

Optical setup for the simultaneous measurement of reflection spectrum and reflected-light direction under an optical microscope

S. Yoshioka, B. Matsuhana, and S. Kinoshita
Graduate School of Frontier Biosciences, Osaka University

E-mail: syoshi@fbs.osaka-u.ac.jp

The measurement of the angle-dependent reflection is one important way to optically characterize structure colors. The quantitative relation between the angle and the wavelength of the reflected light is closely related to the underlying physical mechanisms of reflection. Several optical methods measuring the angle dependence have been reported so far, such as the fiber rotation method, imaging scatterometer, and microscatterometry, which have been summarized well in the literature[1].

We are recently interested in the structural color of fish, since it has a unique optical characteristic that is not observed in the butterfly wing or the bird feather: the microstructure inside the iridophore cell of fish is controlled by the nervous system, and color of the cell can be varied. In fact, it is experimentally possible to induce the color change by immersing the skin of a tropical fish, neon tetra, in potassium-ion-rich physiological saline solution. Since the angle of the light-reflecting platelets is expected to change during the color change[2], it is desirable to simultaneously measure the reflection spectrum and the direction of the reflected light in real time from the small iridophore. For that purpose, we have prepared an optical system that works both as the microspectrophotometer and the microscatterometer[1,3]. In the following, we will describe the principle and the actual set up of the optical system in detail.

An epi-illumination optical microscope, BX-51 Olympus, was basically used, but some other optical components were attached to it. In particular, one beam splitter was inserted in the microscope tube, by which the split light is used to observe the image on the back focal plane of an objective lens. The image corresponds to the far-field pattern of reflection that expresses the direction of the reflected light. In addition, another beam splitter was introduced after the tube lens. One beam is used to observe the real image of the sample and the other is used for the spectral measurement by placing the tip of an optical fiber.

For the angle-dependence measurement, the illuminating beam should be almost collimated on the sample plane. Thus, the aperture stop unit of the microscope was replaced by the special one that has a small pinhole of 100 μm diameter. We have paid particular attention whether the pinhole image is precisely focused on the back (front) focal plane of the objective lens. To realize this, the pinhole unit has the translational degree of freedom along the optical axis.

We have applied the above optical system to the study of the color variation mechanism of neon tetra. It has been confirmed that the direction of the reflected light gradually changes as the color change, clearly demonstrating the usefulness of the system.

References

1. P. Vukusic, and D. G. Stavenga, *J. R. Soc. Interface* 6, s133-s148 (2009).
2. H. Nagaishi, N. Oshima, and R. Fujii, *Comp. Biochem. Physiol.* 95A, 337-341 (1990).
3. D. G. Stavenga, *J. Exp. Biol.* 205, 1077-1085 (2002) .