（別紙様式２）

# フォトンサイエンス国際卓越大学院プログラム（XPS）

# 光科学特別実習　報告書

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Subject of the training

Learning techniques for fluorescent single molecule experiments using motor protein kinesins.

Background

The main topic of my on-going research is the development of a two-photon excitation fluorescent microscope for single molecule imaging. Before conducting the experiments for the proof of the concept, suitable samples need to be prepared in order to prove that single molecule imaging can be done with my microscopy method. Concretely, samples were needed on which fluorescent single molecules (not forming oligomers) were fixed stably or moving linearly near the glass surface for simple analysis. For experiments in which fluorescent molecules are fixed to glass surfaces, I tried a method in which dye solution was poured into a chamber and left to stand, and the dye molecules were absorbed on the glass surface. However, this method was not suitable for my experiments because it was difficult to confirm that the absorbed molecules are monomers, not form oligomers. Therefore, it was necessary to prepare samples using a different method.

Kinesins, a kind of motor proteins, move along microtubule filaments and play a variety of roles in living cells, including miosis, meiosis, and cellular cargo transportation such as vesicles, organelles and chromosomes. Kinesins have been used in in vitro experiments and the property of kinesins like activity and movement velocity has been studied well. By using kinesins and microtubules, suitable experimental conditions can be created not only where single fluorescent molecules move linearly on the glass surface but also where single molecules are stably fixed near the glass surface achieved by protein mutations. Experiments with motor proteins are difficult to start alone, so it is better to learn the secrets from an experienced person. In order to prepare suitable samples using kinesins and microtubules, I decided to learn these techniques from an experienced molecular cell biologist.

Contents and results of the training

This training was hosted by Dr. Taketoshi Kambara, a senior research scientist in the laboratory for cell polarity regulation, RIKEN center for biosystems dynamics research (RIKEN BDR). He is an expert in molecular cell biology who has performed many experiments using motor proteins, so he was well suited for the host of the training, and he kindly accepted the host. I visited to RIKEN BDR Quantitative Biology Buildings, Osaka, worked together with Kambara-san, and learned a lot of things about the experiments using kinesins and microtubules.

First, I worked on microtubule polymerization experiments. The surface of the glass slide was treated properly to ensure stable biding of the microtubule filaments to the glass surface. A flow chamber was created on the glass slide using double-sided tape and cover glass. Purified tubulin was mixed with an appropriate solution to form filaments, then was poured into the flow chamber and placed stationary until it was absorbed on the glass surface. Fluorescently labeled tubulin was mixed, so it could be confirmed with fluorescent observation whether the tubulin filament polymerization was successfully achieved. I used a total internal reflection fluorescence (TIRF) microscope, with which a thin region (< 200 nm) from the surface can be observed, and I checked that microtubule filaments were polymerized on the glass surface. The picture of the TIRF microscopy I used is shown in Fig. 1.

After the microtubule filament polymerization was confirmed, I worked on experiments using fluorescently labeled kinesins. Kinesins were flowed into the flow chamber along with a buffer with an appropriate concentration of ATP. With TIRF microscopy observation, it was confirmed that fluorescent molecules bound to kinesin were moving along the microtubule filaments on the glass surface. After the kinesin movements were observed, I worked on experiments using kinesins with mutation to lose motor activity. After the mutated kinesin was poured into the flow chamber, I observed that the fluorescent molecule bound to the mutated kinesin did not

move and stayed on microtubule filaments.

Fig. 1. TIRF microscopy in RIKEN BDR.

Outlook and closing remarks

Unfortunately, due to lack of time, something has been left to learn. The fluorescent dye used for fluorescent labeling were provided by the host. I planned to be taught the procedure for using fluorescent dye of my choice. I received the protocol of tagging the motor proteins and bind desired fluorescent dyes to the motor proteins. The host and a researcher acquainted with him in UTokyo will support me when I am stucked.

The next step is to show that signal from single fluorescent molecule can be detected with my microscopy. By using the sample preparation methods learned in this training, I will create a sample on which single fluorescent molecules are bound near the glass surface and prove that the target is a monomer by analyzing the fluorescent photon signals.

At the end of this report, I would like to express my gratitude to Dr. Kambara for his kind support, teaching me a lot about experiments during my training course.