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Title	Force Measurement of Kinesin Propelled Microtubules in Swarming Using an Electromagnetic Tweezer [an abstract of dissertation and a summary of dissertation review]
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学 位 論 文 題 名
Force Measurement of Kinesin Propelled Microtubules in Swarming Using an Electromagnetic Tweezer
(電磁ピンセットを用いたキネシンにより推進する微小管の群れにおける力測定)

Swarming of living organisms such as fishes, birds, and ants is a fascinating display of coordinated behavior in nature. It added several advantages such as parallelism, robustness, and flexibility, and permits exhibiting of emergent functions to the organisms. Such attractive features of the swarming have been motivating researchers in material science to mimic the swarming of living organisms by self-propelled objects in artificial environments. Recently our group has successfully demonstrated the swarming of microtubules (MTs) as a self-propelled biomolecular machine, on a kinesin-modified substrate by the association of a DNA single strand that is modified on the surface of MTs. In the presence of ATP, the swarms of MTs exhibited translational and rotational motion due to ATP hydrolysis by kinesins. They have great potential to be a power generator to work for nanotechnological applications, such as molecular machines and molecular robotics. To ensure real applications it is a prerequisite to evaluate the amount of work harnessed from the MT swarms. In this dissertation, I determined the force of the MT swarms generated by the kinesin on the substrate by the force measurement using an electromagnetic tweezer and a magnetic bead conjugated to the MT swarms. Furthermore, the fine structure of the MT ring swarms was investigated by the combination of high-speed atomic force microscopy (HS-AFM) and fluorescence microscopy.

In **Chapter 1**, the purpose of the dissertation and the background of the study have been explained.

In **Chapter 2**, I reported the configuration and setup of a fluorescence microscope and a customized electromagnetic tweezer with a micro-actuator for the fluorescence microscope observation under a controlled magnetic field (Figure 1). The force calibration was performed systematically by applying the magnetic beads in the liquid media by the electromagnetic tweezer. The bead was further attached to the MT swarms in a flow cell, and I observed the trajectory of the MT swarms by applying the magnetic field to the beads on the MT swarms. This study will enrich the understanding of the use of electromagnetic tweezers in biomolecular manipulations and dynamic control over the MT swarms.

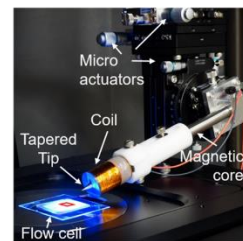


Figure 1: Configuration of the electromagnetic tweezers.

In **Chapter 3**, a new approach was taken to determine the force of kinesin on the substrate to propel the MT swarm with a magnetic bead by utilizing the system described in Chapter 2. The electromagnetic field was applied to the ring-shaped MT swarms (MT ring swarms) attached with a magnetic bead by the electromagnetic tweezers. The continuous circular motion of the magnetic bead on the MT ring swarms allowed the tweezer to apply controlled positive and negative force dependent on the angles between the tweezer's tip and the magnetic bead (Figure 2). The applied force changed the velocity of the swarm ring. After the calibration, I determined the force of the MT ring swarms generated by ATP hydrolysis on the kinesin substrate. I systematically compared the forces of the MT ring swarms with different ring sizes. The force increased with increasing the size of MT ring swarms. This increase in force arose from the larger number of

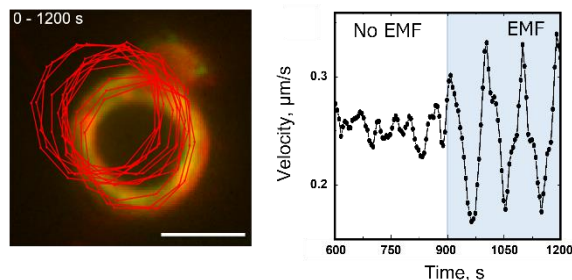


Figure 2: (a) Trajectory of the bead-loaded swarm in the absence and presence of EMF. (b) Velocity of the bead in absence and presence of EMF. Scale bar 10 μm .

active kinesins propelling the MT ring swarms. The estimation of the force of the MT swarms will widen applications of the MT swarm in nanotechnology as well as robotics.

In **Chapter 4**, I investigated the detail of the structures of the MT ring swarms and evaluated the number of MTs in the MT ring swarms by the combined system of HS-AFM and fluorescence microscopy (Figure 3). A combined HS-AFM and fluorescence microscopy in a single machine was used to investigate the packing and alignment of MTs in an aqueous medium. It was found that in MT ring swarms, many MTs self-organized not only horizontally but also vertically to the substrate to form a multiple-layered structure, providing new insight into the structure of the MT ring swarms and the alignment of MTs in the swarms. This will be indispensable in assessing the force associated with the MT swarms and allow for further regulation of the force by designing their structure, which will consequently further their applications.

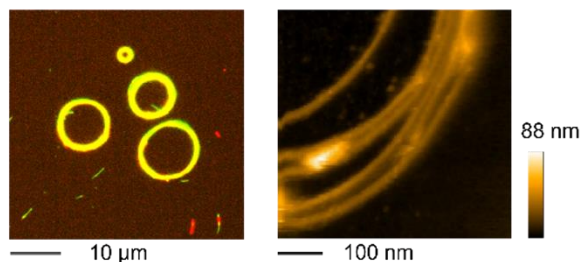


Figure 3: (a) fluorescence microscopy image. (b) and high-speed atomic force microscopic images of the swarm ring

In **Chapter 5**, all the important results are summarized, and future perspectives have been described. In this dissertation, I focused on the investigations of MT swarms. The fluorescence observation system under the controlled electromagnetic field by the electromagnetic tweezer was developed to manipulate the MT swarms with a magnetic bead. The force of the MT ring swarms generated by ATP hydrolysis on the kinesin substrate could be determined by the change of the velocity of the MT ring swarms perturbed by the electromagnetic force between the electromagnetic tweezer and the magnetic bead on the MT ring swarms. As a more detailed structure of the MT ring swarms, the combined system of HS-AFM and fluorescence microscopy revealed that many MTs self-organized not only horizontally but also vertically to the substrate to form multiple layered structures. Consequently, the outcome of this dissertation contributes to the handling and understanding of MT swarms. Especially, the structural study of the MT ring swarms may generate new ideas for designing future biomolecular devices to perform nanotechnology tasks.