STANDARD CMOS PIEZORESISTIVE SENSOR TO QUANTIFY HEART CELL CONTRACTILE FORCES

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ABSTRACT

A MEMS force transducer system, with a volume less than one cubic millimeter, is being developed to measure forces generated by living, isolated cardiac muscle cells. Cell attachment and measurement of contractile forces have been demonstrated with prototype hinged polysilicon devices. A new transducer system has been fabricated using a standard CMOS process with a post-processing XeF_2 etch step. The system consists of a three dimensional oxide structure with aluminum hinges, polysilicon piezoresistive sensor, and CMOS amplifier. System response is 0.45mV/μN. This MEMS force transducer will permit improved resolution of the mechanisms of muscle contraction.

INTRODUCTION

The measurement of contractile force in the μN range from single rat heart cells has proven difficult with current transducer technology. Typically, the force transducers currently used to determine the contractile characteristics from isolated heart cells are positioned outside the cell's saline bath and attached to the cells via complex and relatively massive structures (>200g) which inherently limit frequency response and sensitivity [1-4]. By necessity slender pipettes or needles must enter the bath and are subject to surface tension forces that can be greater than that of the cell's force. Thus, the readings of force development and complex stiffness modulus (frequency dependent stiffness/area) obtained are often restricted in range of frequency response (typically up to 100Hz).

Studies have been done on the passive properties of living rat heart cells by mechanically stretching cells and studying the tensile strength and stiffness of the cellular proteins [4]. In this case, bandwidth is not a critical parameter. However, to study the active properties of living rat heart cells, such as a contraction in response to a stimulus, a higher bandwidth is desirable to resolve dynamic changes in the overall stiffness of the cell due to contractile protein changes. Resolving forces in the range of 50nN - 50μN is preferred. The ends of the cell must be firmly attached to the transducer to prevent slippage during a contraction, and transmission optical microscopy through the cell is highly desirable to visually monitor the changes in protein distribution throughout the cell during a contraction.

We have designed a fully submersible, miniaturized CMOS force transducer less than $(1mm)^3$, which eliminates surface tension artifacts. With this low-mass transducer, we will be able to not only make steady state measurements of direct force, but possibly dynamic measurements such as the complex stiffness modulus from isolated rat cardiac cells at a range of oscillatory frequencies in excess of 1 KHz.

An earlier version of the transducer was implemented in polysilicon with no integrated electronics [5]. By taking advantage of standard CMOS technology, our present design integrates electronics, strain gauges, and three-dimensional structural components that extend past the side of the wafer. This over-hanging design allows transmissive illumination of the cell. In this paper, we describe this CMOS device along with the CMOS and polysilicon prototypes.

PROTOTYPE STRUCTURES

Polysilicon prototype

Prototype microstructures were initially fabricated using a two-polysilicon, two-oxide process [5]. Fig. 1 shows two moveable polysilicon clamps hold-

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ing a fixed single heart cell. A close-up of the right clamp is shown in Fig. 2. This structure was fabricated using the MUMPs fabrication service [6]. In this process, two structural layers of polysilicon are released by etching the sacrificial oxide layers in 49% HF. Using polysilicon hinges, two of the plates are rotated up 90° to the surface [7]. These clamps are suspended 2μm above the surface by 200 × 6 × 2$(\mu m)^3$ support beams anchored at one end. The force of contraction can be estimated using the calculated spring constant and the visually measured deflection in the beams.

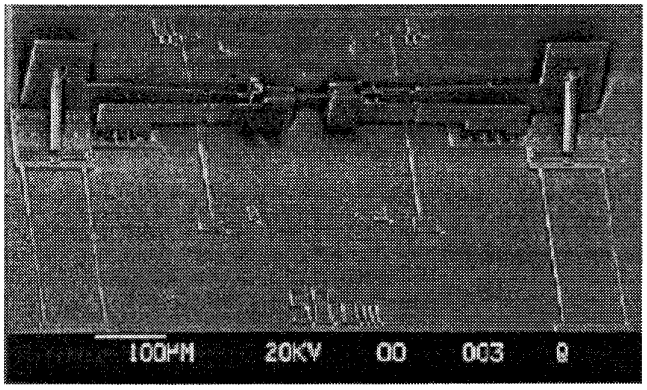

Fig. 1. SEM photo of two suspended polysilicon clamps holding a single heart cell. When the cell contracts, the support beams bend as the clamps move towards each other. For SEM purposes the cell was fixed in glutaraldehyde and has been dried in alcohol and HMDS and has shrunk in size.

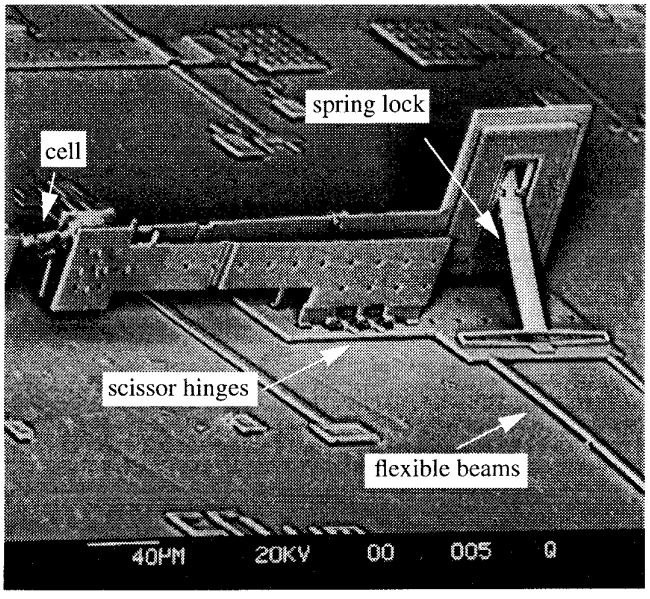

Fig. 2. Close-up of the right clamp shown in Fig. 1. Vertical plates are attached to a movable shuttle. Scissor hinges allow the vertical plates to rotate with respect to each other and translate in response to the cell's contraction. A spring lock supports the back vertical plate at 90°. The entire structure is suspended 2μm above the substrate and is anchored via the long beams (lower right corner). Dried cell is also shown (left side).

Cells have been routinely attached to these structures. In current experiments a live cell is glued between the clamps using a Dow Corning silicone rubber sealant. Allowing the glue to set for about half an hour in solution produces a firm seal around the ends of the cell. The attachment occurs simultaneously with clamp assembly. First, glue is applied to the attachment sites when the structures are dry. Then the cells in solution are introduced to the chip and the structure is assembled around the ends of the cell. A similar method will be used with the CMOS clamps described later. To date we have measured forces up to 16μN generated by living isolated heart cells, which is comparable to the cellular forces measured using macro-based systems [4].

In the polysilicon system, high beam compliance is necessary to facilitate direct visual measurement of spring deflection. However, this is not optimal. Ideally we would like to keep the cell's length relatively constant while it develops force (isometric tension). Additionally, the polysilicon design does not permit transmissive illumination of the cell. Direct illumination is necessary to provide visual observation of protein overlap inside the cell during a contraction.

CMOS prototype

To address some of these limitations, prototype devices were fabricated using a standard CMOS process [8]. All lithography and thin film patterning is performed during this process. When the chips come back from the foundry, they require a single unmasked etch. The primary structural material is oxide, with silicon as a sacrificial layer. Etch windows can be created in a standard CMOS process by patterning successive vias on top of each other but not filling them, thereby leaving the substrate exposed. The silicon is etched in XeF_2 which is selective to oxide and aluminum [9]. This isotropic gas phase etchant is extremely gentle and eliminates the possibility of structural damage due to meniscus forces of liquid etchants. However, liquid etchants such as EDP have also been used to undercut oxide structures [10] [11].

The prototype CMOS structure is shown in

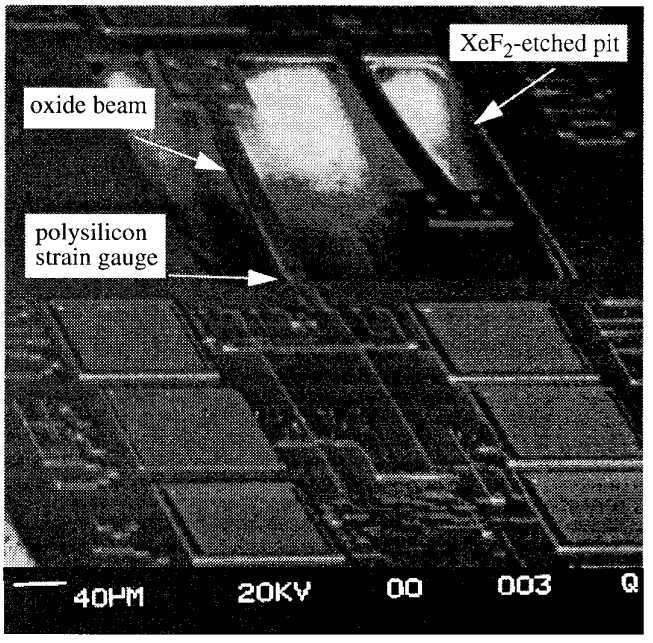

Fig. 3. Prototype CMOS structure consisting of beam with base piezoresistor, Wheatstone bridge, and amplifier. Chip was etched in XeF_2.

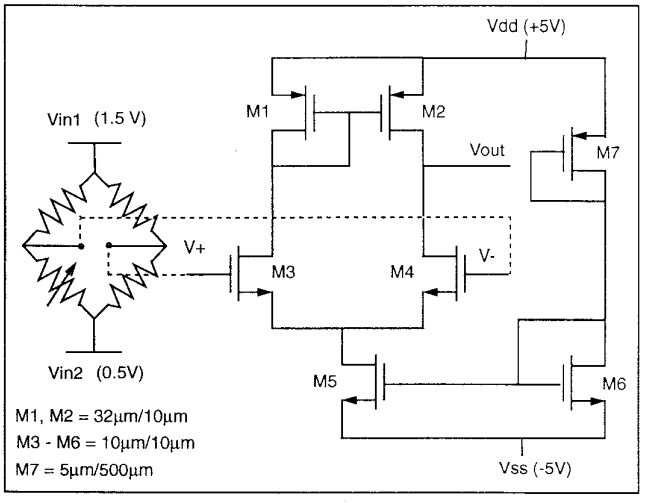

Fig. 4. Schematic diagram of sensor electronics. Wheatstone bridge has one variable resistor located in the base of a flexible oxide beam shown in Fig. 3.

Fig. 3. This structure consists of a beam $200 \times 20 \times 3(\mu m)^3$ with a $50 \times 50(\mu m)^2$ pad at the free end, which was undercut using XeF_2. The beam has a calculated spring constant of 2N/m. A 1kΩ polysilicon piezoresistor is embedded in the base which is part of a Wheatstone bridge attached to an on-chip CMOS amplifier. The circuit diagram is shown in Fig. 4. This simple amplifier consists of differential pair and biasing transistors. The area of the amplifier is approximately 3×10^4 $(\mu m)^2$ and has a measured open loop gain of 65. This amplifier is a "proof of concept" design to show that the voltage signal coming from the bridge in response to the deflection of the beam could be amplified on-chip.

CURRENT CMOS DESIGN

Fig. 5a schematically illustrates the various elements of the current version of the standard CMOS sensor as well as the location of the cell. A scanning electron microscope (SEM) photograph of the actual device is shown in Fig. 5b. As shown, the current design consists of two overhanging oxide clamps, each attached to a $200 \times 20 \times 3(\mu m)^3$ beam. The individual oxide pieces are connected via 1μm thick aluminum strips that can be folded using metal probes. A 90° fold in these aluminum "hinges" produces the clamps and the rotated beam, while a 180° fold in the base hinges flips the entire structure over the edge of the wafer to enable transmissive illumination of the cells. A close-up of the hinges is shown in Fig. 6.

The aluminum hinges also function electrically as interconnects. They can be folded clockwise or counterclockwise -- the direction of folding does not appear to affect electrical continuity. One of the beams in Fig. 5b has a 1KΩ polysilicon piezoresistor in the base that is encased in oxide and connected to the rest of a Wheatstone bridge via the aluminum hinges. A single living cell will be mounted between the clamps. When the cell contracts, the beams will bend. The change in resistance due to deformation will off-balance the Wheatstone bridge and this voltage signal can be amplified using the simple on-chip CMOS amplifier described above.

Fig. 7 shows the structure in Fig. 5b before assembly. The rippling is due to the residual stress in the oxide film after release. To assemble the structure, we first assemble the cell attachment site by making 90° folds in hinges #1 and #2. Then we make a 90° fold in the interconnect hinges (hinges #3). Finally we make a 180° fold in the base hinges (hinges #4) to flip the entire structure over the edge of the chip to complete assembly.

The cells that we are using for these experiments are cardiac cells isolated from rat hearts [1] [2] [4]. These cells are prepared fresh each experimental day from adult rat ventricles using standard 0.1% collagenase perfusion techniques to isolate the cells.

The cells are then placed in a relaxing solution (i.e. low calcium concentration) immediately after digestion. These cells are demembranated for experiments in Triton-X detergent. All experiments are performed with cells less than one day old to eliminate any possible cellular changes due to the cell's removal from the heart.

Before cells are attached to the structures, the aluminum will be passivated by PECVD oxide. Cell attachment to the force transducer will take place during the assembly sequence. The sequence may need to be modified to accommodate the extra glue steps and liquid environment.

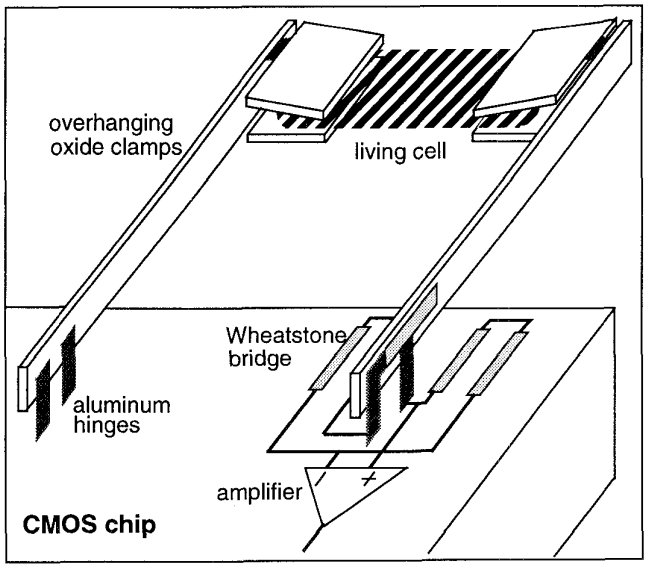

Fig. 5a. Schematic illustration of the components of the sensor system to measure heart cell contractile forces. Flip-over plate and etch pits are not shown. 1μm thick aluminum strips are used as hinges as well as electrical interconnects.

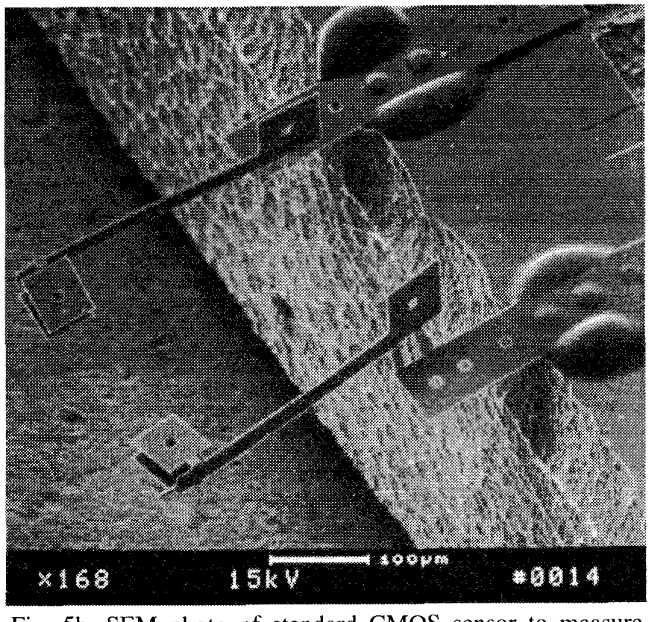

Fig. 5b. SEM photo of standard CMOS sensor to measure heart cell contractile forces. Silicone sealant was used to glue flip-over plates to the substrate the protect the structure in the SEM vacuum. Chip was etched in XeF_2. Top beam has piezoresistor in the base. Bridge and amplifier are connected on this chip but are not shown.

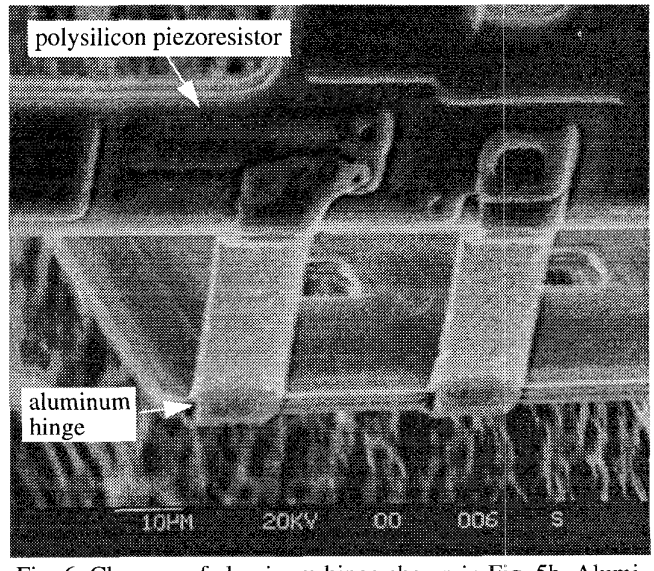

Fig. 6. Close up of aluminum hinge shown in Fig. 5b. Aluminum strip is 10μm wide, 1μm thick. Polysilicon embedded piezoresistor is also shown. This is hinge #3 from Fig. 7.

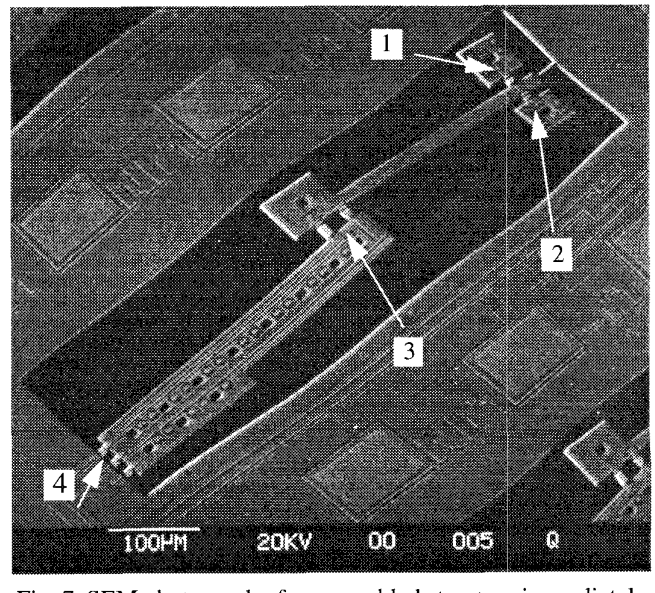

Fig. 7. SEM photograph of unassembled structure immediately after release in XeF_2. Rippling is due to residual stress in the oxide film after release. Each hinge is numbered consecutively in order of assembly.

RESULTS AND DISCUSSION

Aluminum hinges make acceptable mechanical supports provided they are not repeatedly bent through large angles of deflection. In an experiment to characterize the fatigue life of aluminum hinges, they were repeatedly folded back and forth through a fixed angle until electrical failure. Large angle deflections were generated by moving the structures using metal probes attached to micromanipulators. As expected, the results in Table 1 indicate that large angle deflections cause failure sooner than small angle deflections. Failure usually occurs at the aluminum/silicon-dioxide junction. Small deflections of XeF_2-etched aluminum hinges by electrostatic forces does not cause hinge failure even after millions of cycles [11].

Table 1:

Angle of rotation (degrees)	Number of successful folds before failure
180	1 - 2
90	8 - 12
30 - 40	20 - 40
1 - 2	$> 10^6$

The prototype CMOS structure shown in Fig. 3 was electrically tested by vertically deflecting the beam with a probe and monitoring the change in output voltage of the amplifier (Fig. 8). The response is fairly linear with slope 0.45mV/μm. XeF_2 etching has no measurable effect on circuit performance. All aluminum hinges are connected to undercut oxide plates or membranes. Thus, these are not ideal, rigid supports -- when the beams are deflected, a portion of the supporting membrane is also deflected which adds to the deformation of the piezoresistor.

The overhanging beam shown in Fig. 5b was deflected parallel to the surface to simulate a cellular contraction. When released, the beam returned to within 1μm of its original position for deflections up to 25μm, indicating that the hinges are elastic when subject to strains up to 4×10^{-5}. Using the calculated beam spring constant (k = 2N/m), a 25μm displacement corresponds to 50μN of force. A large rat heart cell (approximately 150μm long and 50μm in diameter) can generate up to 50μN of force, so during normal operation these hinges should not experience substantial plastic deformation. During these tests, there was no change in the continuity of the aluminum until breakage, as expected [13].

Electrically, this sensor system shows a linear response with slope 0.9mV/μm (Fig. 9). Dividing by the spring constant of the beam, we expect a 0.45 mV/μN response from this system. With this level of

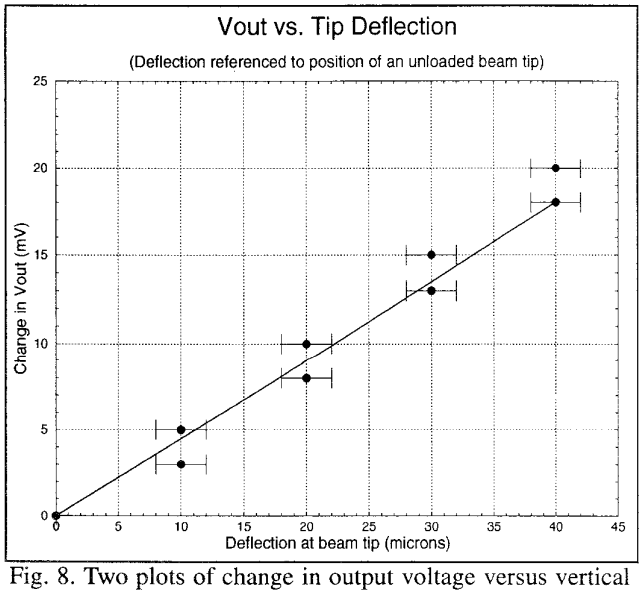

Fig. 8. Two plots of change in output voltage versus vertical deflection for prototype CMOS sensor and amplifier shown in Fig. 3. Slope of the best-fit line is 0.45 mV/μm.

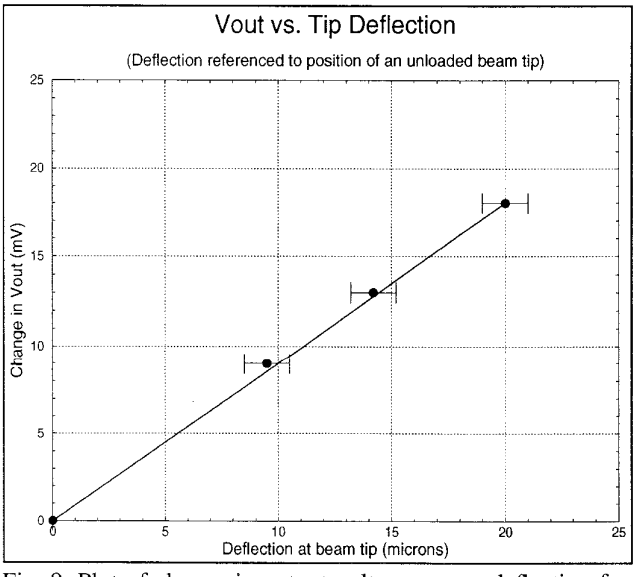

Fig. 9. Plot of change in output voltage versus deflection for three-dimensional sensor system with CMOS amplifier shown in Fig. 5b. Slope of best-fit line is 0.9mV/μm.

response, resolving a 50nN force in a 1 KHz bandwidth will require an amplifier with a noise density of less than 10 nV/(Hz)$^{1/2}$.

CONCLUSIONS

To date, we have fabricated a three-dimensional standard CMOS cellular force transducer system with on-board sensing electronics. The system response is linear with a slope of 0.9mV/μm. Structures incorporate aluminum hinges as mechanical supports as well as electrical interconnects. Hinges allow the entire structure to be rotated over the edge of the chip to permit the necessary transmissive illumination of the cells during contraction. Cell attachment and measurement of contractile forces have been demonstrated with prototype hinged polysilicon devices. With this system, we can begin to uncover the fundamental processes in muscle contraction with a much higher resolution than that offered by currently available technology.

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