Adjuster for Repeatable Targeting of Local Part of Cell at Stage of Microscope for Biochemical Analysis

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ABSTRACT

Biochemical analysis with molecular biological technique at local area of the biological cell is much more informative than that at the entire area of the tissue. A devise of arms for adjusting a position of biological specimen has been designed for repeatable sampling of a local target part of a biological cell at a stage of a microscope. The space for the device is limited to the small area surrounded by a laser irradiator and by a needle for collecting sample. The main part of the manufactured device is made of aluminum, although the screw is made of stainless steel. The device has been applied to the glass plate or to the culture dish on the stage of the microscope. After the local part of the cell was cut and taken off for the sample segment, the specimen was re-adjusted for the next sampling. The local part of the biological tissue on the glass plate has repeatedly been targeted with the designed device within 0.05 mm. The device is available for both glass plates and culture dishes to be re-adjusted on the stage of the microscope to be focused at the same targeted micro area in the advanced laser micro dissection system.

Keywords: Biomedical Engineering, Biological Cell, Microscope, Laser and Local Target.

1. INTRODUCTION

Protein relates to the biological function. Analysis of protein is useful to trace the behavior of biological cells.

Biochemical analysis is useful to detect protein in the biological tissue. The conventional technique, however, is not enough sensitive to detect local distribution of protein within a cell. In the conventional technique, protein in the biological tissue is detected after homogenization.

An aggregate of protein might induce neurological diseases. The aggregation exists at a local part in a tissue. The concentration of the protein decreases after homogenization of the entire tissue, which asymmetrically includes the protein. The low concentration makes difficult to detect protein in the biochemical analysis. The concentration of protein can be accelerated, if the sample is collected from the targeted micro space, which densely includes the protein.

If the position of the micro dissection is traceable, the distribution of protein can be identified. The identification of localization of protein may useful to investigate mechanism of diseases.

The advanced laser micro dissection system [1-3] has newly been designed and applied to biochemical analysis for neurological disease. In the present study, an adjusting device has been designed for repeatable sampling of a local target part of a biological cell at the stage of the microscope of the advanced laser micro dissection system.

2. METHODS

Laser Micro Dissection System

The laser micro dissection system consists of a laser oscillator for dissection, a microscope for observation, and an aspirator for sample collection. In the system, the sample is cut at the perimeter of targeted micro segment with the laser beam under microscopic observation, and specified micro dissection is collected by the aspirator with the micro needle capillary. The dimension of the laser beam focused on the specimen is 0.005 mm.

The space for the adjusting device is limited to the small area surrounded by the laser irradiator and by the needle for the biological micro sample to be collected (Fig. 1).

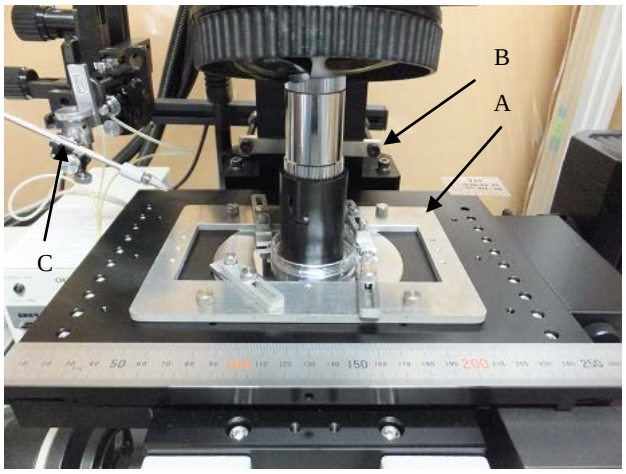

Fig. 1: Adjusting device (A) surrounded by the laser irradiator (B) and by the collector needle (C) on the stage of the microscope.

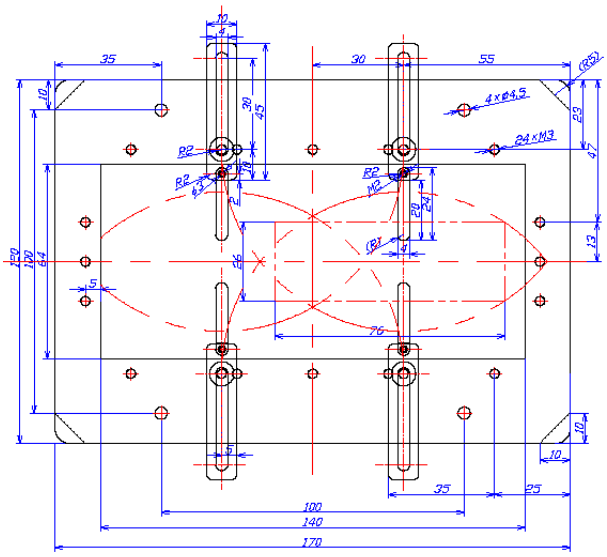

Fig. 2: Device for adjustment. Numbers are millimeter.

Device for Adjustment

The adjusting device consists of a stage and several arms (Fig. 2). The stage is made of aluminum and fixed to the original stage of the microscope with screw of M4. The stage has a rectangular center hole, where a glass plate or a culture dish is placed.

The arms are made of aluminum. They are placed around the hole. These arms are fixed on the stage with screws of M3. Both the internal and the external screws are made of stainless steel to get enough strength for clamping. The internal screw is inserted in the stage of aluminum. Each arm has rectangular hole, through which the screw is inserted. The screw can slide along the hole. The position of fixation of each arm is adjusted with variation of position between the arm and the screw. The position of these arms traces that of the glass slide or the culture dish.

Each arm consists of two rods: a forearm and an upper arm. These two rods are connected with a screw of M2, with which the angle between the two rods varies.

Adjusting Position of Glass Slide

To adjust the position of a glass slide, one of the following methods is alternatively applied: the method with two arms, or the method with three arms.

The method with two arms is as follows. When one of the rims of the glass slide is facing along the rim of the thin forearm of one of the arms, the neighbor rim of the glass slide is touching to the tip of the thin forearm of the other arm.

The method with three arms is as follows. When one of the rims of the glass slide is simultaneously touching to the tips of the thin forearm of two arms, the neighbor rim of the glass slide is touching the tip of the thin forearm of the third arm (Fig. 3).

These thin forearms are useful to save spaces for approaching to the thin glass slide.

Adjusting Position of Culture Dish

Two arms are applied to the culture dishes, which has a notch on the outer cylindrical wall. When the notch is touching to the tip of the short forearm of one of the arm, the other point of the outer cylindrical wall is touching to the tip of the short forearm of the other arm. These two points of contact should not be on the line of the diameter of the dish (Fig. 4). The short arms have enough height to fit to the notch.

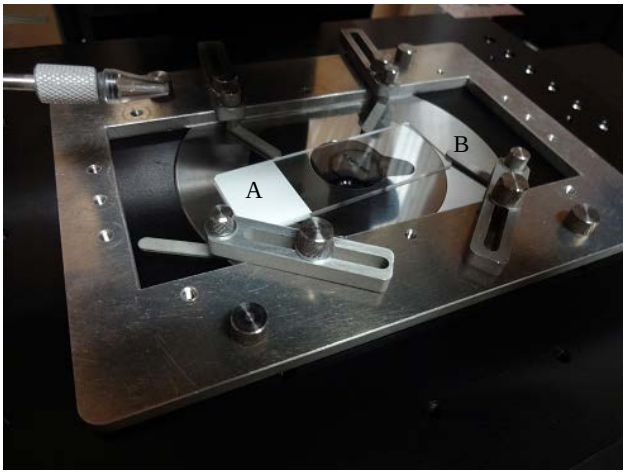

Fig. 3: Glass slide (A) adjusted with three forearms (B).

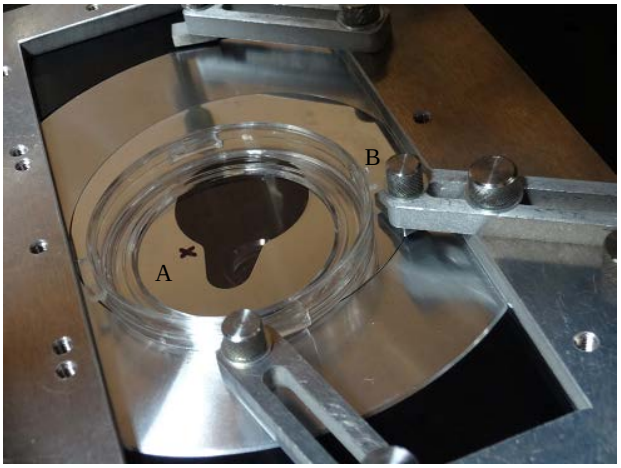

Fig. 4: Dish (A) adjusted with two short forearms (B).

Trial for Adjusting Position

The glass slide with a freeze-dried biological specimen was set with the designed device. Once the position of the arms is set at the first trial, the position of the arms is maintained with tightening the screws of the device. A target area was dissected with the laser beam. A droplet was supplied with the micro pipette to drift the dissected micro sample. The dissected micro specimen was collected with the micro needle capillary. The position of the glass slide was memorized with the position of the arms. With the same position of the arms as the first sampling, the position of the slide was successively readjusted on the stage of the microscope.

By the device, the glass slide was tried to be adjusted to the same position as that of dissection. The displacement between the targeted point at the second trial and the dissected point at the first trial was measured (Fig. 5).

The displacement was measured in several tests. Each test consists of two successive trials of adjustment of the position. The displacements were quantified as coordinates of two dimensions in x-y plane, when the position of the first trial is set as the origin. The x-y plane is defined in parallel to the stage of the microscope. The direction of x axis is defined in parallel to one of the rim of the stage. The direction of y axis is defined in perpendicular to that of x axis, and in parallel to the stage.

3. RESULTS

In Figs. 5-7, the painted dye of red is irradiated by the laser for demonstration.

Figs. 5 & 6 exemplify the microscopic viewing fields of successive adjusting of dish with the designed arms. The same target in the biological tissue is able to be easily traced in the figures.

Fig. 5: Distance between the dissected point at the first trial (circle) and the targeted point at the second trial (square) of successive adjusting of dish. The square shows the target point for laser beam. The arrows show x and y coordinates, respectively.

Fig. 6: Distance between the dissected point at the first trial (circle) and the targeted point at the third trial (square) of adjusting of dish. The square shows the target point for laser beam. The arrows show x and y coordinates, respectively.

Fig. 7 exemplifies the microscopic viewing fields of successive adjusting of dish with the designed arms. The same dissected area (square) is able to be easily traced in these figures.

Data of displacement are summarized in Figs. 8 & 9. Data are scattered and smaller than 0.05 mm. Each datum shows the distance between the cut area and the successively focused area. The local part of the biological tissue on the glass plate has repeatedly been targeted with the designed device within 0.01 mm (Fig. 8). The local part of the tissue on the dish, on the other hand, has been targeted within 0.05 mm (Fig. 9). The dimension is smaller than that of cells.

4. DISCUSSION

Laser has widely been used in a lot of applications: to measure micro movement [4], and to cut the biological tissue [5]. Scanning system is important to trace target in microscopic studies [6]. In the trials of the glass slide, displacement of 0.01 mm is smaller than width of 0.06 mm in the observation area of the microscope. In the trials of the culture dish, displacement of 0.05 mm is also smaller than width of 0.06 mm in the observation area of the microscope. The displacements are smaller than 0.1 mm of the viewing field for microscopic observation.

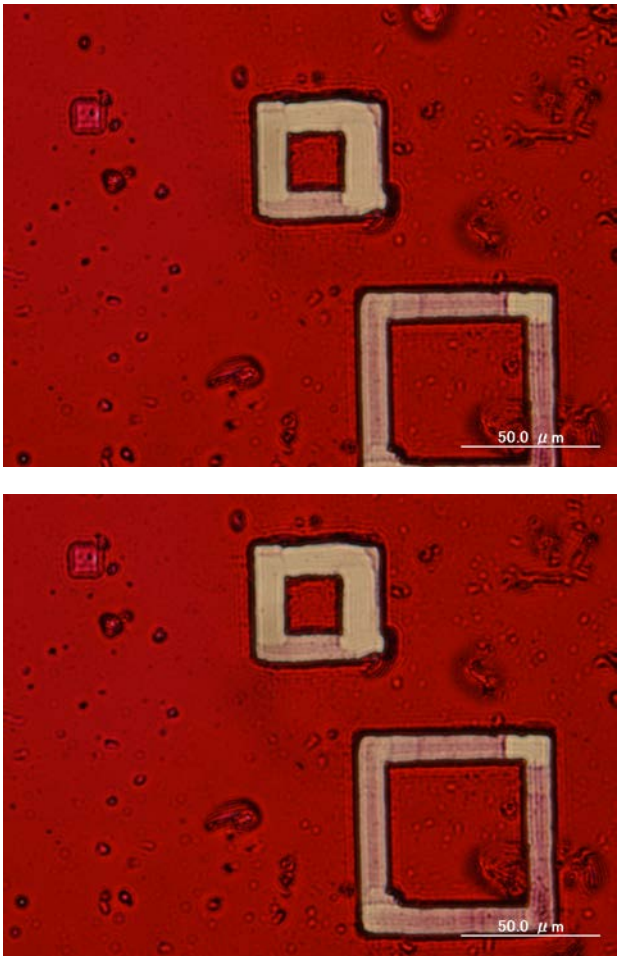

Fig. 7: Microscopic viewing fields of successive adjusting of dish: the first trial (upper) and the second trial (lower).

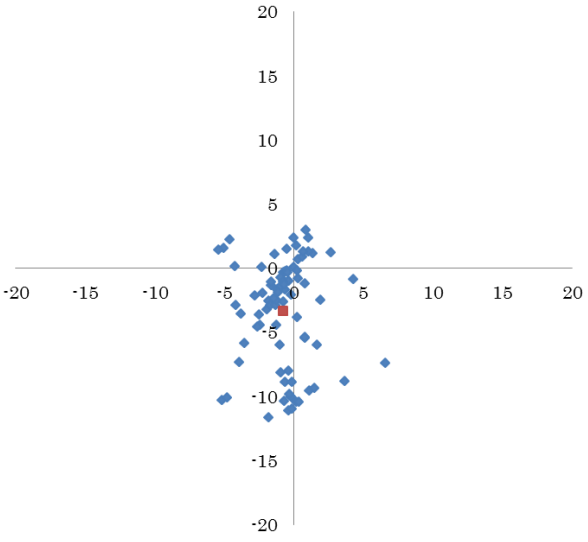

Fig. 8: Distance between the dissected point at the first trial and the targeted point at the successive second trial on the glass slide. Displacements are shown with the combination of x and y coordinates. Numbers show micrometer. The origin shows the dissected point at first trial.

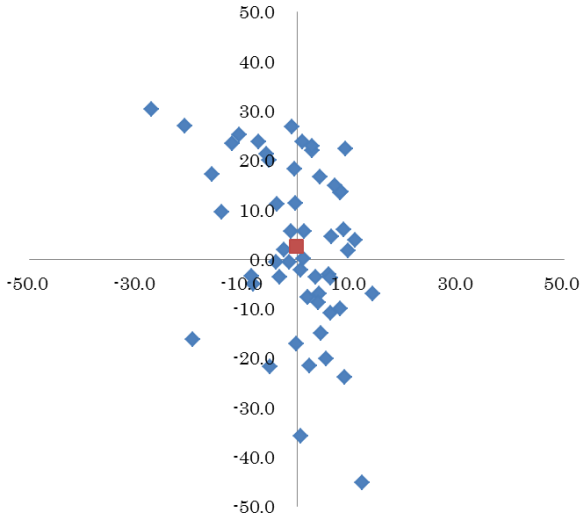

Fig. 9: Distance between the dissected point at the first trial and the targeted point at the successive second trial on the dish. Displacements are shown with the combination of x and y coordinates. Numbers show micrometer. The origin shows the dissected point at first trial.

Micro debris adhered on the rim of the glass slide might cause the micro displacement of the readjusted position. The position of the forearm is not stable, when the friction between screws is low. Stainless steel is selected for the material of the screw to strengthen the morphology of the surface of the screw against strong tightening of the screw. The larger diameter of the screw is selected to get higher stability of the arm. The stability of the arm is improved, when the diameter of the screw increases.

Micro debris adhered on the outer wall of the culture dish might also cause the micro displacement of the readjusted position. The fitting between the notch of the dish and the tip of the forearm affects the preciseness of the adjustment of the position of the dish on the stage of the microscope.

While the micro dissection is collected, the operation produces several forces. When the droplet of liquid is supplied on the glass slide to drift the dissected micro sample, vibration can be occurred on the glass slide. The vibration generates horizontal forces against the arms. When the position of the glass slide is readjusted on the stage, the sliding action produces horizontal forces against the arms. The position of the arms has successfully been fixed with enough strength on the stage of the microscope with the screws (M2, M3, M4) of stainless steel of against these forces.

The micro technology for biochemical analysis contributes to evaluation of behavior of biological cells on engineered surface [7]. The dimension of 0.05 mm for readjustment is the same order of cells, and available for analysis of local part of tissue.

5. CONCLUSION

A devise of arms for adjusting a position of biological specimen has been designed for repeatable sampling of a local target part

of a biological cell at a stage of a microscope. With the manufactured devise, the position of the target point of biological sample has been adjusted in the area within 0.05 mm. The devise is applicable to adjust the position on the stage of the microscope.

6. ACKNOWLEDGMENT

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