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Low-Cost and High-Precision Stepping Motor Controlled Multi-Point Automatic Time-Lapse Microscope

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Abstract **Background:** Presently, time-lapse imaging is widely used in many fields in science. In the field of cell biology, it is frequently used in wound healing assay to access the migration and proliferation of cells. To assess the effect of drugs on cell migration and proliferation, it is important to obtain the time-lapse image of many conditions in one experiment. However, the commercially available motorized stage with the control program is expensive. The aim of this study is to make a hand-made multipoint time-lapse microscope using a stepping motor. **Method and Result:** To control the stage of the microscope, we used the XYZ-stage of a computerized numerical control (CNC) router. Using open-source software GRBL for Arduino®, we could easily control the stage by the stepping motor at a 1.0 μm resolution. We also developed the autofocus function in this system. Finally, we could obtain images of the cells at many different conditions for at least 48 h with good focusing. **Conclusion:** The hand-made multipoint time-laps microscope with open-source software GRBL is a low-cost and good enough precision for cell migration and proliferation study.

Key words: time-laps microscope, stepping motor, motorized stage

Introduction

Time-lapse photography reduces time by playing back pictures taken at regular intervals as a movie. It is used in a wide range of fields such as art and science. It is also used in the field of cell biology for applications such as wound healing assays.[1,2] It is an excellent imaging method that can capture cell division, proliferation, migration, etc. over time. In this study, we established our original time-lapse microscope system, which can install any inverted microscope. Additionally, we implement a function that automatically patrols multiple points and adjusts the focus.

Materials and methods

Multi-point automatic patrol stage

Reproducibility is important for time-lapse imaging. The commercially available motorized stage uses a piezo motor for the high precision motorized stage. We employed the XYZ stage for a computerized numerical control (CNC) router with the stepping motor for our motorized stage. Stepping motors were attached the Proxon's milling machine MF70® (Fig. 1) so that the stage could move the XYZ-axis with micron scale resolution at a lower cost. The XYZ-axis stepping motor was controlled from a personal computer via a microcomputer board (Arduino®) with

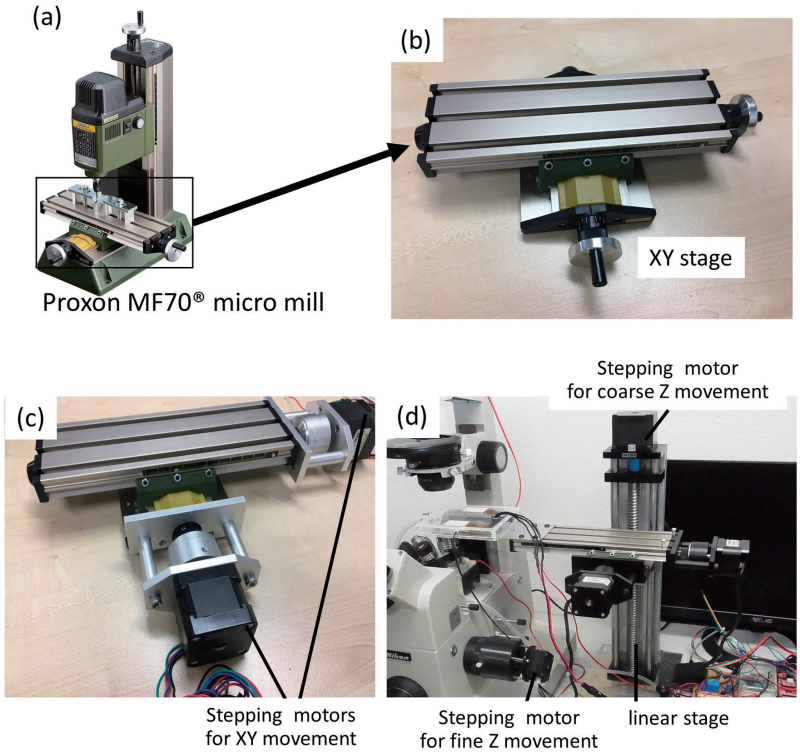

Fig. 1 Building motorized stage.
(a), (b): We used XY stage for Proxon MF70® micro mill. (c): We attached stepping motor to the XY stage. (d): For the Z axis linear stage (a high precision positioning device) with stepping motor was used for coarse Z movement, and the stepping motor attached to microscope fine adjustment knob was used for fine Z movement.

a stepping motor driver (A4988). The open-source general-purpose program GRBL[3] was used for controlling stage, and the GRBL in Arduino® was controlled from the custom made PC software. The Arduino® was equipped with a GRBL shield with an A4988 stepping motor driver, each of which was connected to a stepping motor on the XYZ axes. The GRBL command was transmitted from the PC to the Arduino® via USB. The light source of microscope was replaced with a 5W LED for both the phase difference and fluorescence so that it would only light up during shooting. This control was also conducted from a PC via Arduino®. Fig. 2a shows a conceptual diagram of this system.

Incubation chamber on the stage

A culture chamber was created on the XYZ stage. Acrylic board with a thickness of 3 mm was cut using a laser cutting machine to assemble a culture chamber (Fig. 3). Regarding the CO_2 supply into the culture chamber, CO_2 was placed in a gas sampling bag and the solenoid valve was controlled by an Arduino® and a relay. When the gas sampling bag is filled with gas, high pressure is applied at first. Therefore, a program was set to shorten the supply time and gradually increase it. A PTC heater was used to control the temperature inside the culture chamber. The humidity was controlled by installing a container filled with water in the chamber and evaporating the water at the temperature of the heater. The water vapor evaporated by the heat of the heater becomes water droplets and adheres to the lid of the chamber. These water droplets not only darken the field of view, but also prevent the phase contrast from working well. Therefore, by installing two heaters on the lid on the top of the chamber, water droplets were prevented (Fig. 3).

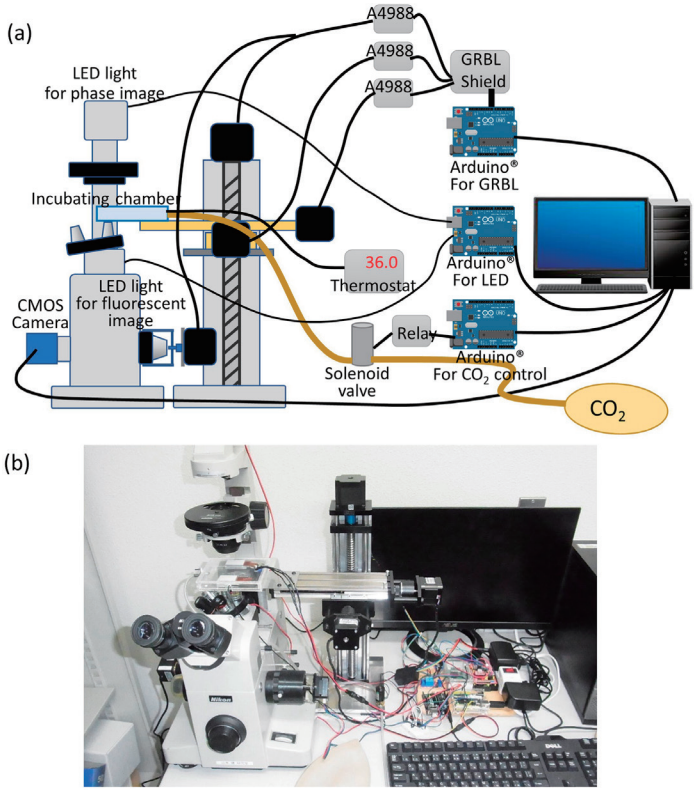

Fig. 2 Schematic diagram of the entire microscope system and whole image of the system.
(a): We use 3 Arduino®s. Each Arduino® is connected to PC by USB2.0 cable. First Arduino® is for stepping motor control through A4988 motor driver, second Arduino® for LED light source control, and third Arduino® is for CO_2 control by opening solenoid valve. CO_2 is supplied from the gas collecting balloon connecting to the solenoid valve. (b): Our stage was set to Nikon Diaphot®. The XYZ motion of the stage can be controlled by the PC. GRBL:open-source firmware for Arduino® to control stepping motors of XYZ axis originally developed for CNC router.

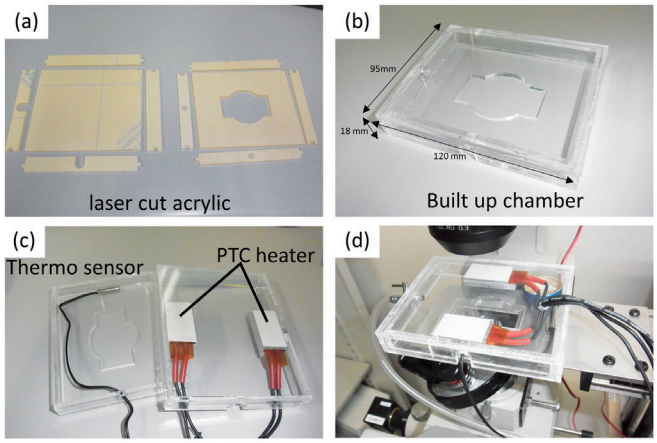

Fig. 3 Building incubation chamber.
(a): An acrylic board with 3 mm thick was cut by the laser cutter. (b): Laser cut acrylic parts were build up. (c): On the lid, two PTC heater was set and thermo sensor was set at the bottom. (d): The chamber was set on the XYZ stage. PTC heater: a heater with positive temperature coefficient which has a characteristic that the electric resistance of the heater increases as the temperature rises.

CMOS camera

A C-mount CMOS camera with a SONY IMX322® sensor was installed on the microscope. The camera was connected to the PC by a USB cable. The highly sensitive IMX322® COS sensor makes it possible to obtain the fluorescent image of the cells.

Dish selection

To obtain many different conditions of the cells by this microscope, we tried the 4-well chamber. However, we could not obtain a good image with a 4×objective phase contrast with the regular 4-well chamber. The deterioration and uneven brightness is caused by the meniscus.[4,5] Therefore, we choose the anti-meniscus 4-well chamber produced by ibid® (Fig. 4).

Focus tracking

To improve the out-of-focus, we took multiple pictures that were shifted in the Z-direction in a preset step. The most focused image was automatically selected from the pictures. Fig. 5 shows the mechanism for automatic focus tracking. First, seven photographs were taken in the vertical direction in the preset step set with the Z-coordinate registered first as the center. In this study, the distance between the photographs in the Z direction was set as 1 μm. This group of photographs in the vertical direction is referred to as the Z-stack. Next, the focus value indicating the degree of focus in each photograph was obtained using a method referred to as Canny Edge Detection.[6] In this method, a Gaussian filter is applied to the image to remove high-frequency noise; edge detection is used for high-frequency noise. Because it is sensitive, it is possible to detect edges by removing noise. Next, we calculated the intensity and direction of the brightness gradient in the image using a Sobel filter; the peak was detected as an edge. When the image was in focus, many edges were detected. The total edge in the image was quantified and used as the focus value. For each photo of the Z-stack, the focus value was calculated by the method described above.

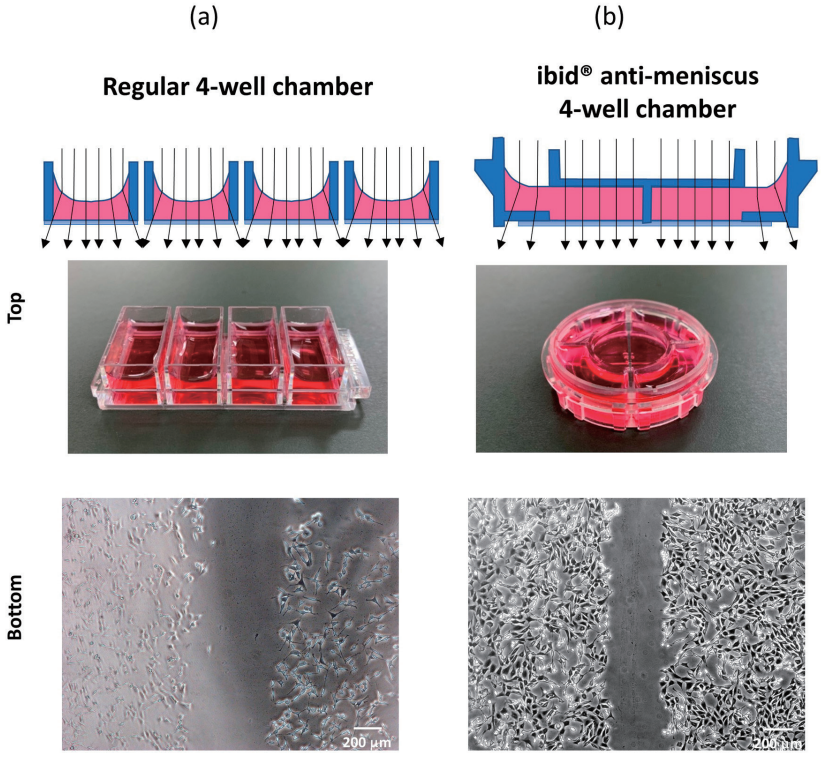

Fig. 4 Influence of meniscus on phase contrast image of cells.
Top: (a) In regular 4-well chamber, both ends have a raised liquid level. (b) However, ibid® anti meniscus 4-well chamber shows flat liquid level at least in the center of the dish. Bottom: Phase contrast image is much better in ibid® dish.

The image with the highest focus value was automatically selected and saved in a folder. The rest of the photos were also saved in a separate folder for backup. When patrolling other shooting points and then shooting the same point, seven Z-stacks were shot vertically around the Z-coordinate, which had the highest focus value the previous time. Subsequently, by repeating this operation, we added a function to automatically track the in-focus Z-coordinate (Fig. 5).

Cell for the experiments

The mouse aortic vascular smooth muscle cell line (MOVAS) was used in this study. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with high glucose (Wako) supplemented with 10% fetal bovine serum (BioWest S1400-500) at 37℃ in a 5% CO_2 humidified atmosphere. The cells were grown to 70% sub-confluent condition and then starved to synchronize for 2 h in DMEM containing 0.2% FBS before wound assay.

Data analysis

All data were shown with mean ± SD.

Results

The proposed microscope system is shown in Fig. 2b. By controlling the stage using a XY stage for CNC router with stepping motor, it became possible to register multiple points and shoot while moving the registered points. The registered point can be returned from anywhere, and an unlimited number of shooting locations can be set. The cost of the system is approximately 30,000 yen, which is approximately 1/50 that of a commercially available electric stage. Using the 4-well cover glass chamber, it became possible to shoot under four conditions at the same time.

pH and temperature stability of the hand-made incubation chamber

The stability of the pH and temperature of our chamber was examined. Fig. 6a shows the result of the temperature and the pH of medium in the chamber. At a minimum of 48 h, the pH and temperature were stable. However, the gas balloon size was limited, making it necessary to replace the gas balloon every 24 h.

Reproducibility of the mapping position

The mapping position reproducibility was examined. More than 90% of all the four positions was accurate with an accuracy of 1 μm (Fig. 6b).

Auto-focus function

Fig. 7 demonstrates the focus value of the Z-stack seven pictures taken every 15 min for 24 h. As shown in Fig. 7, focus tracking worked excellently.

Discussion

The major finding of this study is that using open source GRBL, which was developed for the CNC router, we could successfully build a low-cost and high-precision stepping motor controlled by a multi-point automatic time-lapse microscope. There are few studies

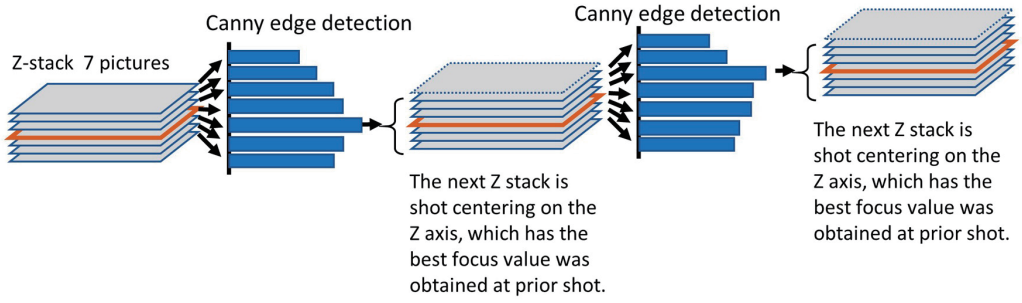

Fig. 5 Focus tracking argolism.
Using Canny edge detection algolism we caliculated focus value of 7 Z-stack pictures. In the next shot of same mapping point, 7 Z-stack was taken with the central Z axis set at highest focus value in the prior shot.

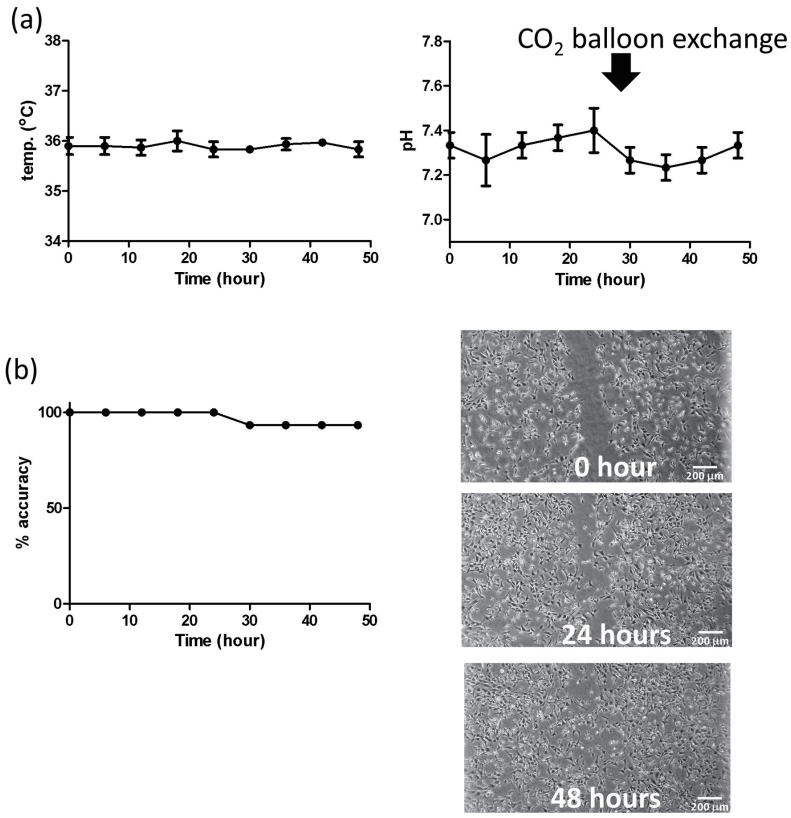

Fig. 6 Chamber temparature and pH of the incubation medium and the accuracy of the returing to the mapping position.

(a): The temparature of the chamber was stable at the set temparature. The pH of incubation medium tends to go up when the CO_2 gas balloon contents approach empty. Data was obtaied three times experiments for 48 hours. The data was shown as mean ± SD. (b): Taking picrure at 4 mapping points for 48 hours was done 15 times. Returning to the mapping position within 2 μm shift thought to be accurate and accuracy was calculated.

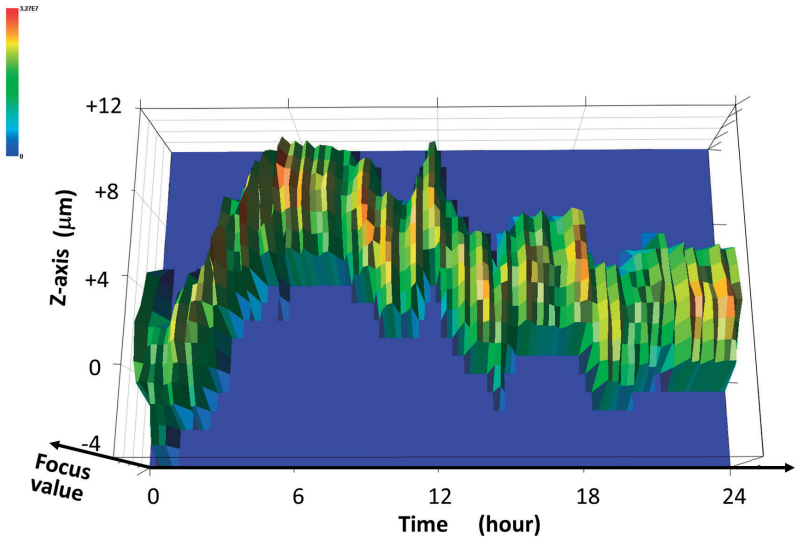

Fig. 7 Result of the focus tracking.

The 3D graph of the focus value of 7 Z-stack images. As shown, when the focus shifted the Z-stack catches up the focus shift, so that we can take the pictures with good focus.

on a low cost scanning motorized microscope. Guver et. al.[7] reported a low cost and high-precision scanning electrochemical microscope. They used a custom built stage with 3D printed gears. Campbell et. al.[8] also reported A Low-Cost Motorized Microscope Stage. They used a ThorLabs PT1® linear translator with a micrometer for the XY stage. In our study, we used the XY stage of the Proxon's milling machine MF70®, which costs only 8,000 yen, but it has very high-precision with a stepping motor. For the control of the XYZ stepping motor, we employed Arduino® and GRBL. It was originally developed for the CNC router or the 3D printer. Because of the recent development of this low cost technology for 3D printers, it is easy to make high-precision XYZ movement.

This machine can be used as a virtual slide scanner. We tested the scanner using a HE stained slide and connected them in one large image by free panorama software. Thanks to the focus tracking, we can easily obtain the 60 times, 60 full HD resolution image automatically with good focus.

This machine can also be used for tele-pathology. If the PC is connected to the internet, we can control the stage and observe the slide from a different position. However, at this point, it is difficult to change the objective lens. This may be the next issue of this machine.

Limitations

At this point, the microscope does not have motorized turret for objective lens. Motorized objective lens is the next function to improve. One more limitation is limited supply of CO_2. Large gas cylinder may allow us longer time-laps imaging.

Conclusions

Using the XY stage of the CNC router and the open-source GRBL program, we established a low-cost and high-precision stepping motor controlled by a multi-point automatic time-lapse microscope. The machine was especially useful for multi-condition live cell imaging.

Conflict of Interest

The authors declare no conflict of interest.

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