

BIOLOGICAL MEASUREMENTS OF *C. ELEGANS* TOUCH SENSITIVITY WITH MICROFABRICATED FORCE SENSORS

Joseph C. Doll¹, Nahid Harjee², Nathan Klejwa², Ronald Kwon¹, Sarah M. Coulthard¹, Miriam B. Goodman³, and Beth L. Pruitt¹

¹Department of Mechanical Engineering, Stanford University, Stanford, CA, USA

²Department of Electrical Engineering, Stanford University, Stanford, CA, USA

³Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA, USA

ABSTRACT

This paper presents biological measurements performed with a microfabricated force-post fabricated from SU-8 and metal on quartz wafers. The transparent device is used to measure the touch sensitivity of nematodes (*Caenorhabditis elegans*) during normal locomotion. We measure forces in the $0.5\mu\text{N}$ - $10\mu\text{N}$ range for wild type and *mec-6*, a touch insensitive mutant, and observe a measurable difference between the two strains.

Keywords: *C. elegans*, Force Sensor, Mechanotransduction, Locomotion

1. INTRODUCTION

The sensation of touch guides complex organism behavior such as obstacle avoidance and locomotion. Many organisms have highly specialized touch cells that are responsible for the rapid transduction of force into electrochemical signals. Several touch receptor neurons located in *C. elegans* are responsible for the avoidance light touch. Ionic currents in response to touch have been measured [1], however the range of force that mediates a response in normal behavior is not known and the relevance of the applied forces to normal animal behavior has not been quantitatively verified.

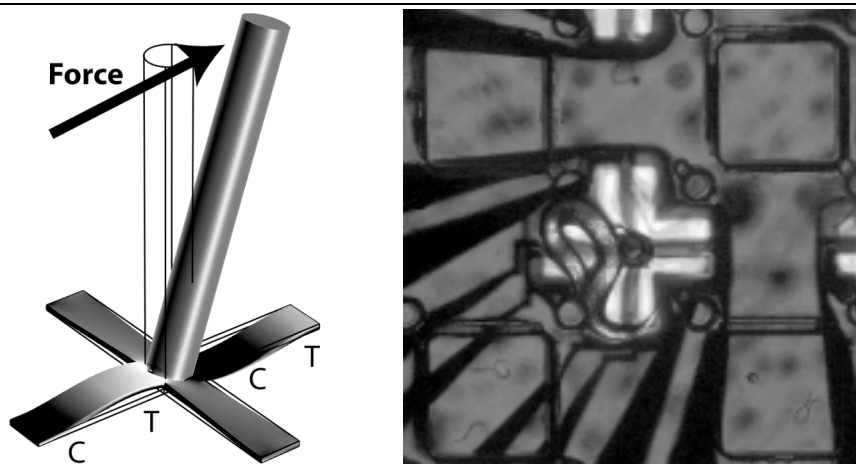


Figure 1. A finite element simulation of force post deflection illustrating the tensile and compressive stresses in the cantilever beams (left) and low magnification image of *C. elegans* interacting with force post (right).

Microfabricated devices have been used in the past to study the forces generated and sensed by small organisms. Previous efforts in studying microscale cell and organism locomotion have utilized both optical and electronic transduction mechanisms. Polydimethylsiloxane (PDMS) has been molded into pillars and visually tracked during deflection to study cell motility [2].

This paper reports new biomechanical measurements using a microfabricated force post system with direct electronic readout [3]. The device consists of four fixed-guided cantilever arms with a vertical pillar rising from the central junction. Forces applied at the tip of the pillar induce strains in the cantilevers that are sense by metal strain gauges. Differential resistivity measurements are taken along the two in-plane axes aimultaneously to calculate the force.

2. THEORY

The ratio of output voltage to force applied at the pillar tip can be approximated from the bridge bias voltage V_{in} , amplifier gain A , straing gauge Poisson ratio ν , pillar height h , cantilever arm Young's modulus E , width w , and thickness t :

$$\frac{V_{out}}{F} \approx \frac{3V_{in}A(1+2\nu)h}{4Ewt^2}. \quad (1)$$

3. EXPERIMENTAL

Briefly, the device was fabricated from SU-8 photoresist and gold strain gauges on a quartz wafer. Fabrication details and device characterization have been reported in detail previously [3]. Devices with 300 μ m x 65 μ m cantilever beams and 300 μ m tall force posts were used for all measurements.

For measurements, the strain gauges along each axis were connected in a balanced Wheatstone bridge with two-stage amplification for a total of 10,000X gain (TI INA103 and AD622). Calibration was performed with a piezoresistive silicon cantilever. A calibrated force was applied to the pillar tip and the cantilever and force sensor outputs were recorded. A force sensitivity of 0.376 V/ μ N was measured at a bias voltage of 1V. An optically transparent cover was placed over the sample to shield air currents and data was typically recorded over a period of less than 60 seconds.

C. elegans cultures were prepared on standard growth plates seeded with OP50 and raised at room temperature for several days to obtain adults. Worms were then replated on a clean agar plates and a small piece of agar was cut out and placed on a glass slide. The slide was inverted and attached to the end of a three-axis micromanipulator to position the worms over the force sensors for measurements. Two strains of worm were studied, wild type and *mec-6* (u247), a strain with a defect in the touch sensitive ion channel complex that leads to vastly reduced touch sensitivity [4].

4. RESULTS AND DISCUSSION

The mean contact force for wild type and *mec-6* was 2.5 and 4.7 μ N, respectively. A typical force-time plot and histogram of measured forces is shown in Figure 2. The contact force applied by the worms to the pillar during locomotion was typically less than 10 μ N. Interactions varied from brief nose touches followed by immediately backing up to wrapping around the post for several seconds. The latter interaction was more common and

the worms appeared to prefer the tight spaces afforded by the posts and spacer blocks rather than shying away from them.

It is likely that the structure of the environment affects locomotion in *C. elegans* and other organisms. The small difference between wild type and touch insensitive mutants suggests that the measured forces are more indicative of locomotion forces than touch sensitivity thresholds.

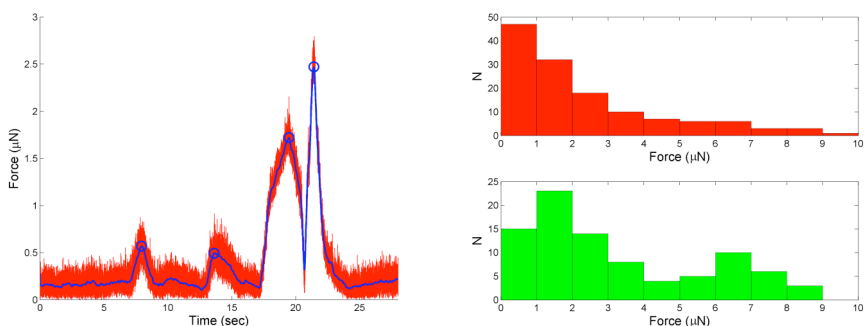


Figure 2. Typical measurement of force over time (left). In post-processing, the force measurements from the x- and y-axes are summed to obtain the total force. The total force is smoothed and the peak forces are identified. These peaks are then plotted in a histogram (right) for wild-type and *mec-6* strains.

5. CONCLUSIONS

We have measured the nose and body touch sensitivity of *C. elegans* during natural locomotion with a microfabricated force sensors made from SU-8 and metal strain gauges.

ACKNOWLEDGEMENTS

Fabrication work was performed in part at the Stanford Nanofabrication Facility (a member of the National Nanotechnology Infrastructure Network) supported by the NSF under Grant ECS-9731293, its lab members, and the industrial members of the Stanford Center for Integrated Systems. This work was supported by the NSF CAREER Award ECS-0449400. The authors thank S.J. Park for device calibration assistance and J. Cueva for the *mec-6* strain.

REFERENCES

- [1] R. O'Hagan, M. Chalfie, M. B. Goodman, *The MEC-4 DEG/ENaC channel of Caenorhabditis elegans touch receptor neurons transduces mechanical signals* Nat Neurosci, 8, 43-50 (2005).
- [2] J. L. Tan, J. Tien, D. M. Pirone, D. S. Gray, K. Bhadriraju, C. S. Chen, *Cells lying on a bed of microneedles: an approach to isolate mechanical force* Proc Natl Acad Sci U S A, 100, 1484-1489 (2003).
- [3] N. Klejwa, N. Harjee, R. Kwon, S. M. Coulthard, B. L. Pruitt, *Transparent SU-8 Three-Axis Micro Strain Gauge Force Sensing Pillar Arrays for Biological Applications* Proceedings of Transducers 2007, In Press,
- [4] G. Gu, G. A. Caldwell, M. Chalfie, *Genetic interactions affecting touch sensitivity in Caenorhabditis elegans* PNAS, 93, 6577-6582 (1996).