LOW-COST MICROMANIPULATOR DEVICE FABRICATION FOR *IN VITRO* STUDIES

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Abstract. Biomedical research often requires the use of precise equipment for micromanipulation, particularly in cellular biology. However, relatively inexpensive devices for cellular manipulation with electrophysiological methods or local chemical application with micrometer accuracy are rarely available in the equipment market. In this study, we present a method to develop a micromanipulator device based on stepper motors that is controlled by a microcontroller via a gamepad. This micro-motion system can be easily produced in any laboratory for various scientific experiments that require the movement of the electrode or pipette with a precision of several micrometers.

Keywords: micromanipulation device, neural electrical stimulator, cellular biology, low-cost engineering.

List of Abbreviations
DC – direct current
LED – light-emitting diode
CAD – computer-aided design
USB – Universal serial bus

Introduction

In recent decades, the emergence of inexpensive microelectronics, programming interfaces, and affordable 3D printing technologies has significantly improved the quality of low-cost engineering. There is a growing number of scientific articles focusing on the low-cost design and production of precise instruments for specific scientific studies, such as perfusion systems (Wijnen *et al*., 2014; Kujawa *et al*., 2021; Lupinski *et al*., 2021), microfluidics (Filatov *et al*., 2021), dispensers (Baden, 2014; Baden *et al*., 2015), tumor detection (Islam *et al*., 2019), and many others. Researchers can now design and perform complex experiments without requiring deep knowledge in the field of engineering.

Many scientific studies in biology require manipulation with the cells and living tissues with precision of several micrometers or even nanometers. Commercial micromanipulators, such as those offered by Scientifica (UK) and Kleindiek Nanotechnik (Germany), provide a sufficient degree of accuracy of a few nanometers. Existing micromotion systems based on various devices such as servo-actuators, stepper motors, ferroelectric drives and piezomotors (Ouyang *et al*., 2008). Stepper motors and servo drives have become very popular for robotics applications. The accuracy of these types of motors can provide several micrometers which is suitable for a wide range of tasks (Schreurs *et al*., 1974; Sonnhof *et al*., 1982). However, relatively cheap manipulators are not available in the laboratory equipment market. Developing an inexpensive, simple, and precise micropositioning system would greatly simplify most of the experiments with cellular manipulation.

In this study, we present a device for precise micromanipulation of biological objects. The system can precisely position a microelectrode or micropipette on the surface or within the depth of several cells (50–100 μm) through precise manipulation. The manipulator can be used in any scientific or technical application where lightweight objects need to be moved with high accuracy (3–20 μm). Our manipulator is low cost, easy to produce, mobile, and can be placed on the subject table of most microscopes. We used 3D printing methods, along with widely available open-source software and hardware to engineer individual components.

Materials and Methods

We used a DC stepper motor (4-6V/500mA) and a stepper motor from a commercial CD-ROM drive with a control voltage of 5V to position an object. The control unit for the stepper motors was developed based on the Arduino MEGA microcontroller (Atmega168) and low-voltage DRV8834 stepper motor drivers. Note, driver TMC 2209 with similar characteristics and lower noise levels can be used. The motions of the stepper motors were controlled by a joystick from a game console (Dendy/8-bit) via COM port. The program settings were displayed on an LED display. We used the Liquid Crystal software library for the LED display and the NESpad software library for the joystick to program the microcontroller. The system was powered by a single 5V external switching power supply (Robiton EN2250S, supply voltage 3-12V, 2.25A). The 3D model for the stepper motors' fastening was developed using the AutoCAD computer-aided design software and implemented with PLA material in 1.75 mm (REC) on a Rep-Rap Prusa I3 3D printer (BQ, Italy). External bipolar electrodes with a diameter of 50 μm were made of stainless steel in a Teflon shell with total weight less than 50 g.

The travel range and movement speed of the micro tool (metal microelectrode) were evaluated using a Supereyes Best Digital USB microscope with 500 magnification and a micrometer stage with divisions of 100 μm. The experimental data were analyzed using STATISTICA and MS Excel software.

The methodological details of the experiment with neural cultures can be found in our previous study (Pimashkin *et al.*, 2016).

Results

The main objective of the developed micromanipulator (Fig. 1, A, B) was to move objects within a travel range of several micrometers. Such objects can include metal microelectrodes, micropipettes for applying reagents, and other items. We have developed a reliable construction for the micromanipulator, with the aim of accommodating a variety of micro-tools and establishing a micromanipulation system on microscope tables (Fig. 1, D).

For the task, we selected two stepper motors: a commercial stepper motor 4-6B/500mA (No.1) and a stepper motor embedded in the base of a commercial CD-ROM drive (No. 2). The anchor turn step was adjusted using the DRV8834 driver under the control of the ATmega168 microcontroller (Arduino). Our micro-motion system implemented two action modes: Precise mode for slow and precise positioning and Travel mode with fast speeds. Both modes were performed in microstep mode - 32 microsteps per full step. The stepper motor moved incrementally, with the rotor rotating to a certain angle and the moving platform with the micro-tool fixed on it moving linearly to a certain distance. The angle of rotation of the rotor is determined by the number of control electric pulses delivered to the motor. The position of the stepper motor's rotor between the incoming pulses is static for a certain time interval. The pulse repetition rate algorithms are programmed accordingly.

The micromanipulator was controlled through a joystick (8-bit/Dendy) connected to the control unit, allowing linear movement of micro-tools fixed on the stepper motors and switching between full steps and microsteps. The "START" button on the joystick was used to turn the system on and off. The pulse repetition rate for both modes of action of the stepper motors was selected using the buttons located to the right and left. These buttons allowed switching between full-step or microstep modes, with the "SELECT" button used to confirm the selection. The up and down buttons controlled the linear movement of stepper motor № 2, while the buttons to the right and left controlled stepper motor № 1. The "Cancel" button automatically switched the action mode of the stepper motor. The system parameters, such as action mode and the period between pulses, were displayed on the LED monitor.

The stepper motors were connected by plastic parts (Fig. 1, B, 6 and 7), which were previously modeled in AutoCAD and printed on a 3D printer (Fig. 1, B) (see Methods). The elements of the micro-motion system were connected using bolts, screws, nuts, and glue. The lower plastic base (Fig. 1, B, 7) was glued to the

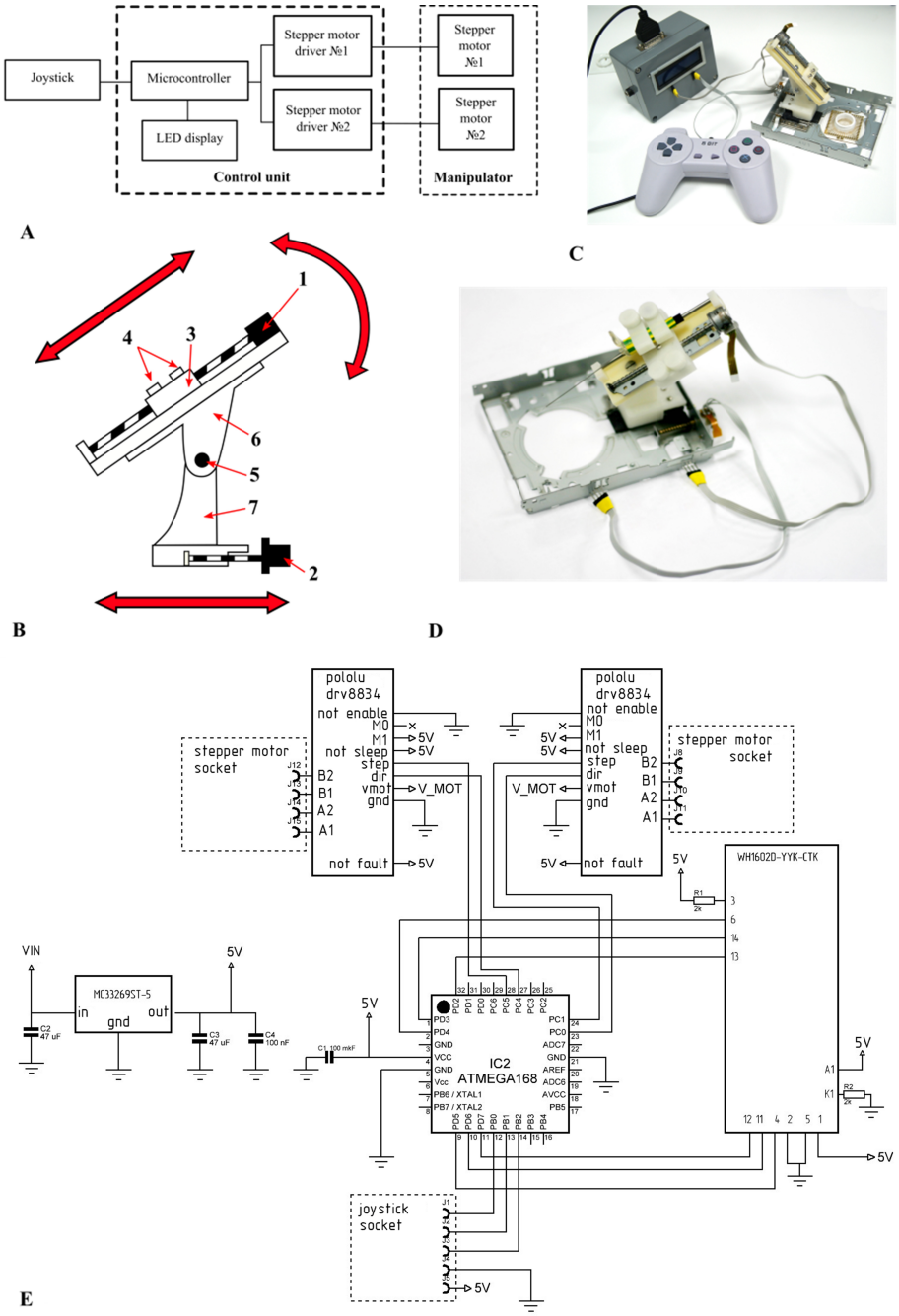

Fig. 1. The Micromanipulator scheme. (A) Block diagram of the micro-motion system. (B) Schematic representation of the manipulator indicating individual elements (side view): 1 – Stepper motor № 1, moving the platform with the object; 2 – Stepper motor №2, providing horizontal movement of the object; 3 – Platform for placing the object on the stepper motor; 4 – Screws for fixing the object; 5 – Screw for adjusting the angle of inclination of stepper motor № 2; 6 – The upper plastic base of the micromanipulator; 7 – The lower plastic base of the micromanipulator. The arrows indicate possible directions of movement. (C) General view of the micro-motion system. The control unit is on the left, the input device (joystick) in the center, and the micromanipulator on the right. (D) A manipulator with a microelectrode attached to it. (E) Electric circuit scheme of the control unit

movable platform of the CD-ROM drive and fixed through screws to the prepared grooves. Stepper motor №1 (Fig. 1, B, 1) was fixed with a screw on the upper plastic base (the screw head was countersunk into the body of the stepper motor so that it does not interfere with the

movement of the object). Nuts were glued into special grooves in the upper plastic base to form the thread for the bolt. The bolt (Fig. 1, B, 5) in turn fastened the two plastic parts of the system. When the bolt was loosened, the angle of inclination of the upper plastic base with the stepper motor fixed to it changed and consequently, the direction of movement of the object was changed during the experimentation process. Stepper motor № 2 (Fig. 1, B, 2) moves stepper motor № 1 (Fig. 1, B, 1) along the horizontal axis. Stepper motor № 1 (Fig. 1, B, 1) moves only the platform (Fig. 1, B, 3), which is fixed with screws (Fig. 1, B, 4). Thus, the microelectrode or any other fixed object can be accurately moved within the same plane.

We measured the time during which the electrode attached to the moving platform was moved in the microstep mode by 1 mm while holding the joystick button. The measurement was conducted using a USB microscope and micrometer stage with a grid of 100 μm in increments (Fig. 2, A, B) at periods between pulses of 50, 150, 250, 350, and 450 (n = 6). We calculated the number of pulses received by the stepper motor controllers during the test (the ratio of the time the electrode traveled by 1 mm to the time between the pulses) to estimate the linear movement step in the microstep mode. Then we quantified the electrode displacement to the number of pulses during the measured time to get the value of one microstep. The smallest linear movement step in the microstep mode for stepper motors № 1 and № 2 was achieved with a minimal period between pulses of 50 ms and reached 3.77 ± 0.28 μm and 5.10 ± 0.12 μm, respectively (Fig. 2, C, D).

We estimated the dependence of speed in the microstep mode on the time between pulses (Fig. 2, E). The highest average speed for stepper motor №1 was 75.26 ± 5.43 μm/s, and the lowest was 9.84 ± 1.41 μm/s. For stepper motor № 2, the highest average speed was 103.51 ± ± 2.32 μm/s, and the lowest was 12.30 ± ± 0.19 μm/s. As the time between pulses increased, the speed of linear motion of the microtool decreased non-linearly. Between pulse times of 50–150 μs, the rate sharply declined, and changes were insignificant beyond that range.

In addition, we estimated the linear movement step and speed in the Travel mode. We measured the effective distance that the platform moved for stepper motor №1 and the lower plastic base of the micromanipulator for stepper motor №2. The displacement distance for stepper motor №1 was 63 mm, and for stepper motor №2, the displacement distance of the plastic base was 15 mm. We estimated the travel time with the pulse frequency of 4000 Hz in microstep (1/32) mode for both engines (n = = 6). The linear movement step was measured similarly to the Precise mode test. The magnitude of the linear movement step was 2.77 ± ± 0.05 mm for stepper motor № 1 and 3.38 ± ± 0.3 mm for stepper motor № 2. In Travel mode, as expected, pressing the button of the joystick greatly reduced the accuracy of the movement of the microtool. Additionally, the motor did not move smoothly but in jerks, and therefore the movement was not particularly accurate in the order of millimeters. We tested how carrying weight affects positioning accuracy. Several various microelectrodes with average weight less than 50 g demonstrated the same accuracy for stepper motor № 1. Stepper motor № 2 carried additional weight of stepper motor №1 and the plastic platform with overall weight of 75 g. and its accuracy with various microelectrodes was also the same.

To test micromanipulator efficiency we performed an experiment with electrical stimulation of neuronal cells *in vitro* using an external bipolar electrode with a diameter of 50 μm made of stainless steel in a Teflon shell. The culture of dissociated hippocampal cells grown on a microelectrode array (MEA) generated bursts of spikes lasting for 500–1000 ms in response to an electrical stimulus of 800 μV amplitude and 500 μs duration (Pimashkin *et al.*, 2016). Neural network spiking activity was recorded through planar microelectrodes of the MEA. The bipolar electrode attached to the movable platform of the micromanipulator was positioned close to the cell culture surface at a height of less than 50 μm, and an electrical pulse was generated. The stimulus depolarized the cells, and the microelectrodes of the MEA recorded a stimulus artifact. Then, a series of

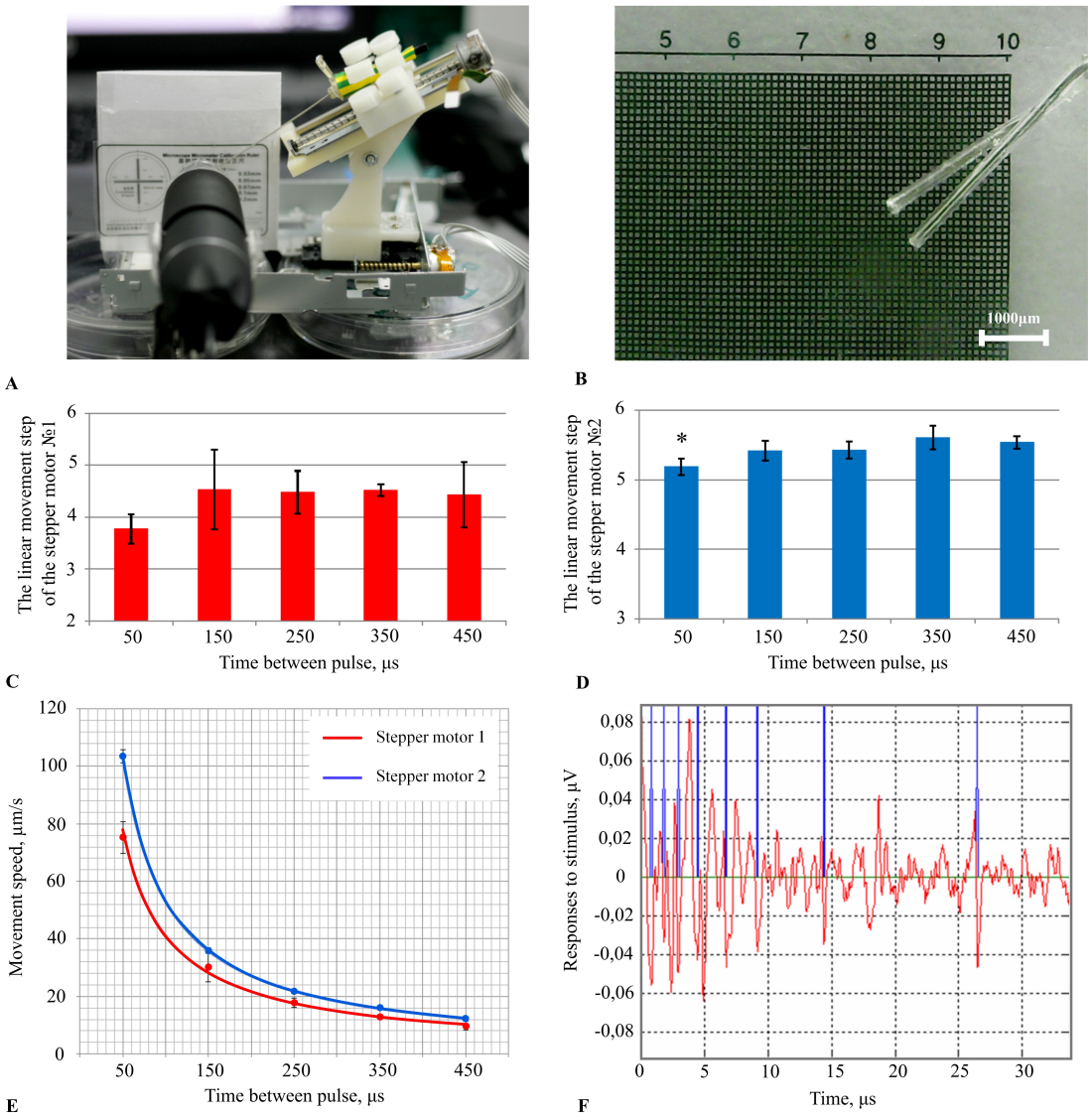

Fig. 2. Evaluation of micromanipulator performance. (A) A micromanipulator with an electrode attached to the platform of the stepper motor. The USB microscope is mounted opposite the electrode so that the electrode and the micrometer stage are in focus. (B) The electrode under a USB microscope. (C) The linear movement steps of stepper motor №1 in microstep mode. (D) The linear movement steps of stepper motor №2 in microstep mode. (E) Speed of movement of stepper motors in microstep mode. The red line represents the stepper motor №1, while the blue line represents the stepper motor №2. (F) A single stimulus and response in the form of the burst of spikes on a single electrode of the MEA. The red line indicates electrode potential, while the blue lines indicate detected spikes of the neurons in response to the stimulus

pulses was repeated 30 times to assess reproducibility and stability of the responses (Fig. 3, F). On average, the stimulus evoked 20.4 ± 13.3 spikes in each of 60 electrodes, which were comparable to the stimulation through the planar microelectrodes of the MEA. The electrode was stably positioned above the culture cells and did not damage the tissue during the experiment.

Discussion

In this paper, we present a simple and affordable way to design and assemble a micromanipulator for research studies. The device consists of stepper motors and components produced on a 3D printer. The rotation of the stepper motor screw results in the movement of the motor platform along the axis with an accuracy of several micrometers. We have demonstrated that

such a micromanipulator can be used to conduct experiments involving electrical stimulation of neural cells using a microelectrode. The manipulator allows for precise positioning of the electrodes onto the neural culture grown on a simple coverslip or a plastic cup without using planar electrodes. Live imaging techniques such as transfection, transduction, and Ca-imaging, including postsynaptic density labeling, can be used to evaluate the effects of stimulation. Additionally, the micromanipulator can be adapted for experiments involving the application of chemical reagents through a micropipette to cultured cells or brain slices. The efficiency of cell stimulation using this device was evaluated using microelectrode arrays that recorded spiking activity of the hippocampal neurons in vitro (Fig. 2F).

In neurobiology, localized electrical stimulation of the neurons for several minutes is necessary to study synaptic plasticity and memory formation. Expensive manipulators or microelectrode arrays are often used for this purpose. However, such stimulation can be carried out simply and at a lower cost with the proposed micromanipulator, allowing several experiments to be conducted simultaneously. This is particularly relevant in cases where the experiment is conducted on brain slices of expensive transgenic mice.

Furthermore, the micromanipulator can be adapted for experiments involving the application of chemical reagents through a micropipette to cultured cells or brain slices. Such experiments often require bringing the pipette close to the tissue surface for a short application and then withdrawing it to change the pipette or solution, which requires an accuracy about 10 μm per step. The micro-motion system can also be installed to a microscope for visual control of micromanipulation.

An increasing number of publications in scientific journals in the field of laboratory equipment development using simple microelectronics and 3D-printing technology indicates (Wijnen *et al.*, 2014; Baden *et al.*, 2015) the relevance of such research and significantly increases the accessibility of precise experiments due to the simplicity of production and low cost. The method presented in this paper for micromanipulator development broadens the experimental possibilities of any laboratory specializing in biological or other research where precise manipulation and positioning are necessary. Additionally, this study may be useful in educational institutes specializing in engineering and microelectronics in laboratory and practical studies.

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