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Clear Images of Neuronal Cells Cultured on a Plasmonic Dish Observed with the Inverted Fluorescence Microscope

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1. Introduction

A plasmonic chip, a periodic structure coated with thin metal films, was fabricated [1] and it was developed to the plasmonic dish by combining with a cell cultivation dish. The plasmonic chip can provide an enhanced electric field based on the grating-coupled surface plasmon resonance (GC-SPR) [2] and therefore, the enhanced fluorescence can be detected. In our previous talk, a plasmonic dish fabricated was applied to the cultivation of neuronal cells and the bright epi-fluorescence images of neurons were observed with the upright fluorescence microscope. In this study, the clear epi-fluorescence images of neuron dendrites cultivated on the plasmonic dish were observed with an inverted fluorescence microscope [3].

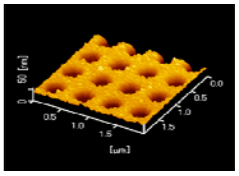

Fig. 1 AFM image of a plasmonic dish

2. Experimental

Fabrication of a plasmonic dish

A plasmonic chip was prepared by the sputtering depositions of silver and SiO_2 on a UV imprinted replica formed on a resin (TOYO GOSEI, PAK02-A). A mold of a quartz glass with a two-dimensional periodic structure (NTT-AT, JAPAN) was used. The pitch and hole depth of replica were 500nm and 30nm, respectively (Fig. 1). Finally, the plasmonic chip was attached to the cultivation dish after removing a cover glass from the bottom.

Culture of neurons

After all the accessories were sterilized by UV irradiation, polyethyleneimine solution was dropped into the well and incubated [4]. Hippocampal neuronal cells were separated from the 18-day-old embryos of a Wistar rat and scattered [4]. After cultivation more than 7 days, immunostaining was performed to fluorescently label the cultured neuronal cell. As for the primary and secondary antibodies, anti-microtubule-associated protein 2 (MAP2) rabbit-IgG, and Alexa Fluor 633-labeled anti-rabbit IgG antibody were used. Finally, it was sealed in a soft mount.

Fluorescence microscopy

Neuronal cells were observed with an inverted fluorescence microscope (Olympus) equipped with a Hg lamp, an electron multiplying charge coupled device (EM-CCD) camera (Luca-r, Andor), and 10x (NA = 0.3) and 40× (NA = 0.75) objective lenses.

3. Results and Discussion

Fig.2 shows the epi-fluorescence image of neurons cultured on the plasmonic dish taken with a 10x objective lens, in which two kinds of fluorescence images of cells cultured on the grating area and on the flat area were included. At a glance, the neuron's images on the former were brighter than those on the latter. The enhanced fluorescence was considered to be due to the enhanced electric field effect based on the GC-SPR. The fluorescence image of neuron dendrites will be further analyzed in the points of space resolution and fluorescence enhancement, and the signal-to-noise ratio of fluorescence images on the plasmonic dish will be also discussed compared with those on the conventional cultivation dish,.

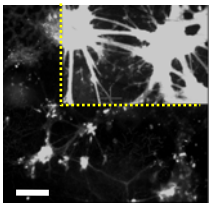

Fig. 2. Epi fluorescence image of neurons cultured on the plasmonic dish. The bar corresponds to 100μm. Dotted line shows the edge of a grating field.

4. Conclusions

Neuronal cells were cultured on the plasmonic dish for several weeks and the clear fluorescence images of neuron dendrites were observed with an inverted fluorescence microscope.

Acknowledgements

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