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# Usefulness of Electromagnetic Induction Type of Force Transducer and Actuator for Myofibril Mechanics

Kazushige Kimura, Takahiro Abe, Kien Nguyen Phan and Takakazu Kobayashi

*Abstract*— **A high performance device for measuring force and length change during myofibril contraction is fabricated. The principle of a device depends on the law of electromagnetic induction. Homogenized myofibrils were attached between two wires exposed in the uniform magnetic field by silicon adhesive under an inverted microscope. The purpose of this study is to examine performance whether the electromagnetic induction type of device actually works. Sensitivity and time resolution of force transducer was 50nN and 1ms respectively. Working displacement and time resolution of actuator as length transducer was 1-20 μm and 1.2 ms. We confirmed the performance of the device by showing appropriate force response to changes in length during myofibrils contraction, and possibility of application of the device to myofibril mechanics is discussed.**

## I INTRODUCTION

Over the past 25 years, in *in vitro* experimental systems to extract the actin and myosin molecules, it has been measured the displacement of the nm order and the force of the pN order that occurs between the cross-molecules by the techniques to very sophisticated, such as micro-glass needle and optical laser trap. However, the phenomenon *in vivo*, in fact, occurs in the state of actin and myosin array has maintained a three-dimensional structure. There are huge findings about research of muscle contraction, but some part of mechanism is still unknown. The findings obtained *in vitro* experimental system is not always match to those obtained in physiological experiments. Therefore, the experiments to perform a minimum contractile unit system maintained physiological condition / myofibril is meaningful. Until now, mechanical study using single skeletal muscle fibers has been performed in detail using a commercial servo motor (General Scanning P100) and force transducer (Horten AME801). In mechanical study using the myofibrils there is the difficulty in the fabrication of experimental devices, almost experiments have been mainly associated with measurement of isometric force using the micro-glass needle. Force transducer to measure the force of skeletal myofibril or single cardiac myocyte during contraction, have been devised by using of the glass micro- needle [1, 2], the deflection of glass micro-needle with UV marker [3], opto-electronic deflection of suction micropipette [4], an electromagnetic induction [5], nano-fabricated cantilever[6], laser deflection of steel foil with needle[7], a bimorph cantilever [8] and MEMS related polysilicon plate [9]. However, the majority of these force transducers are mainly used to measure isometric force of myofibril or single myocyte, there is little force transducer can be applied to a dynamic mechanics experiment of myofibril.

High speed electromagnetic induction type of the force transducer was developed by Iwazumi [5], and in his paper it can be applied to dynamic mechanical experiments during myofibril contraction. It works in the same magnetic field, one wire end serves as the force transducer, and the other does as displacement actuator. He has been successful in isometric force measurements of myofibril, however, there are no reports about dynamic mechanical experiments such as force response to a change in length during isometric contraction in myofibril. The purpose of this study is to examine and verify whether the electromagnetic induction type of device can be really applied to the myofibril mechanical experiments, and we discussed about its application and limit.

K.Kimura and T.Abe are with Graduate School of Engineering and Science, Shibaura Institute of Technology and Science, Tokyo,135-8548 Japan (phone and fax:+81-3-5859-8321, e-mail: MA11058@sic.shibaura-it.ac.jp and MA11009@sic.shibaura-it.ac.jp )

K.N.Phan is with Department of Electronics Technology and Biomedical Engineering School of Electronics and Telecommunications, Hanoi University of Technology, Hanoi,Vietnam (phone+84-4-3-8682164; e-mail: knguyenp@gmail.com)

T.Kobayashi is with Department of Electronic Engineering, Shibaura Institute of Technology, Tokyo, 135-8548 Japan (phone and fax:+ 81-3-5859-8321; e-mail: kobataka@sic.shibaura-it.ac.jp).

Correspondence should be sent to T.Kobayashi.

## II METHODS

### Operating principle of device as force transducer and actuator

Our device is a simplified version compared to Iwazumi's device. The device consists of two wires, these are put in a uniform magnetic field in the center of the ring magnet (outer and inner diameter, thickness: 39×19×7mm). The magnetic flux density in the central part of the ring was 150mT. The material of the wires is the nonmagnetic copper-nickel alloy, is not affected by magnetic field. One wire is used as a force transducer, and the other is done as an actuator. The image of the wire is projected onto the center of dual photodiodes having two light detecting areas (Hamamatsu Photonics S5870). When the myofibrils are $Ca^{2+}$-activated, the image of the wire is slightly shifted from the central position. The wire is pulled by the force produced during the myofibril contraction, is pushed back to original central position by Lorentz force generated by the wire current and the magnetic flux density of the ring magnet. On the other hand, the actuator is

driven by the current from the current driver, and the current wave form was made by an arbitrary waveform generator (Tectronics AFG3021B) to apply length change during the myofibrils contraction. In the case of isometric contraction, the current driven by the actuator was constant or switched off. We used 30 μm diameter of compliant wire as the force transducer and 70 μm diameter of stiff wire as the actuator.

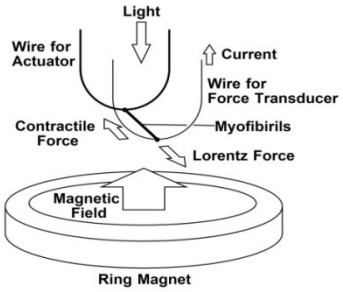


Figure 1. Principle in measurements of force and length change in myofibril contraction. One wire is served as force transducer, and the other was done as an actuator. One wire is pulled by the force produced in myofibril contraction, is pushed back to original position by Lorentz force generated by the wire current and magnetic flux density of ring magnet.

**Experimental set up**

An inverted microscope (Olympus IX-71) was used for observing image of myofibrils and for measuring force and length change of myofibrils. The collimated light from a 100 W halogen lamp passes through a ring magnet that put a vertical magnetic field at the myofibril. A neodymium magnet was used, it was put to the central position of the optical axis on the stage of inverted microscope. Two wires were placed in parallel at the plane perpendicular to the magnetic field. The transmitted light through the myofibril was split by a beam splitter, and half of light is reflected toward the CCD camera (Panasonic DMC-GH1). The other half is passed through the beam splitter and projected onto dual photodiodes.

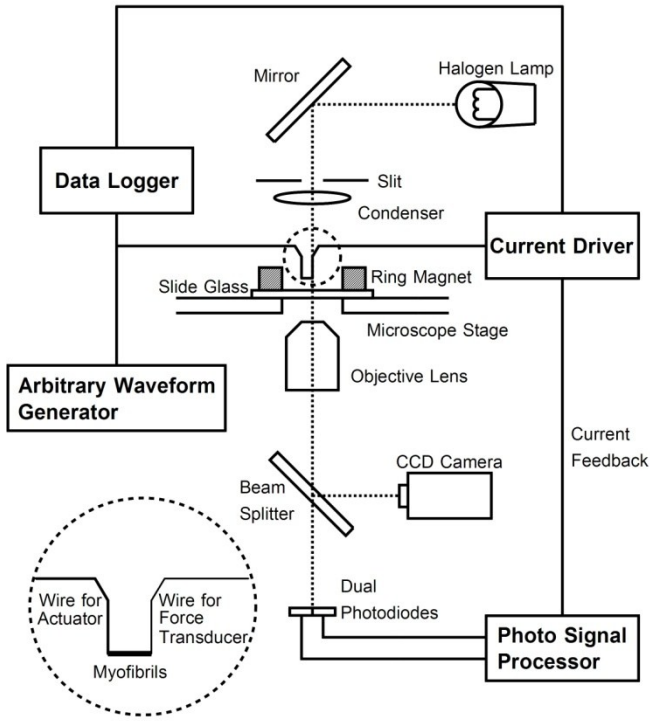


Figure 2. Optical arrangement. White light from a halogen lamp is condensed and focused on the myofibrils. A small fraction of transmitted light from objective lens is divided by the beam splitter, and focused onto the dual photodiodes and CCD camera.

**Feedback circuit for wire position control**

It is very important to detect wire position because the resolution of the force and actuator is determined. The image of the wire for force detection is projected onto the center of a dual photodiodes. When the wire position is shifted from the center, a photocurrent of one photo diode increases the other decreases. The difference of the two photo-currents is proportional to the wire position deviation. The difference is divided by the sum of the two photo-currents for normalization with respect to total light intensity. An electronic circuit was made based on this idea. The electronic circuit consists of current-voltage converter, subtractor, adder and divider. We used first stage operational amplifiers having low noise, low input bias current and moderate frequency response (AD8662), and also used high precision divider (AD538).

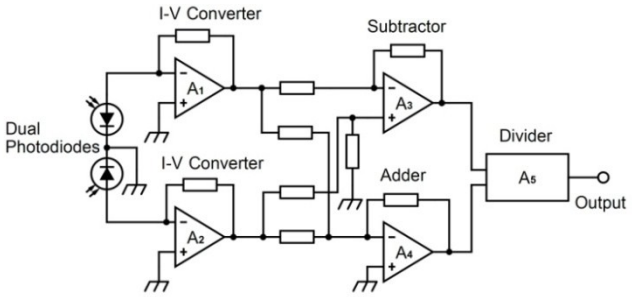


Figure 3. Electronic circuit for detecting the wire position. Current signal from the dual photodiodes are amplified by the current to voltage (I - V) converters respectively (op-amps Al and A2). Difference and sum of signal from right and left diode is calculated by op-amp A3 and A4, and divided the summing signal to difference signal using divider A5.

**Preparation**

Bundles of muscle fibers (2-3 mm in diameter) were dissected from rabbit psoas muscle and tied to glass rods, kept in a 50% glycerol solution containing 5 mM potassium phosphate and 2 mM EGTA (pH7.0) at 0°C for overnight, then stored into the freezer at -27°C after the solution was changed. Small strips of muscle fibers were dissected from the small bundle of muscle fibers (2-5 fibers, 3mm long), put into the $Ca^{2+}$ free rigor solution of 10 ml in a test tube, and homogenized with a homogenizer for 10 s at speed of 13,500 rpm (IKA ULTRA –TURRAX (R) T25). The myofibrils were then kept in the $Ca^{2+}$-free rigor solution at -27°C and used within a same day. Rigor solution containing the myofibrils was sucked up by the 30μl volume of micropipette, and dropped onto the slide glass in the center of the optical axis of the microscope. After selection of suitable 2-3 myofibrils, the myofibril was held horizontally in an experimental trough with a pair of wires by the silicon adhesive (Down Corning 3145RTF). The two

wires with glue were lowered slowly by three dimensional micromanipulators, it was attached to the myofibrils under the microscope. Length of myofibrils is 200-250 µm. Initial sarcomere length of myofibrils were adjusted to 2.4 µm from the image of the CCD camera, myofibrils showing non-uniform sarcomere were excluded from the experiment.

**Solution**

Relaxing solution: KCl: 125mM, $MgCl_2$: 4mM, ATP: 4mM, EGTA: 4mM, PIPES: 20mM (2) Contracting solution: added $CaCl_2$: 4mM to relaxing solution, (3) Rigor solution: subtracted ATP: 4mM from relaxing solution. pH was adjusted to 7.0 by KOH. All the experiments were carried out at room temperature (20-22°C).

**Protocol**

Relaxing solution was exchanged for $Ca^{2+}$-activating solution. The solutions were perfused to the trough by gravity from a syringe set at approximately 100 mm above the microscope stage. After force had attained at the steady level, 1) the quick release was applied to the contracting myofibril by passing current through the wire of actuator, 2) fast stretch following slow stretch and 3) sinusoidal length change were also applied. Images of myofibrils were obtained from CCD camera via the 20× objective lens, and recorded with a memory card. To apply length change to myofibrils, the wire was connected current driver amplifier controlled by an arbitrary wave form generator. Length and force were recorded to the data logger (Graphtec LOGGER mini GL900) and then data were transferred to personal computer.

**Calibration**

Calibration of force was carried out using the glass micro-needle with known stiffness. We have measured the deflection of the tip of the 1st glass micro-needle by applying a weight (0.05-0.2mN) under the microscope, by this stiffness of the tip of the 1st glass micro-needle was determined. The 1st glass micro-needle is touched with the 2nd compliant glass micro-needle, the stiffness of 2nd glass micro-needle was determined. Repeat in this procedure, made a glass micro-needle with proper stiffness of force measurement range. Calibrated glass micro-needle is touched to the center of the wire where the myofibrils are glued, and from the deflection of the glass micro-needle when the current passes the wire, current-force relation was determined. This relation was linear up to 40 µN. A sensitivity and time resolution of force transducer were 50 nN and 1 ms. Actuator was also calibrated by the same way as the force transducer. Current-displacement relation was linear up to 20µm. Frequency characteristics of length actuator is shown in figure 4C , high cut off frequency was around 800Hz.

**Performance of the device/ force transducer and actuator**

Figures 5 A-D show the examples of myofibril mechanical experiments. A shows $Ca^{2+}$ activated myofibril isometric force was taken by dropping activating solution from the syringe. There is artifact at rising phase of contraction. B shows force response to quick length change, The quick release was made by application of length change from the rectangular pulse of the generator. The myofibrils force

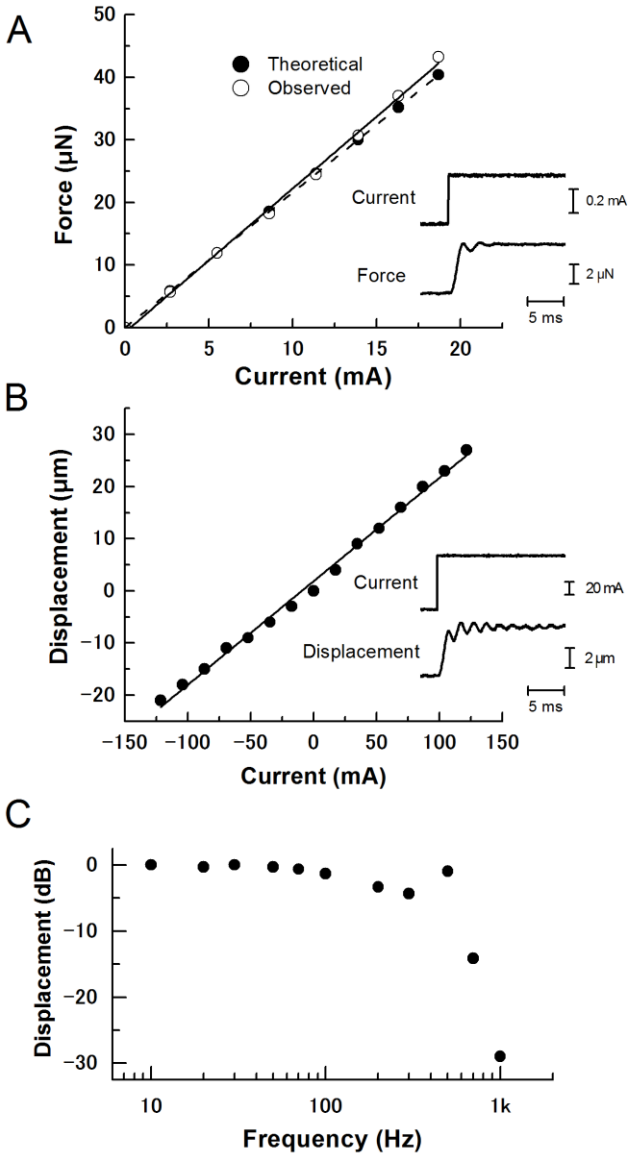


Figure 4. Calibration of force transducer and length actuator. A: Current-force relationship of force transducer, the inset shows force response to step change in current, B: Current-displacement relation of length actuator, the inset shows displacement response to step change in the current and C: Frequency characteristics of the length actuator.

decreases suddenly and then recovers toward to steady level. C shows force responses to moderate fast ramp stretch ( 0.25 $L_0$/s) following slow stretch (0.025 $L_0$/s) during myofibrils contraction. Concerning dual stretch, myofibril force shows sudden decrease at the time of changing from slow to fast stretch velocity. D shows force response to sinusoidal length change (0.8 %$L_0$, 0.5 kHz), myofibrils bared fast vibration. It is important to glue tightly myofibrils and the wires, so that we tried to use many kinds of adhesive such as corrosion, cyanoacrylate glue, and found that the silicone glue having proper viscosity was most suitable in this myofibril experiments.

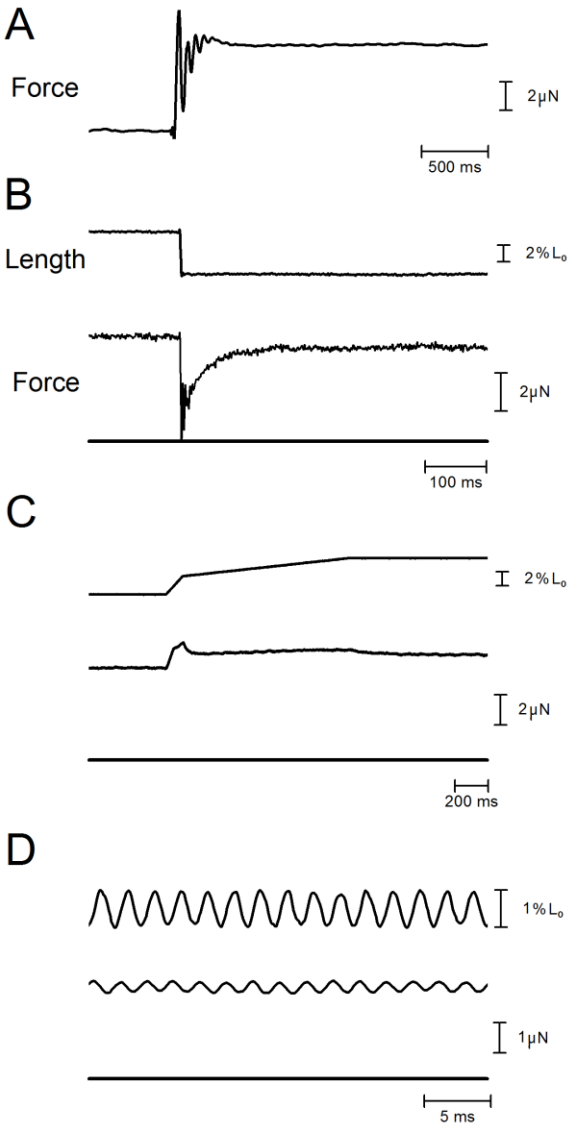


Figure 5. The examples of myofibril mechanical data. A: $Ca^{2+}$- activated isometric force of myofibrils measured by the wire force transducer, B: Force response in quick released myofibril, C: Force response stretched myofibril at dual velocity and D: Force response imposed sinusoidal length change to myofibrils during isometric contraction.

## Ⅲ DISCUSSION

We fabricated practical device for measuring force and length change in myofibrils contraction, and succeeded to measure the force change to changing in the length in $Ca^{2+}$ activated myofibrils. This device can also be used to the mechanical experiments in single cardiac myocyte. It is available to measurement of instantaneous stiffness applied change in small sinusoidal length [10] and measurement of force-velocity relationship applied change in hyperbolic length at once. Since diffusion of the solution in the myofibril is very fast compared to that in the single muscle fiber, it may be possible to measure high-speed ATPase activity using NADH-NAD reaction by using 340nm UV light [11] . In any case, the future, various applications of measurements can be expected. About improvements of the device, we were initially intended to make the device for measuring with much high-speed and high-sensitivity, the wire of the force transducer should have a certain amount of flexibility, so the response speed of device is lowered to about 1ms. The wire of the actuator should be somewhat stiff to maintain the isometric condition. We can improve the response speed of the device by passing a large current to the wire and using a fine wire. In this study, experiments are carried out in the bundle of 2-3 myofibrils, but it is possible to measure the single myofibril because of having enough sensitivity and low noise level. In this experiment, a large artifact occurred during the solution exchange, this can be avoided by photolysis of caged substances such as caged ATP or caged Ca. Timing of the contraction and starting of mechanical change also can be controlled by using caged substances. In addition, although we used the force transducer and the actuator as length clamp mode, it is also possible to use that as the force clamp mode in the basic principle.

The demerit of this device is to be very difficult to perform mechanical experiments using single myofibril with 10-20 sarcomeres. In this case, the size of wires should be made small enough in diameter. To make a fine metal wire is very difficult, and not easy to handle. To resolve this problem, the force transducer on one side of the nano-fabricated levers deviced by X. Liu et.al., if the other side to the other electrostatic actuators, may be possible. In any, the electro-magnetic induction type of device for myofibril mechanics is useful for comparing with other devices.

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