HIGH-PRECISION CHARACTERIZATION OF EMBRYO POSITIONING FORCE USING MEMS OPTICAL ENCODER

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Abstract

In this paper we describe the characterization of positioning and immobilization forces on Drosophila embryos in 2-D microfluidic self-assembly positioning arrays. The forces are measured using a surface micromachined optical-encoder force sensor operating in reflection. The average positioning force on embryos immobilized on 100μm x 250μm bonding pads was estimated to be 8.9μN±1.3μN, in reasonable agreement with earlier controlled-fluidic force measurements, but with much smaller measurement variance. Measurements of the positioning force as a function of displacement of the embryo on the self-assembly sites show that the self-assembly force can be modeled as a linear spring with a corresponding quadratic variation of the potential energy with respect to embryo displacement.

Keywords: Drosophila embryo, force sensor, optical encoder, self-assembly

1. Introduction

Micromachining technologies for injection [1], sorting [2], and positioning of single cells and embryos are becoming increasingly important for biological and genetic studies. Recently a technique for positioning and immobilization of Drosophila embryos at specific sites through the use of surface tension on patterned self-assembled monolayers (SAMs) was developed [3]. This technology enables 2-D arrays of immobilized embryos for high-throughput microinjection of genetic material. Earlier we reported measurements of the adhesion force of embryos on bonding sites performed by observing the volumetric flow rate required for detaching the embryos [4]. This method yields measurement results with a large force variance (±60% from the mean value). In this paper we describe more accurate measurements that allow us to characterize the self-assembly force and its displacement dependence in detail. The objective is to optimize the force and potential energy profile so that fluidic-self assembly arrays with high alignment yield can be designed.

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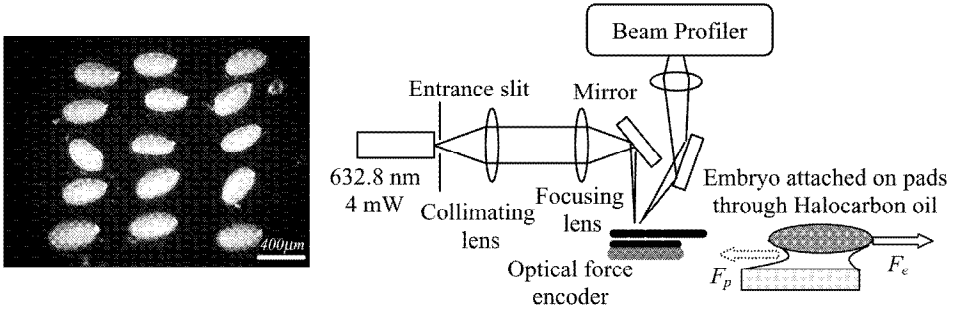
2. Experimental Design and Results

Arrays of Drosophila embryos were positioned on gold pads coated with SAMs and oil. The size of the individual pads was 100μm x 250μm and the array pitch was 500μm x 1000μm (Figure 1a). Relatively small pads were chosen to decrease the adhesion force and demonstrate high-resolution force sensing by the optical encoder. The measurement set-up is shown in Figure 1(b). The force sensor was illuminated by a HeNe laser with a spot size of 60 μm. The power of the first-order diffracted mode was measured with a photodiode placed 5 cm above the encoder.



(a) (b)

Figure 1. (a) 3x5 array of embryos positioned on a SAM chip. Gold pad size: 100μm x 250μm, pitch: 500μm x 1000μm. (b) Positioning force measurement setup (not to scale). The optical force encoder has an 85μm probe, dual gratings with 20 μm pitch and 2 μm vertical separation. The supporting beams have a measured spring constant of k=1.8 N/m.

The principle of the force sensor is shown in Figure 2. An optical phase encoder operated in reflection mode is integrated with the probe [5]. Both gratings of the encoder had 20 μm pitch period, and their vertical separation was 2 μm. With no lateral force applied, the gratings of the encoder are aligned. When the probe applies a force to an embryo, the counter force displaces the index grating, which is attached to a mechanical spring of known stiffness. The displacement and therefore the force are accurately determined by measurement of the diffracted light intensity.

Figure 3(a) shows the measured power of the first diffraction mode as a function of the total displacement of the stage. The total stage displacement is the sum of the displacement of the embryo on the bonding pads, the bending of the springs of the force sensor, and the bending of the embryo membrane. The membrane bending is relatively small due to the weak bonding force generated by the positioning sites. Earlier results show that the membrane deflection can be modeled as a linear spring with a spring constant of 0.9 N/m [5], which means that the maximum membrane bending is less than 9 μm.

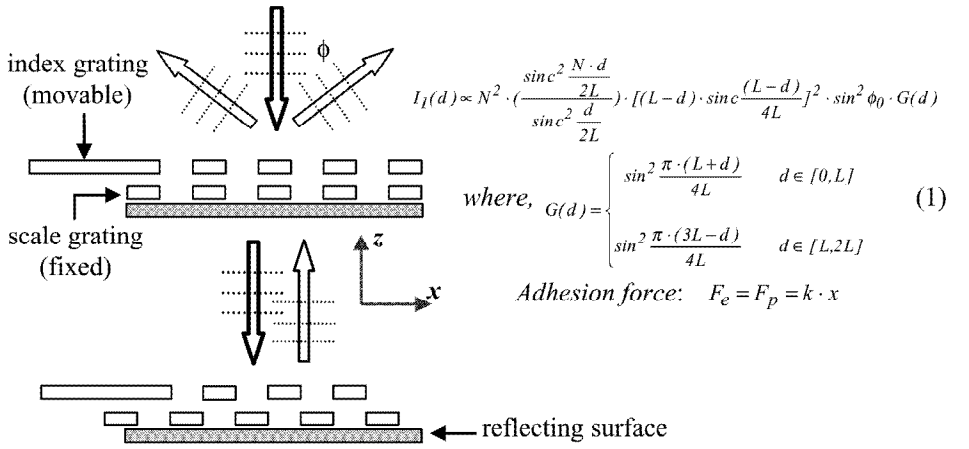
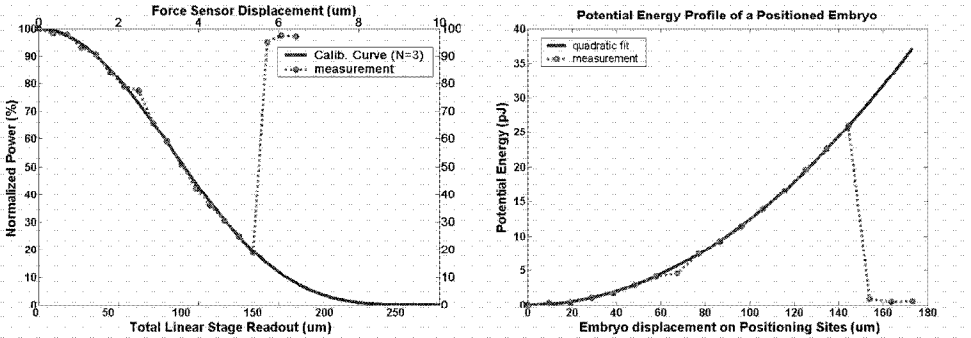


Figure 2. Force measurement using MEMS optical encoder in reflection. The two extreme cases of maximum diffraction (top) and maximum reflection (bottom) are shown. $I_1(d)$ is the intensity in the first diffraction order, N is the number of illuminated grating periods, $\phi_0(x)$ is the phase-delay for each grating element, 2L is the period of the grating with 50% duty cycle, d is displacement of the probe, and k is the spring constant of the force sensor.



(a) (b)

Figure 3. Characterization of the positioning force on Drosophila embryos on SAM binding pads using optical force encoder. (a) First diffraction mode power vs. probe displacement (N=3, L=10 μm, k=1.8 N/m). Eq.1 is fitted to the data to give the force sensor displacement. The detachment force is estimated to be 9.65 μN. (b) Potential energy of immobilized embryo is a quadratic function of displacement.

The force sensor displacement can be found from the known 20 μm period of the diffraction response. Using this calibration and the reported membrane spring constant, we calculate the positioning force and the potential energy as a function of embryo displacement (Figure 3b). In a series of experiments, an average detachment force of 8.9μN±1.3μN was found for the immobilized embryos. This is in reasonable agreement with earlier controlled-fluidic detachment force measurements, but with much smaller variance (±14.1% vs. ±60% of the mean). The quadratic variation of potential energy with respect to the embryo displacement clearly shows the linear behavior of the positioning mechanism in these experiments, and demonstrates that the force encoder has the required sensitivity to accurately measure the positioning force profile.

3. Conclusions

Massive parallel micro self-assembly is emerging as an efficient and low-cost biotechnology for high throughput embryo and cell manipulation on-chip. We have developed a surface micromachined optical encoder-force sensor for high precision analysis of fluidic self-assembly forces on Drosophila embryos as a function of displacement. The self-assembly sites that have been measured show a linear relationship between the centering force and displacement corresponding to a quadratic potential energy profile. The objective of our continued work is to design and experimentally verify site geometries that lead to optimized centering and alignment of the self-assembled embryos.

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