Micromachined silicon force sensor based on diffractive optical encoders for characterization of microinjection

X.J. Zhang, S. Zappe, R.W. Bernstein, O. Sahin, C.-C. Chen, M. Fish, M.P. Scott, O. Solgaard

Department of Electrical Engineering, Stanford University, Stanford, CA 94305, USA
Department of Microsystems, SINTEF Electronics & Cybernetics, Oslo, Norway
Department of Developmental Biology, School of Medicine, Stanford University, Stanford, CA 94305, USA

Received 11 November 2003; accepted 21 November 2003

Abstract

We present a micrograting-based force sensor integrated with a surface micromachined silicon-nitride probe for penetration and injection into Drosophila embryos. The probe is supported by springs of a known spring constant, and the penetration force is determined from displacement measurements using a high-resolution, miniaturized optical encoder that is designed to only be sensitive to axial deflections of the probe. The optical-encoder force sensor exhibits configurable sensitivity and dynamic range, allowing monitoring over a wide range of forces. The periodicity of the encoder response can be used for calibration of the injector displacement and to obtain information about the localized elastic properties of the target. We used an force sensor with a measured spring constant of 1.85 N/m for penetration experiments on Drosophila embryos, and found a penetration force of 52.5 ± 13.2 N and a membrane displacement of 58 ± 5.2 μm.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Force sensor; MEMS optical encoder; Microinjection; Drosophila embryo

1. Introduction

Localized and accurate microinjection of genetic material into biological model systems, such as Drosophila, will enable a variety of studies of developmental biology and genetics [1]. For such studies to be carried out in vivo, the damage caused by the injection must be minimized. We have developed surface micromachined silicon-nitride injectors [2] with integrated force sensors for measurements of the penetration force and needle-membrane interactions under various physiological conditions.

The force sensor is an optical encoder based on transmission phase gratings integrated with the injector. Precise displacement measurements using diffraction gratings is an established technology [3], and optical encoders have been developed for precise measurements of displacement and revolution angle for a variety of applications. However, the large size and expensive manufacturing of conventional encoders make them unsuitable as integrated sensing devices. Recently, there has been significant renewed interest in using diffractive micro-optical elements as displacement sensors in atomic force microscopes (AFM) [4], MEMS capacitive ultrasonic transducers [5] and accelerometers with nanog resolution [6]. For optical encoders, Sawada et al. demonstrated a hybrid integrated encoder with a single grating on silicon [7]. Hane et al. designed a dual-grating miniaturized displacement sensor using grating imaging [8]. These advancements in microfabricated diffractive grating optics enable integrated optical encoders for sensing and microscopy of embryos and single cells.

2. Operational principles and design

As shown in Fig. 1, the force encoder consists of two identical constant-period transmission phase gratings that are vertically aligned when no force is applied. Phase gratings are used because they have higher optical diffraction efficiency than amplitude gratings [9]. When a force is applied to the injector (not shown in Fig. 1) in the x-direction, the upper index grating is displaced with respect to the bottom grating. This changes the diffraction efficiency of the phase grating, and the relative position of the two gratings can be determined by the intensities in the diffraction orders. The diffraction characteristics of the dual transmission
The phase delay profile \( \phi(x) \) for one grating period \( 2L \): 0 < \( d \) < \( L \).

\[
I_1(d) = I_0 N^2 \left( \frac{\sin^2(\alpha d/(2L))}{\sin^2(d/(2L))} \right) \left[ (L - d) \sin \left( \frac{(L - d)}{4L} \right) \right]^2 
\times \sin^2 \phi_0 G(d) \quad \text{for } d \in [0, L] 
\]

\[
G(d) = \begin{cases} 
\frac{\pi(3L - d)}{4L} & d \in [L, 2L] 
\end{cases} 
\]

where \( I_0 \) is the illuminating light intensity, \( N \) the number of grating periods under illumination, \( \phi_0(x) = \frac{2\pi}{\lambda} (n_1 - n_2)x \) is the phase delay over the thickness of one grating finger, \( 2L \) the period of the grating, \( d \) the displacement of the injector modulus \( 2L \). We define the force-sensor sensitivity as the change in the intensity of the first diffraction mode with respect to a unit displacement of the upper grating. The dynamic range is defined as the total range of motion over which the position can be unambiguously determined from the diffraction pattern. From Eq. (1) we see that the sensitivity and dynamic range of the sensor can be tuned by changing the number of grating fingers, \( N \), that are illuminated and/or by changing the grating period, \( 2L \).

The encoder is designed to be sensitive to translation in the \( z \)-direction, while the sensitivities to the other 5 degrees of freedom of motion are minimized. Translation in the \( y \)-direction can be neglected because it is small (\( k_y \gg k_z \)) and has little effect on the optical readout. Likewise, rotation about the \( z \)-axis does not affect the diffraction of the gratings and can therefore be ignored. Motion in the \( x \)-direction is also inconsequential, because the weak reflections from the grating elements lead to only small variations of the phase shift through the encoder as a function of the separation of the gratings in the \( x \)-direction. To ensure weak reflections, the grating elements must be designed such that the fields reflected from their fronts and backs interfere destructively. We achieve destructive interference by using grating made of silicon nitride with a refractive index of \( n_1 \approx 1.9 \) and a thickness of \( t = 1.5 \mu m \). Thus the phase shift (\( 4\pi L_0 n_2 t \)) associated with traversing the grating film twice is approximately an integer multiple of 2\( \pi \) at HeNe wavelength \( \lambda = 633 \text{ nm} \). Combined with the \( \pi \) phase shift of the internal reflection at the nitride/air interface, this leads to destructive interference of the two parts of the reflected field, and therefore the reflection from the grating elements is minimized. Accurate control of the film thickness can be achieved by monitored etch-back after film deposition. Silicon nitride is well suited for our gratings, because its index allows us to minimize the back reflections and at the same time achieve a relatively high value (\( \approx 0.5 \)) for the factor \( \sin^2(\phi_0) \) that determines the diffraction efficiency (see Eq. (1)). The remaining degree of freedom of motion is rotation about the \( z \)-axis. The encoder is sensitive to such rotation, so it must be minimized. The encoder therefore has maximally separated, straight suspensions to create a large spring constant for rotation about the \( z \)-axis.

The accuracy of the force measurements depends on the spring constant of the movable index grating structure. The
Table 1: Optical-encoder force-sensor design parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index micro-grating</td>
<td>Period: 22 (10), 30 (15) μm</td>
</tr>
<tr>
<td></td>
<td>Thickness: 1.5 μm</td>
</tr>
<tr>
<td>Supporting beam (spring)</td>
<td>Thickness: 1.5 μm</td>
</tr>
<tr>
<td></td>
<td>Length: 850 μm</td>
</tr>
<tr>
<td></td>
<td>Width: 8, 15, 30 μm</td>
</tr>
<tr>
<td></td>
<td>Spring constant (N/m): 2.2, 14.5, 115.2</td>
</tr>
<tr>
<td></td>
<td>Resonant frequency (kHz): 13.9, 35.8, 100.8</td>
</tr>
</tbody>
</table>

*Spring constants and resonant frequencies are simulated for a laterally loaded spring structure (see Fig. 3) with beam widths W = 8, 15, and 30 μm, thickness h = 1.5 μm and probe with length L_0 = 80 μm and tip sidewall area A = 10 μm² and θ = 60°.

The spring constants were simulated for the geometry shown in Fig. 3. A one-dimensional elastic model was used for the doubly supported beam. For a center-loaded doubly clamped beam, the relation between the applied force F and the deflection along x-direction is composed of both linear and non-linear terms. The small-deflection linear bending term is proportional to the beam moment of inertia hW³, while the non-linear stretching term is proportional to hW, so thicker beams have more linear characteristics than thinner beams. For our force sensor, the transition from bending-dominated behavior to stretching-dominated behavior occurs when the deflection is about 10W. Thus for our penetration experiments, the sensor is expected to operate mainly in the linear range. With normally applied pressure over the two sidewalls of the probe tip, the stress and displacement vector distribution across the device were simulated using the finite element method (FEM) for supporting beams of width 8, 15 and 30 μm. The calculated spring constants and resonant frequencies are listed in Table 1.

3. Fabrication process

As shown in Fig. 4, the fabrication starts with the deposition and patterning of a 0.5 μm silicon-nitride layer.
on the oxidized silicon wafer to form the fixed scale grating (10–30 μm periods). Then 2 μm low temperature oxide (LTO) is deposited as the sacrificial layer to allow release of the upper index grating with the integrated injector. A second 1.5 μm silicon-nitride layer is deposited and patterned to form the upper index grating, and an array of anti-sticking dimples is formed by oxygen-plasma etching of the LTO before deposition of the second nitride layer. The injection force characterizations presented in this paper were acquired using optical encoders with probes without hollow channels, but integrated hollow injectors can be realized by embedding sacrificial LTO layer between the two silicon-nitride films of the probe. A deep reactive ion etch (DRIE) step is performed on the backside of the wafer to form the optical interconnect for illuminating the gratings. Finally, the force-sensor chip is removed from the wafer and released using buffered HF followed by critical point drying (CPD) to avoid unintended adhesion of the released structure to the substrate.

Fig. 5 shows scanning electron micrographs (SEMs) of the injector with the integrated optical-encoder force sensor. We selected silicon nitride (Si3N4) as the grating material due to the need for stress-optimized thin films of good optical quality. The optical transmission of 1.5 μm thick silicon nitride, deposited under NH3-rich conditions, was measured to be approximately 83% at 633 nm. The gratings therefore only have weak amplitude modulation, and the transmission encoder can be considered to consist of pure phase gratings.

4. Results and discussion

For DC calibration of the force sensor, we used a SINTEF® piezoresistive microscale with a sensitivity of ~150 μg/mV to measure the injection force into Drosophila embryos. The injections were performed both with a traditional, commonly used drawn-glass needle with a typical 75 μm diameter tip and with the silicon-nitride force encoder probe with a 30 μm tip. The calibration set-up is shown in Fig. 6. The MEMS probe requires ~40 μN to penetrate the newly hatched embryo. This is about four times less than the force needed for penetration with the conventional glass needle. In addition, the nitride probe needs a shorter traveling distance to reach penetration. Dynamic operation of the MEMS injector by off-chip piezoelectric actuation [10] is expected to further improve injection speed and cause less damage to the embryo.

The spring constant k of a force encoder (W = 8 μm, L = 10 μm) was measured using the same set-up. The measured value was 1.85 N/m (±8.65%), in reasonable agreement with the simulation results. The discrepancy is assumed to be due to over-etching of the springs supporting the movable upper grating.

Fig. 7 shows the measurement set-up for the integrated force sensor. The sensor was illuminated by a HeNe laser (633 nm/4 mW) with spot sizes ranging from 60 to 160 μm. The power in the first-order diffracted mode is measured with a photodiode (embedded in a Coherent® Beam-View Analyzer) placed 5 cm from the force encoder. Spatial filtering was performed to minimize the crosstalk.

1 Drosophila embryos 50 min after hatching are dechlorinated in 60% bleach for 1.5 min and then rinsed thoroughly with water (20°C). Properly staged embryos are selected and desiccated for 15 min in a sealed glass jar containing calcium sulfate (CaSO4) desiccant. Finally, embryos are covered in Halocarbon 700 oil (Aqua-Air Industries Inc., Harvey, LA) and ready for injection.
between diffraction orders. Fig. 8 shows the measured power of the first diffraction mode as a function of absolute displacement of the injector. This displacement includes both the relative displacement of the gratings and the displacement of the embryo membrane. The grating displacement can be found from the known 20 \mu m period of the diffraction response. Using this calibration and a spring constant of 1.85 N/m, we find an injection force of 63 \mu N. This assumes that the membrane behaves like a linear spring under small deformation. The sensor as tested here has a significant sensitivity around \( d = 2L \), but, since its output is ambiguous around the displacement where penetration takes place, it cannot be used to verify the assumption that the membrane deformation is linear in the force. The solution is provided by illuminating fewer periods of the force encoder. As shown in Fig. 9, the same force sensor illuminated by a laser spot size of 60 \mu m (\( N = 3 \)), has an improved dynamic range (45% increase), at the cost of lower sensitivity (18%/\mu m reduction). In this case, the diffraction is not ambiguous around the penetration displacement, so both the penetration force (48 \mu N) and the embryo membrane deformation (57 \mu m) at penetration can be determined. In a series of experiments, we found an average penetration force of 52.5 \mu N (\( \pm 13.2\% \)) and an embryo deformation of 58 \mu m (\( \pm 5.2\% \)). The measurements are in reasonable agreement with the piezoresistive-scale calibration data, demonstrating that the optical MEMS encoder force sensor has sufficient sensitivity and dynamic range for monitoring penetration and injection force dynamics in Drosophila embryos.
Fig. 9. Force sensing with large dynamic range with $N = 3$, $L = 10 \mu m$ and $K_y = 1.85$ N/m. The measured embryo injection force is $F = 48 \mu N$.

5. Conclusions

We present an integrated optical-encoder force sensor with configurable sensitivity and sufficient dynamic range for monitoring penetration and injection force in Drosophila embryos. The encoder is based on transmission phase gratings to optimize optical throughput and it is designed to have low sensitivity to rotation and cross axis translation. Tunability of the sensor can be achieved by either using arrays of integrated optical encoders with various pitch periods or varying the size of the optical illumination window on a fixed period encoder. The periodicity of the encoder gratings can be used for sensor self-calibration. These advantages of the integrated optical MEMS encoder force sensor make it a versatile tool for studying and controlling cell-membrane penetration, and give it the potential to facilitate development of microinjection and microsurgery instrumentation for a wide range of applications.

Acknowledgements

This work was funded by the DARPA [Bio:Info:Micro] program (MDA972-00-1-0032). The authors wish to thank Professor Calvin F. Quate for helpful discussions and the technical staff at the National Nanofabrication Users Network (NNUN) facilities and Edward L. Ginzton Lab at Stanford University for their support.

References


Biographies

X.J. Zhang received his BSc degree in precision electronic instrumentation and biomedical engineering from Shanghai Jiao Tong University, China in 1995 and his MSc degree in electrical engineering from University of Maine, Orono in 1998. His industrial experience includes working at Hewlett-Packard (later, Agilent Technologies) on the design of parallel optical interconnects and technical consulting for Cisco Systems on the evaluation of IEEE802.3ae 10Gb/s optical transceivers. He is currently a PhD candidate in electrical engineering at Stanford University. His dissertation research includes diffractive optical microelectromechanical systems (Optical MEMS) and Bio-MEMS design, fabrication and characterization, with focus on developing integrated microphotonic sensing interfaces for in vivo single cell and embryo manipulation, force microscopy and near-field imaging.
S. Zappe received his Diploma degree in electrical engineering from the Berlin University of Technology, Germany, in 1996. From 1996 until 2001 he worked as a PhD student at the Microsystems and Actuator Center at the Berlin University of Technology. In February 2001, he joined the Stanford Microphotonics Laboratory at Stanford University, CA, USA as a Postdoctoral Researcher. His research activities include microfluidic systems for cell- and embryo-handling, sorting and micro-injection; biology of fruit fly development; gene silencing by means of RNAs (RNA interference); micro-arrays for DNA sequencing; microfluidic systems-based re-usable arrays for DNA sequencing; fluid dispensing, micro-droplet injecting and device cooling, for which he holds three related patents.

M. Fehr received his BS degree in biology from San Jose State University, San Jose, CA, in May 1991 and his MS degree in molecular biology from San Jose State University in May 1999. He is currently employed by the Howard Hughes Medical Institute, working at Stanford University, Stanford, CA in the Department of Developmental Biology. His research work focuses on the function of a novel, developmentally regulated gene in Drosophila melanogaster.

M.P. Scott received his BS and PhD degrees in biology from MIT. He did postdoctoral research at Indiana University and then joined the faculty at the University of Colorado at Boulder. In 1983, he moved to Stanford University School of Medicine where he is now Professor of Developmental Biology and of Genetics. He has published more than 130 papers and three patents. His research areas are developmental genetics and cancer research, particularly the roles of signaling systems and transcriptional regulation in embryonic development. His research employs genetics, genomics, cell biology, and molecular biology in exploring how cells acquire their fates and are patterned. He is an editor of Current Opinion in Genetics and Development and of the Proceedings of the National Academy of Sciences. He is a past president of the Society for Developmental Biology, a member of the American Academy of Arts and Sciences, and a member of the National Academy of Sciences. He is presently chairing Stanford’s Bio-X program, which is designed to accelerate the coming together of engineering, physics, and chemistry with biology and medicine.

O. Solgaard received the BS degree in electrical engineering from the Norwegian Institute of Technology and his MS and PhD degrees in electrical engineering from Stanford University, California. He held a post doctoral position at the University of California at Berkeley, and an assistant professorship at the University of California at Davis, before joining the faculty of the Department of Electrical Engineering at Stanford University in 1999. His research interests are optical communication and measurements with an emphasis on semiconductor fabrication and MEMS technology applied to optical devices and systems. He has authored more than 100 technical publications, and holds 13 patents. He is a co-founder of Silicon Light Machines, Sunnyvale, CA, and an active consultant in the MEMS industry.