

Brewster angle microscopy with high spatial resolution

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A Brewster angle microscope is widely used for investigation of thin film and single-molecular layers on a substrate. In this microscope illumination light of p polarization is incident to a sample at a Brewster angle and reflected light is observed at the angle of reflection. Although most of light does not reflect from the sample at the Brewster angle, occasionally small portion of light is reflected back due to refractive-index change of a sample and/or light scattering from sample grains. Those reflected light can be imaged with the Brewster angle microscope.

We have developed a novel type of Brewster angle microscope based on a conventional epi-illumination microscope to investigate live cells. In our microscope, illumination light is incident through a high-numerical aperture objective at the Brewster angle and reflected light from a specimen is collected and imaged through the objective. The advantage of this microscope is as follows: 1) the objective lens is always positioned perpendicular to the specimen, accordingly the specimen can be observed with high spatial resolution. 2) Reflected light from the specimen is efficiently collected because of high numerical aperture. We have measured the equivalent reflectance of the actual setup as $\sim 10^{-5}$. As a consequence we expect that the reflection from the sample down to the equivalent reflectance can be imaged. We have confirmed that a single particle of 28 nm diameter could be observed with this microscope.

We have observed live cells with this microscope. We found that observed images changed with the incident angle of illumination. At the Brewster angle, very low level of reflected light from grains inside a cell could be imaged. The image was similar to a fluorescence image, but no fluorescence molecule was introduced in the specimen. We could observe that these grains were moving in the cell by taking time-laps images. At the angle of slightly smaller than the Brewster angle, the image was like a differential image, but the image contrast was much higher. We could observe much smaller structure in the cell. We expect that this microscope will introduce new type of imaging modality in modern microscope technology.