

A Microsystems Approach to Cellular Manipulation and Interaction

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*ABSTRACT: The sense of touch is poorly understood, partly due to the difficulty in making combined mechanical and electrophysiological measurements on individual touch receptor neurons. In this work, we used micromachined silicon force sensors to measure the force sensitivity of the nematode *Caenorhabditis elegans* to nose touch. The touch sensitivity of wild-type worms and three mutants were measured and analysis is ongoing.*

INTRODUCTION

This research was funded by the International Research and Education in Engineering (IREE), a supplement to the existing NSF CAREER Award (ECCS-0449400) to Prof. Beth Pruitt at Stanford University. Research supported by the CAREER award includes the development of sensors and actuators for studying the biomechanics of individual cells, electrostatic systems for operation in ionic media, and the patterning and surface functionalization microfabricated sensors and actuators. In particular, we are developing micromachined silicon force sensors using the piezoresistive effect in silicon for the study of touch sensitive neurons in the nematode *Caenorhabditis elegans*.

Numerous other groups study microelectromechanical systems (MEMS) with an emphasis on biological measurements. The IREE allowed us to collaborate with the Institute of Robotics and Intelligent Systems (IRIS) at the Swiss Federal Institute of Technology (ETH) in Zurich, Switzerland headed by Prof. Bradley Nelson. They have developed sensors and actuators based upon changes in the electrostatic capacitance of a structure due to an applied force. The IREE enabled the exchange of knowledge and experience in the design and characterization of microscale force sensors, as well as biological applications and experimental configurations.

Joseph (Joey) Doll was chosen as the IREE visiting early-career researcher. Joey is an experienced clean room user and utilizes cell culture, piezoresistive force sensors, atomic force microscopy and light microscopy in his research on mechanotransduction in touch sensitive neurons. Furthermore, he had been making measurements on the sensitivity of *C. elegans* to forces applied to the nose at Stanford, and the force sensors developed at IRIS suggested another approach to measuring the nose touch forces. Joey is a second year graduate student at Stanford University pursuing his PhD.

The research data anticipated from the collaboration with IRIS was the touch sensitivity of *C. elegans* and several mutant strains. It is also expected that the general exchange of research and educational knowledge will be valuable to both laboratories. Joey visited IRIS for three months, from March 1st, 2008 to May 30th, 2008.

RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

At ETH, Joey used the microfabricated silicon force sensors designed and fabricated by IRIS. The technology has been commercialized by Femtotools, and features nanonewton (nN) force resolution with > 100 μ N range over a 300 Hz bandwidth. The electrostatically actuated, electrostatically sensed gripper is shown in Figure 1 and the experimental setup is shown in Figure 2. The force is measured by a differential

change in capacitance between the movable combs attached to the end effector and the stationary combs attached to the substrate. The resulting signal is amplified and filtered on the printed circuit board (PCB) before being digitized with a 14-bit DAQ card (National Instruments). Data was typically sampled at 10 kHz and downsampled to 1 kHz. Force resolution was typically 10 nN. Video is captured with a camera attached to the microscope (Basler Technologies), and a synchronized video of the video and force data are recorded to aid later analysis of the interactions between a worm (*C. elegans*) and the force probe. Worms were sent to Joey at ETH (courtesy of the Goodman Lab at Stanford).

Force measurements consisted of manually controlling the z-position of the force sensor and the x- and y-movement of the motorized stage that the worms sit on. The sensor was manually positioned in front of a worm and left stationary until the worm bumped into the probe and backed up. The force vs. time was recorded for later analysis.

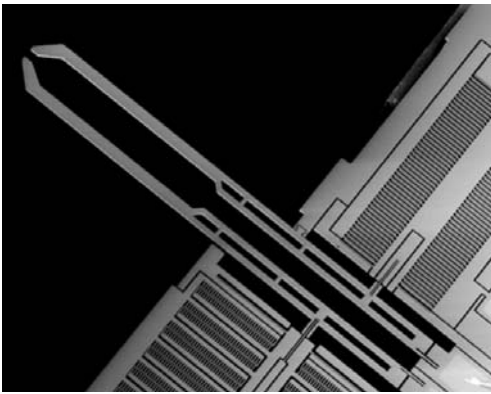


Figure 1. MEMS force sensor used in this work. The gap between the grippers is 50 μm , for scale. The actuator is on the left side and the sensor is on the right. Both the sensor and actuator utilize interdigitated comb electrodes. Only the sensor was used in these experiments; the actuator was manually broken off.

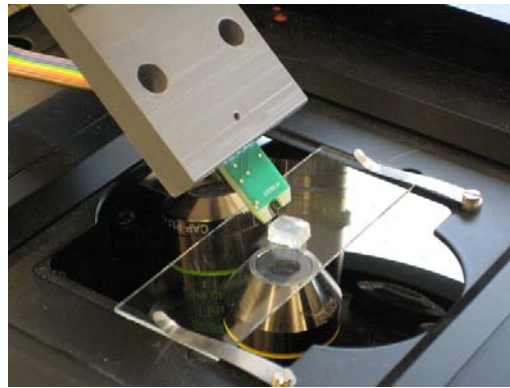


Figure 2. Experimental setup. The force sensor is mounted on a PCB and mounted on a three-axis manipulator. *C. elegans* is grown on agar gel, and a small section is cut off and placed on a glass slide, which is then placed on a two-axis motorized stage. The entire setup is mounted on an inverted microscope.

The nose touch sensitivity of *C. elegans* was measured for wild-type animals as well as three mutant strains: *ha-1134*, *osm-9* and *ocr-2*. The latter two mutants have defects in proteins believed to form an ion channel complex required for touch sensitivity. The *ha-1134* strain expressed green fluorescent protein (GFP) in the sensory neurons (ASH) responsible for sensing nose touch and osmotic stress, and was not expected to show a difference in touch sensitivity compared with the wild-type animals.

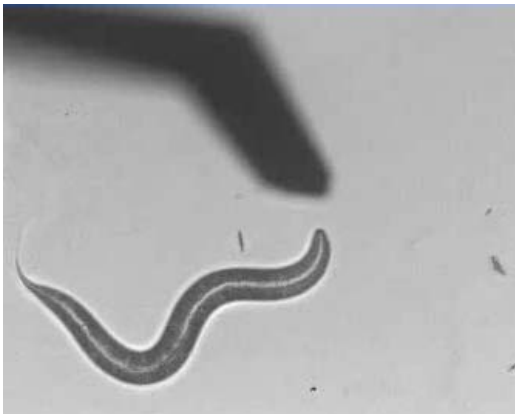


Figure 3. Force measurement probe positioned

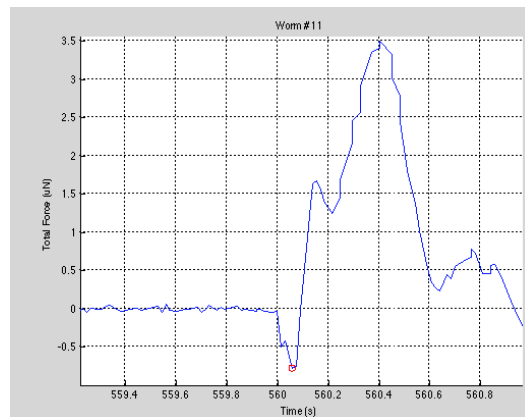


Figure 4. Example force vs. time plot. The worm

above the agar surface with a worm in the field of view. A low magnification object (10x, 100x total) was used to view the experiments through the agar gel with the inverted microscope.

runs into the probe, registering a force of approximately $-0.7 \mu\text{N}$. The worm quickly senses the force and backs away, pulling the probe with it generate the $3.5 \mu\text{N}$ force in the other direction.

The experiments performed at ETH were based upon applying microfabricated sensor technologies to the study of biological phenomena that had never been quantified to date. The research is directly related to our existing research on mechanotransduction under the current NSF CAREER award. At ETH Joey worked most directly with Felix Beyeler and Simon Muntwyler, two Ph.D. students who developed the force sensors and provided them for the worm experiments. He also met with Prof. Nelson to discuss results and shared an office with Karl Vollmers, who he exchanged ideas with. In the lab, Joey had complete access to equipment and performed biological work in a wet bench laboratory, machined mounting pieces and performed experiments in a quiet room and vibration isolation table.

As noted earlier, experimental data was collected by placing a small force sensor in the path of a moving worm and measuring the force required for the worm to sense the force and back away. A view through the microscope is shown in Figure 3. The sensor is slightly above the surface and is still blurry. The diameter of the worm is approximately $35 \mu\text{m}$ and the flat surface of the probe that the worm runs into is $50 \mu\text{m} \times 50 \mu\text{m}$. The force is measured with time (Figure 4), and the results of many worm collisions are recorded and analyzed. Analysis is performed by manually reviewing the combined recording of force and the view from the microscope. After selecting good force interactions and recording their times, an automated script (MATLAB R2007B) is used to extract features such as peak amplitude. The peak force recorded for wild-type *C. elegans* (N2 strain) is plotted in Figure 5. Future analysis of the data will include duration and rise-time, in order to correlate genetic alterations in *C. elegans* with phenotypic changes in touch sensitivity.

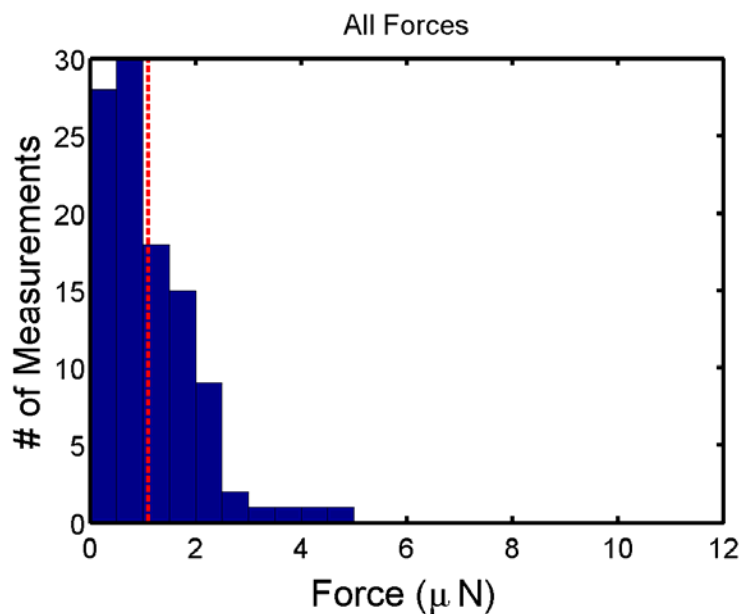


Figure 5. Peak forces measured for wild-type *C. elegans* (N2 strain). The red line is the mean of the data (8 worms, 106 measurements).

BROADER IMPACTS OF THE INTERNATIONAL COOPERATION

While in Switzerland, Joey also had the opportunity to visit other universities working similar projects. He gave a talk at the University of Neuchatel in Neuchatel, CH entitled “MEMS for Studying

Mechanotransduction”, and discussed research with students in the group of Prof. Nico de Rooij. He also visited Ecole Polytechnique Federale de Lausanne (EPFL) in Lausanne, CH to meet the research group of Prof. Juergen Brugger and share ideas.

The IREE award expanded the scope of the CAREER award by applying new force sensor technologies to problems investigated with other sensors under the existing award. Additionally, the IREE supplement promoted diversity by sharing cultural experiences and research knowledge between researchers from different backgrounds. While he was living in Switzerland, Joey had the opportunity to visit a handful of other countries with different languages and cultures, and also was able to observe differences in the structure of academic laboratories and technology companies, for example the differences in startup funding sources.

DISCUSSION AND SUMMARY

The IREE program succeeded in fostering new international collaboration in microscale force sensing applied to biological systems. We applied MEMS force sensors fabricated by the host lab (IRIS at ETH, Zurich) to measure the force sensitivity of the nematode *C. elegans* to nose touch. This measurement has been performed qualitatively for the past 20 years, and we are in the early stages of applying quantitative tools to better characterize the phenotype of genetic mutations that affect touch sensitivity. Experiments were performed on four strains of worms and analysis of the data is ongoing. Early results indicate that wild-type *C. elegans* responds to forces on the order of 1 μ N in magnitude.

In future iterations of the IREE program we suggest scheduling the planning meeting for earlier in the year. By the time it was scheduled (for early May), the visiting IREE researcher was already at the host lab and not able to come to the planning meeting.

ACKNOWLEDGEMENTS

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BRIEF BIOGRAPHIES OF RESEARCHERS

Joseph C. Doll received the B.S. degree in Mechanical Engineering from the University of California, Berkeley in 2006. He is currently pursuing his M.S. and Ph.D. degrees in Mechanical Engineering from Stanford University. His research interests included microfabricated force sensors and other microscale devices for quantitative biological studies.

Beth L. Pruitt received the BS degree in Mechanical Engineering from MIT, Cambridge, MA in 1991, the MS degree in Manufacturing Systems Engineering from Stanford University in 1992, and the PhD degree in Mechanical Engineering from Stanford University in 2002 supported by both the Hertz Foundation Fellowship and the Stanford Future Professors of Manufacturing Fellowship. She served as an officer in the US Navy with tours as an engineering project manager at Naval Reactors in Washington, DC and as an engineering instructor at the US Naval Academy and is certified as a professional engineer. She is presently an assistant professor at Stanford University. Her research interests include micro-electromechanical systems (MEMS), particularly materials characterization, manufacturing and design for packaging, systems integration, biomedical devices and biological measurements. She is a member of the American Society of Mechanical Engineers, Institute of Electrical and Electronics Engineers, and Materials Research Society.