Optically driven micro-robot for measuring three dimensional adsorption force of cells

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Introduction: The optical tweezers or optical trapping effect has widely utilized for manipulation and mechanical measurement of cells [1-2]. This effect has two advantages in that it can be used both for the manipulation of transparent object including cells in the largeness range of 5nm up to several µm as well as for mechanically measuring interaction force in pN order. Therefore, optical tweezers are preferably suited for exerting forces on biological systems and for observing interactions [3-4]. For instance, the interaction forces between bone cells [4] and implant surfaces and observation of fluorescence during manipulation of cell [3] were investigated.

Although the optical tweezers has conventionally maneuvered micro-beads or cells for the above measurement and observation, it has two problems: a low degree of freedom in designing shape and irradiating strong IR laser close to cell. These are significantly disadvantages. Hence, we have working on “optically driven micro-robots” as an innovative device for manipulating and mechanically measuring cell by the optical tweezers. Our micro-robots are made of transparent photocurable plastic fabricated using two-photon micro stereolithgraphy with in submicron resolution. The micro-robot’s advantages lies in that the micro-robot can change contact-area and the micro-robots are designed so that IR laser irradiate far from target cells. Furthermore, in previous work, we have developed a method which modified tip of the micro-robot with cell anchoring molecules such as oleyl group [5]. By this method, we can measure interaction force between cell and some materials. Conventional measurement system, however, cannot mechanically analyze three dimensional (3D) adhesion force.

In this work, we aimed at 3D observation and mechanical measurement system using our micro-robots for cell biology. No method has simultaneously realized both high-speed observation and mechanical analysis of cells in 3D. Hence, it is difficult to accurately measure interactions of the cells in order that cells are 3D structures. By realizing 3D measurement system, we can measure 3D interaction force such as interaction between human bone cell and medical implant materials.

Tapered Markers for 3D Tracking: Firstly, in order to acquire the 3D location and posture of the micro-robot through a single 2D image from a fixed camera, we worked out a new method which micro-robot is attached tapered cylindrical marker triplets. The markers were located in the same plane, and not in a straight line. Additionally, the markers were made of quantum dots incorporated fluorescent photocurable resin for high-speed image processing, where quantum dots are utilized as a fluorescent marker equipped with minimum photobleaching characteristics. The 6 dimension of freedom position of the micro-robot could be acquired from the markers diameter observed through the microscope image. Linear correlation of the markers between the marker size and vertical position is -2.00[µm/pixel]. Moreover, the rate between two different vertical positions will not be changed even when photobleaching progresses, preserving the effectiveness of the observation for up to 60 minutes. Thus, this technique can mechanically analyze the cell for a long time.
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**3D Force Measurement System:** Secondly, we developed a real-time 3D cellular reactive force sensing and 6-D.O.F. tracking systems by aforementioned method. Previously, the micro-robot’s user-friendly 3D image was displayed by digitally uniting a 3D coordinate data acquired from the above method with the predesigned micro-robot 3D data on computer. Subsequently, applied force to the cell was calculated by monitoring displacement between the laser focus and trap-point center captured by optical tweezers on the micro-robot. The relationship between the two has been calibrated in previous reports. In the case of this report, the 3D position of the trap-point was calculated in reference to the three markers described above. Furthermore, the system was analyzed and displayed with close to none time lag, meaning that it can be adapted to various applications with few restrictions.

**Demonstration:** Finally, we demonstrated cellular reactive force measurement in 3D by an experiment using a yeast cell with the aforementioned system. In this demonstration, we used specialized optically driven microrobot for pushing a single living cell. Before measurement, the surface of the micro-robots needed to be hydrophilized by a surfactant since the micro-robots are originally hydrophobic and cannot be driven in liquid. Then, the micro-robot is placed in a liquid suspension which contains the target cell. In the experiment, a single yeast cell was repeatedly compressed and the applied force was measured to analyze the mechanical characteristics of the cell. The actual frame rate was between 30-50 fps and can be enhanced with improved equipment. According to this experiment, when optically driven microrobot strongly pushes to cell, we understand that the microrobot runs upon the yeast cell from Z-axis reactive force. This result cannot get from conventional methods.

**Summary:** Owing to the simple methods, the new developed system was successfully established. The fact that it only requires a single plane image to specify the location and posture of the micro-robot, and that the observation is carried out through a conventional microscope setup with a single camera, the method has proved to be versatile and widely applicable.

**References**


