiles of pale green nacre showed large variations. In particular, the layer thickness of nacre D showed a large variation from 450 nm to 690 nm. The reflection peaks in the measured spectra could be explained by Bragg theory and the results of SEM observation. Furthermore, the reflection spectra calculated by transfer matrix using measured thickness profiles
qualitatively agreed with the experimental results. At the present stage, light scattering in nacre cannot be analyzed well. If light scattering could be simulated, the color of nacre of Akoya oyster would be reproduced in calculation. We believe that this study will contribute to the development of pearl farming and grading of pearls.

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