

# LINEAR MICRO-ACTUATION SYSTEM FOR PATCH-CLAMP RECORDING

Ilya Kolb<sup>1\*</sup>, Gregory L. Holst<sup>2\*</sup>, Max A. Stockslager<sup>2</sup>, Suhasa B. Kodandaramaiah<sup>3</sup>,  
William Stoy<sup>1</sup>, Edward S. Boyden<sup>3,4</sup>, Craig R. Forest<sup>2</sup>

<sup>1</sup> Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta GA, 30332 USA

<sup>2</sup> George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta GA, 30332 USA

<sup>3</sup> Media Lab, Massachusetts Institute of Technology, Cambridge MA, 02139 US

<sup>4</sup> McGovern Institute, Massachusetts Institute of Technology, Cambridge MA, 02139 USA

\*authors contributed equally

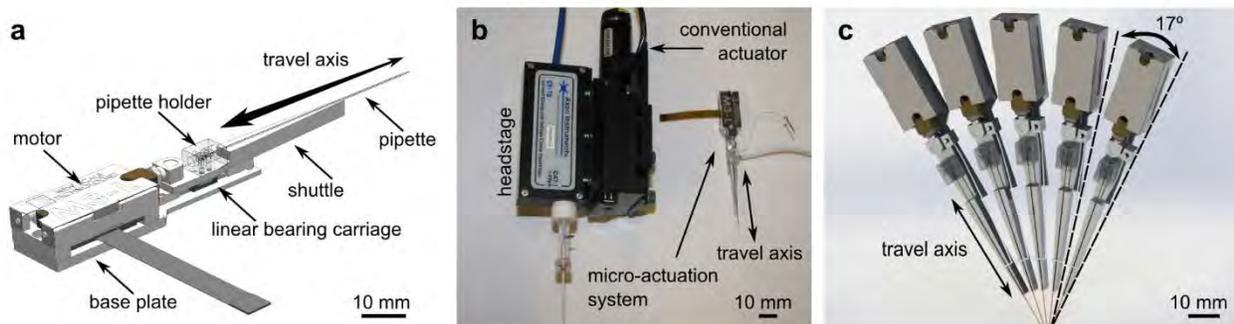
## ABSTRACT

Measuring the electrical activity of neurons is essential for understanding how they encode and transmit information in the brain. Using a technique known as patch-clamping, the electrical activity of single neurons can be reliably recorded by pressing a small glass pipette filled with electrically conductive and pneumatically controlled solution against the neuron's membrane. This requires accurate and repeatable mechanical control of pipette position, typically necessitating a bulky actuation system and thus making it difficult to position several pipettes around a tissue specimen to record from multiple neurons at once. We have developed a linear micro-actuation system for patch-clamping that exhibits high positional accuracy ( $< 150 \mu\text{m}$  on-axis error over full travel), high repeatability

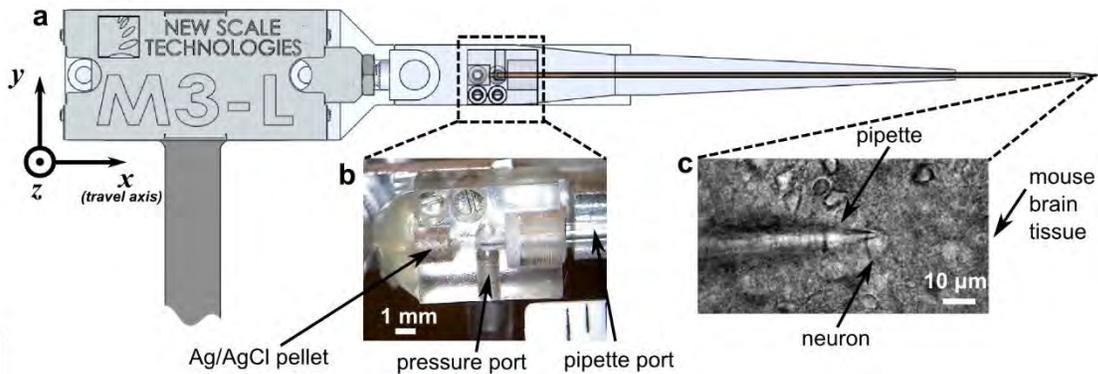
(on-axis  $\sigma = 33 \mu\text{m}$  for full travel;  $\sigma = 0.71 \mu\text{m}$  for  $15 \mu\text{m}$  travel) and low drift ( $0.61 \mu\text{m}/\text{hour}$ ). The system was designed and fabricated to patch-clamp onto neurons in a mouse brain slice. The miniaturized device presented here makes it possible to position up to 21 actuators around a  $5 \times 5 \text{ mm}$  tissue sample and thus record intracellularly from a large number of neurons.

## INTRODUCTION

Neurons are electrically active due to the controlled flow of ions through pores in their membranes. Patch-clamping, the gold standard technique for measuring trans-membrane voltages and currents, involves delicately resting a  $1 \mu\text{m}$  diameter pipette against a cell to create



**FIGURE 1.** Micro-actuation system for patch-clamp recording. (a) CAD model. (b) Conventional actuator, pipette holder and headstage [left] and our micro-actuation system [right]. (c) Proposed CAD design of micro-actuation array. The small ( $\sim 17^\circ$ ) angular profile a single micro-actuation system can enable high-channel recording from small ( $\sim 5 \text{ mm} \times 5 \text{ mm}$ ) tissue specimens (not shown).



**FIGURE 2.** Detailed design and testing of the linear micro-actuator for patch-clamp recording. (a) Micro-actuation system based on the M3-L Linear Actuator (New Scale Technologies; Victor, NY, USA) (b) Micro-machined pipette holder. (c) Differential interference contrast (DIC) image of a patch-clamping experiment. A neuron in a mouse brain slice has been patch-clamped using a pipette mounted on the linear micro-actuation system.

an intimate electrical and mechanical connection between the pipette tip and the neuronal membrane. One can then apply suction to ‘break-in’ to the neuron to attain a ‘whole-cell’ configuration in which the contents of the cell are directly accessible [1]. From there, it is possible to record essentially interference-free single-neuron “spikes” of membrane voltage. These spikes are the primary method of inter-neuronal communication in the nervous system.

The size of conventional pipette micro-actuators is the current limiting factor when attempting to record from many neurons simultaneously in a brain slice with a  $100\ \mu\text{m} \times 100\ \mu\text{m} \times 10\ \mu\text{m}$  region of interest. Commercial systems have been outfitted to support up to 12 independent pipette actuators [2] but due to their large size, this is near the physical limit.

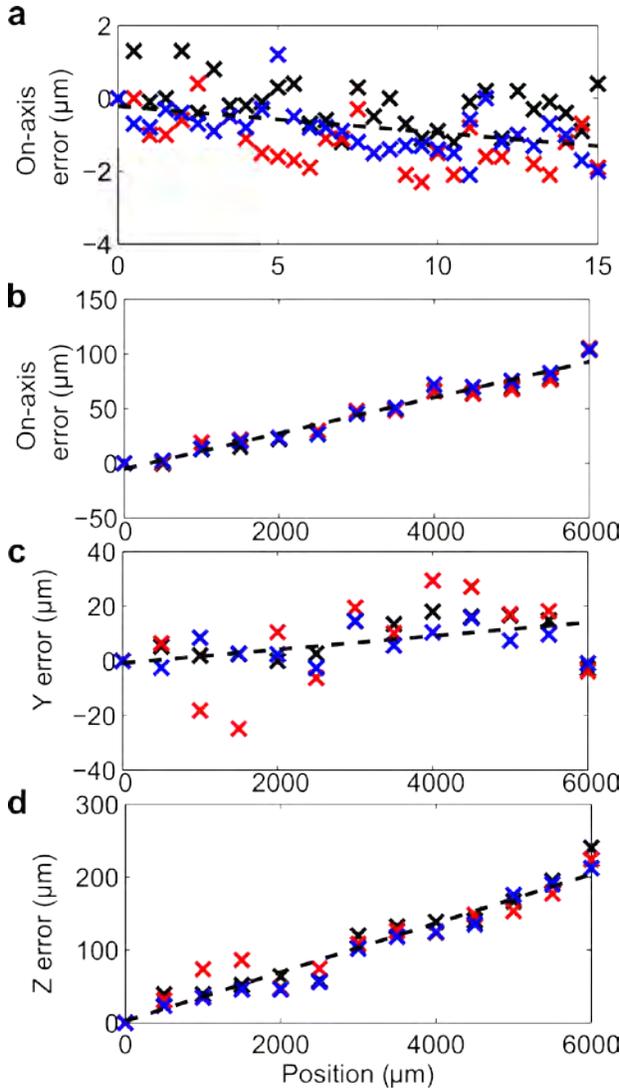
Here we present a novel linear micro-actuation system (Fig. 1a) that exhibits mechanical characteristics sufficient to perform patch-clamping. Our system has a volume that is less than 1% and a weight that is only 1.4% of the conventional actuator and holder (Fig. 1b). This miniaturization of actuation systems will enable the use of many closely packed electrodes (Fig. 1c) that can simultaneously access many neurons, as is desirable for high-channel probing of neural circuits [3].

## MATERIALS AND METHODS

### Detailed design

The linear micro-actuation system in Fig. 2a is based on the M3-L motor (NewScale Technologies; Victor, NY). The motor has a travel range of 6 mm and a motion resolution of 500 nm. The motor pushes a custom-made aluminum shuttle mounted on a miniature linear ball bearing carriage. The carriage rides on a 60 mm long guide rail (McMaster-Carr 8381k27).

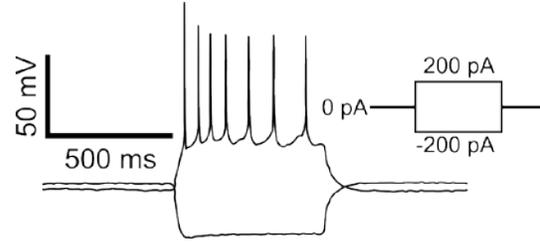
A custom pipette holder (Fig. 2b) was machined out of cast acrylic and mounted on the shuttle. Three ports were drilled in the pipette holder: (1) a port to house an Ag/AgCl pellet for electrical connectivity with the pipette, (2) a pressure port to connect to an air pressure controller, and (3) a port to constrain the pipette. The three ports are interconnected and are filled with physiological saline to ensure electrical connectivity between the Ag/AgCl pellet and the pipette. The guide rail and the motor are mounted on a custom-made base plate. The base plate and shuttle were machined out of 6061 aluminum plate using a HAAS OM-1A CNC office mill. The pipette is pulled from 1 mm OD / 0.5 mm ID capillary glass using a Flaming/Brown P-97 micropipette puller (Sutter Instrument Company; Novato, CA) to a  $\sim 1\ \mu\text{m}$  diameter tip (Fig. 2c).



**FIGURE 3. Accuracy measurement.** (a) On-axis accuracy over a 15  $\mu\text{m}$  travel distance. (b) On-axis and (c), (d) off-axis accuracy over full travel 6 mm. Three trials (black, blue, red) were performed. The error in motion was nearly linear (dashed line).

### **Benchtop testing**

The accuracy, repeatability and drift of the micro-actuation system was evaluated. A pipette was fit into the pipette holder and clamped to the shuttle with an o-ring. The motor was mounted flat on a motorized XY stage (resolution = 100 nm) under a microscope equipped with a motorized 40x objective (UPLFLN 40X, NA = 0.75, Olympus; Center Valley, PA) and differential interference contrast (DIC) optics (Scientifica Ltd, East Sussex, United Kingdom). This metrology system



**FIGURE 4. Electrical activity recorded from the neuron shown in Fig. 2c in response to positive and negative current stimulation (inset) includes action potentials (large positive spikes) as well as passive resistive/capacitive components, both of which are typical electrophysiological characteristics of a patch-clamped cell.**

has a resolution of approximately 500 nm. Pipette position in three dimensions was determined by reading out motor encoder values of the XY stage and objective when the pipette tip is centered and focused over a stationary on-screen crosshair.

Actuator accuracy is important when it is necessary to target specific structures in the brain. For instance, layers of the mouse cortex are tens to hundreds of micrometers deep in the brain [4], making positioning within  $\sim 10 \mu\text{m}$  necessary for reliably targeting them. To test accuracy, the motor was first moved over 15  $\mu\text{m}$  in 500 nm increments and subsequently over its full range of travel (6 mm) in 500  $\mu\text{m}$  increments. The movement error was the difference between the actual pipette position and the commanded position as measured using the XY stage encoder averaged over  $n = 3$  trials.

Repeatability of actuator motion is vital for algorithmic finding of cells based on electrical resistance [5]. It is also important for the ability to repeatedly target a brain structure for patch-clamping. The repeatability of motion was evaluated by commanding the motor to approach the middle of its travel (3 mm mark) from both directions in 1 mm increments. It was quantified by computing the standard deviation ( $\sigma$ ) of the differences between the commanded position

and actual position of the pipette tip over  $n = 5$  trials.

Positional drift of the pipette tip after a whole-cell configuration has been established is detrimental to the stability of the membrane-pipette interface and ultimately worsens the signal-to-noise ratio of the intracellular recording. Therefore a system with minimal drift ( $< 1 \mu\text{m}/\text{hour}$ ) is required. Motor drift was measured with the pipette acting as the load. Measurements were taken every 30 minutes for 2.5 hours.

### **In-vitro testing**

We performed patch-clamp experiments in mouse brain slices [6] under DIC optics (Fig. 2c) by mounting the micro-actuation system on a three-axis manipulator. Electrical signals were recorded using a standard patch-clamp amplifier and data acquisition software (Molecular Devices; Sunnyvale, CA). Power to the motor was disconnected during the recording to minimize electrical interference.

## **RESULTS AND DISCUSSION**

### **Mechanical performance**

In benchtop testing, the micro-actuation system exhibited on-axis accuracy down to  $2 \mu\text{m}$  in small movements and  $150 \mu\text{m}$  in full-travel movements (Fig. 3a,b). The large full-travel on-axis error was systematic and linear ( $R^2 = 0.96$ ) and can therefore be predicted and compensated with a linear slope correction of  $m = 0.01639 \mu\text{m}$  adjustment per  $1 \mu\text{m}$  of on-axis travel to satisfy the  $10 \mu\text{m}$  accuracy requirement for patch-clamping.

The off-axis accuracy of the system reached  $14 \mu\text{m}$  in the  $y$  direction and  $93 \mu\text{m}$  in the  $z$  direction (Fig. 3c,d). This is insufficient for targeting specific areas in the brain so additional motion constraints will need to be placed if highly accurate motion is needed.

After linearization, the repeatability of small actuator displacements (up to  $15 \mu\text{m}$ ) is  $\sigma = 0.71 \mu\text{m}$  which is sufficient for algorithmic finding of cells based on electrical resistance. The on-axis repeatability over the full range of travel was within  $\sigma = 33.1 \mu\text{m}$ . This is sufficient for repeatable targeting of brain structures such as distinct neuronal layers in the cortex of a mouse.

The off-axis repeatabilities were within  $\sigma = 203 \mu\text{m}$  and  $\sigma = 154 \mu\text{m}$  in  $y$  and  $z$  directions, respectively.

The pipette mounted on the micro-actuation system drifted a total distance of  $4.22 \mu\text{m}$  over 4 hours. The average drift was  $0.61 \mu\text{m}/\text{hour}$ . Based on average recording times of  $\sim 1$  hour, this drift is negligible.

### **In-vitro experiment**

We tested the usability of a single micro-actuation system by performing patch-clamp experiments in mouse brain slices. Representative intracellular electrophysiology traces (Fig. 4) are qualitatively indistinguishable from those obtained using commercially available pipette holders and actuators.

## **CONCLUSION**

We have designed, manufactured, and tested a linear micro-actuation system that has sufficient mechanical characteristics for performing patch-clamp experiments. It was subsequently validated in an in-vitro patch-clamp experiment.

The system presented here uses many off-the-shelf components and can therefore be easily replicated in order to increase the number of signals that can be simultaneously obtained from brain tissue. The angular profile of the entire assembly is only  $17^\circ$ , which potentially allows for a circular arrangement of, conceivably, up to 21 single-axis actuators around a tissue sample. This would theoretically enable simultaneous recording of up to 21 intracellular signals, and eventually more after additional miniaturization. These technical advances can open the door for performing high-fidelity simultaneous recordings from larger groups of neurons than previously possible.

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