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Interfacial imaging between cell and substrate by localized surface plasmon microscopy toward live observation of cell adhesion sites

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1. Introduction

Cell adhesion sites, which play essential roles in activating cellular functions, such as signal communication, embryonic morphogenesis, and cell proliferation [1] have been intensively studied through microscopic observations. Although fluorescence microscopy is useful for these imaging, fluorescent labeling potentially affects behaviors of cells. Therefore, development of a method to image the adhesion sites without labeling is important.

Surface plasmon microscopy, which images refractive index distribution on the surface of metal is supposed to be a useful method because fluorescent labeling is not required. By employing a technique to localize surface plasmons in the optical diffraction-limited-region on the metal surface [2], spatial resolution required for the imaging of adhesion sites can be satisfied, while the conventional surface plasmon microscope based on the prism-coupling setup has insufficient spatial resolution due to propagation of surface plasmons.

In this presentation, we report on localized surface plasmon microscopy of the cell adhesion sites grown at the interface of cell and substrate. Toward live imaging of the adhesion sites, adaptability of the observation method to aqueous environment is also studied.

2. Microscope

Figure 1 shows the optical setup of localized surface plasmon microscope. Light from a He-Ne laser operated at the wavelength of 632.8 nm is expanded and given quasi-radial polarization. The illumination light is focused on the gold thin-film with the thickness of 47nm. The reflected light is recorded by using a CCD device placed at the optical conjugate plane of the exit pupil of objective lens to obtain a reflected spatial-frequency-spectrum. The recorded spectrum is processed to extract reflected light-intensity for a component of illumination light having a certain magnitude of spatial frequency, and the extracted intensity is given for a pixel value.

3. Experimental Result

Figure 2 shows the observed image of a mouse myoblast cell line (C2C12) on the substrate in dry condition. Spot like objects that are supposed as adhesion sites are observed around a converging point of fiber like structures.

Although spot and fiber like structures are successfully imaged, the same structures are not clearly imaged when the cell is observed in aqueous environment. It is because difference of refractive index between cellular structure and surroundings becomes smaller. For the imaging of this sample, it is required to increase the sensitivity of refractive index measurement. Therefore, we evaluate a substrate using a silver film. Since silver surface can be damaged quickly, the surface is coated by silica. From theoretical comparison, the substrate with silver film performs ~3 times higher sensitivity than the one with gold film. Experimental evaluation of the substrate fabricated by spattering and imaging trial with the silver substrate will be shown in the presentation.

References

[1] A. J. Ridley *et al*. Science, **302** (2003) 1704.
[2] K. Watanabe *et al*. Appl. Opt. **46** (2007) 4985.

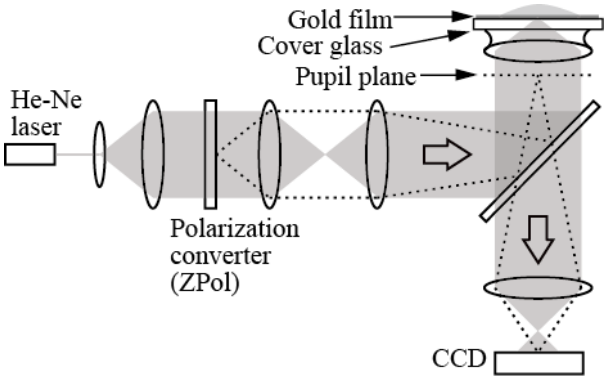

Fig. 1 Optical setup of scanning localized surface plasmon microscope.

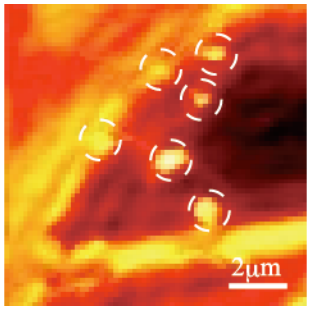

Fig. 2 Observed image of cell/substrate interface by localized surface plasmon microscopy. Spot like objects are observed around a converging point of fiber like structures.