Piezoresistive cantilever force-clamp system

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We present a microelectromechanical device-based tool, namely, a force-clamp system that sets or “clamps” the scaled force and can apply designed loading profiles (e.g., constant, sinusoidal) of a desired magnitude. The system implements a piezoresistive cantilever as a force sensor and the built-in capacitive sensor of a piezoelectric actuator as a displacement sensor, such that sample indentation depth can be directly calculated from the force and displacement signals. A programmable real-time controller operating at 100 kHz feedback calculates the driving voltage of the actuator. The system has two distinct modes: a force-clamp mode that controls the force applied to a sample and a displacement-clamp mode that controls the moving distance of the actuator. We demonstrate that the system has a large dynamic range (sub-nN up to tens of μN force and nm up to tens of μm displacement) in both air and water, and excellent dynamic response (fast response time, <2 ms and large bandwidth, 1 Hz up to 1 kHz). In addition, the system has been specifically designed to be integrated with other instruments such as a microscope with patch-clamp electronics. We demonstrate the capabilities of the system by using it to calibrate the stiffness and sensitivity of an electrostatic actuator and to measure the mechanics of a living, freely moving *Caenorhabditis elegans* nematode.

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I. INTRODUCTION

Systems capable of applying controlled loads to a sample are a powerful means to calibrate nN– μN devices, to characterize viscoelastic materials, and to understand fundamental biophysical processes.¹ For example, control of applied force facilitates indentation studies (how much a sample is deformed by a given force), eliminating the need for viscoelastic corrections of displacement signals.² Viscoelastic properties depend on the force loading rate; thus, a system to control the force loading speed or force profile is required to fully characterize viscoelastic materials. The role of force in biophysical processes at scales ranging from single molecules to whole organisms can be monitored with force-clamp systems, providing quantitative mechanical descriptions such as stiffness, frequency response, or force sensation threshold.

Techniques such as optical tweezers, magnetic tweezers, and atomic force microscopy (AFM) can be used to control applied force.¹ These tools operate in the pN to nN force range but few are appropriate for the relatively larger forces required in mechanical studies of multicellular systems or microscale devices (often μN). In addition, although the optical detection methods used in these tools often have excellent resolution, optical detection is not ideal where optical access is not possible or is inconvenient, or in cases where laser induced thermal disturbances can disrupt the sample.^{3,4}

Piezoresistive cantilevers are commonly used for force sensing^{5,6} and have comparable force resolution with AFM: sub- μm thick cantilevers can measure sub-pN forces.^{7,8} A piezoresistive loop at the root of the cantilever exhibits a

change in resistance when strained, thus enabling direct measurement of cantilever deflection and applied force. Advantages of piezoresistive force sensors include a large dynamic range, relatively small size, simple fabrication, and straightforward signal conditioning circuitry. A piezoresistive cantilever integrated with a high speed piezoelectric actuator and a high-bandwidth feedback control system can be a stand-alone force-clamp system that can be integrated with other measurement equipment without geometrical or electrical constraints. In addition, piezoresistive cantilevers can be tuned to have the optimal force and displacement range for a given application via optimization techniques previously reported.^{8,9}

Here, we present the design and characterization of a piezoresistive cantilever-based force-clamp system that can apply nN to μN forces. The system, which has recently been used for characterization of a biomaterial³ and a microsystem,^{10,11} is characterized here to demonstrate its high dynamic range (0.5 nN – 50 μN) and high speed response (up to 1 kHz, limited by the piezoelectric actuator). In addition, we show two additional applications to demonstrate that the system can be easily integrated with optical microscopes and other instruments and can be used to apply force to a sample in any direction.

II. APPARATUS

The force-clamp system (Fig. 1) consists of a piezoresistive cantilever, a piezoelectric actuator with a built-in capacitive displacement sensor, and a real-time controller. The position of the cantilever is controlled by a piezoelectric actuator monitored by a built-in displacement sensor. To correct the error between the voltage signal from the

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FIG. 1. (Color online) Force-clamp system: (a) consisting of a piezoresistive cantilever, a piezoelectric actuator, and a real-time controller. The real-time controller calculates and adjusts the driving voltage of the piezoelectric actuator with a 100 kHz feedback loop. The controller is triggered by a digital signal and can operate in force or displacement-clamping modes to hold scaled force or displacement at a desired magnitude. (b) The force-clamp system is a stand-alone system, and can be easily integrated with additional systems such as computer vision-based x-y stage. (c) A piezoresistive cantilever glued on a printed circuit board has a piezoresistor at the root of the cantilever and three additional piezoresistors in the base for temperature compensation. A 10 μm -diameter glass bead is attached at the cantilever tip to provide a controlled contact geometry. (d) Signal conditioning circuitry for the piezoresistive cantilever consists of a Wheatstone bridge, an instrumentation amplifier, and a low pass filter to amplify the resultant voltage signal while filtering high frequency noise.

piezoresistive cantilever and a desired setpoint, the real-time controller calculates and adjusts the driving voltage of the piezoelectric actuator with a 100 kHz feedback loop, utilizing proportional, integral, derivative (PID) control. The details of each component are as follows.

A. Force probe: Piezoresistive cantilever

Piezoresistive cantilevers convert mechanical strain into a change in resistance using a semiconductor piezoresistor typically oriented at the root of the cantilever in a single loop to maximize strain. When strained, a piezoresistor undergoes a change in interatomic spacing that shifts the bandgaps to raise or lower resistivity depending on the material, dopants, and orientation of the piezoresistor. This results in a change in resistance proportional to strain which can be converted to a voltage signal using a Wheatstone bridge circuit. Piezoresistive cantilever performance is governed by three primary parameters: stiffness (k_c), resonant frequency (f_0), and sensitivity (S_c for displacement sensitivity). The cantilever stiffness is primarily a function of device geometry,

$$k_c = \frac{E w_c t_c^3}{4 l_c^3}, \quad (1)$$

and is related to the resonant frequency, f_0 as

$$f_0 = \frac{1}{2\pi} \sqrt{\frac{k_c}{0.24 m_c}} = \frac{t_c}{2\pi l_c^2} \frac{E}{\rho_s}, \quad (2)$$

where E is the Young modulus, m_c is the cantilever mass, ρ_s is the cantilever density, and l_c , w_c , and t_c are the length, width, and thickness of the cantilever, respectively. Displacement sensitivity can be described by the following proportionality:

$$S_c \propto \frac{3 E t_c (l_c - 0.5 l_p)}{l_c^3} V_{\text{bridge}}, \quad (3)$$

where V_{bridge} is the voltage applied across the Wheatstone bridge and l_p is the length of the piezoresistor. Force sensitivity is related to displacement sensitivity by the cantilever stiffness as S_c/k_c .

Microfabricated single-crystal Silicon piezoresistive cantilevers optimized for nN– μN force sensing were fabricated as previously reported.⁹ The piezoresistors are formed by Boron implantation, with the cantilever and piezoresistor oriented in the $\langle 110 \rangle$ crystallographic direction to maximize longitudinal piezoresistivity. Aluminum bondpads provide a means to electrically connect to the piezoresistor postfabrication. Devices are released by deep reactive ion

etching. Individual cantilevers are adhered to custom printed circuit boards (PCB) with epoxy (Devcon, Glenview, IL) and wirebonded with aluminum wires to form electrical connections to the measurement circuit [Fig. 1(c)]. The PCB (5 mm wide, 29 mm long, 0.8 mm thick) made with Flame Retardant 4, FR4 (Young's modulus: 11 GPa) is designed to be very stiff compared to the cantilevers ($k_{\text{PCB}} = 300 \text{ N/m}$, $k_c < 16 \text{ N/m}$). To isolate the piezoresistors from ionic media, we cover the aluminum wirebonds with epoxy and deposit 600 nm of parylene N (Specialty Coating Systems, Indianapolis, IN) on each device. Parylene N deposition also minimizes the cantilever-sample attractive forces. Because thin parylene N has a low Young's modulus (2.4 GPa), it increases stiffness by only 2.5% and 0.9% for 3 and 7 μm -thick cantilevers, respectively.

We use a Wheatstone bridge (1/4-active, full bridge configuration), an instrumentation amplifier, and a low pass filter (8 kHz) to convert resistance changes proportional to tip deflection into voltages and to amplify the resultant signal while filtering high frequency noise [Fig. 1(d)]. For an instrumentation amplifier, we use AD8221 (Analog Devices, MA) for piezoresistors with high resistance and INA103 (Texas Instruments, TX) for piezoresistors with low resistance ($<200 \Omega$). INA103 has very low noise (1 nV/ $\sqrt{\text{Hz}}$), but is not suitable for high input impedances because of its low input impedance (200 Ω). We located the signal conditioning circuit as close to the cantilever as possible.

We use four piezoresistors in the Wheatstone bridge to compensate for thermal drift, which is the main source of low frequency noise. Only one piezoresistor is strained by the cantilever deflection. This configuration leads to a precise and repeatable measurement for long-term experiments without requiring frequent bridge rezeroing.

Here we use the piezoresistive cantilever as both a force probe and a deflection sensor. Then we can relate cantilever deflection (x_c) to the applied force (F) by the cantilever stiffness (k_c) as $F = k_c x_c$.

B. Actuator

The force probes adhered to PCBs are rigidly fixed to a piezoelectric actuator using a custom mechanical fixture at a small angle (θ) relative to the surface of the actuator. The tilt can affect the apparent stiffness of the cantilever as

$$k_c = \frac{k_{c,0}}{\cos^2 \theta \left(1 - \frac{3D}{4l_c} \tan \theta\right)}, \quad (4)$$

where $k_{c,0}$ is the cantilever stiffness with 0° angle and D is the diameter of the glass bead attached at the cantilever tip to provide a controlled contact geometry.¹² We typically use a fixture with $\theta = 10^\circ$, resulting in $k_c \approx 0.97k_{c,0}$. Depending on the desired force range and the cantilever in use, one of the two actuators was used. The first, P-622.ZCD (Physik Instrumente, Germany), has a long travel length (250 μm) but limited bandwidth. The second, P-841 (Physik Instrumente, Germany), has a short travel length (15 μm) but higher bandwidth. We utilized the built-in displacement sensor (capacitive sensor for P-622.ZCD, strain gauge for P-841) to measure the moving distance of the actuator (x_a). The sample inden-

tation depth is then calculated as $x_s = x_a - x_c - x_0$ using the signals from the two displacement sensors (actuator and cantilever) and indentation start position, x_0 . This configuration allows us to apply a force to a sample while simultaneously monitoring changes in the sample indentation depth.

C. Real-time controller

To achieve fast feedback control, we used a field programmable gate array (FPGA)-based system [CompactRIO, National Instruments, detailed in Fig. 1(a)] to define the custom measurement hardware circuitry. The control system, initiated by a digital trigger signal, produces the required actuator command voltage by correcting the error between a desired setpoint and voltage signals proportional to the actuator travel distance and applied force with digital PID logic.

One advantage of an FPGA configuration is that we can implement multiple independent control loops in parallel. FPGA control systems can operate at MHz frequencies for digital signals and a few hundred kHz for analog signals (limited by analog-digital conversion). Capitalizing on these advantages, we built five feedback control loops operating in parallel such that they can operate at unique frequencies optimal for trigger detection, PID control, analog to digital conversion (ADC), digital to analog conversion (DAC), and parameter updating. Using a fast digital module, we made the critically timed loop which can operate at 500 kHz to detect a trigger signal from the parent program. This loop synchronizes all of the modules and initiates the PID control loop. The PID control loop is operated at 100 kHz. The analog loops (ADC and DAC) operate at the same frequency and are designed to update analog signals such as the applied force (F), moving distance of the actuator (x_a), and actuator driving voltage (V_{out}). Finally, a slow loop operates at 500 Hz, updating control parameters, such as PID gains and feedback mode selection, and communicating with the other systems through an ethernet cable.

The force-clamp control requires the use of very small digital PID gains. Thus, we used large digital buffers for PID gains: a 16-bit buffer for proportional and differential gains ($3\text{E}-5$ to 1) and a 64-bit buffer for integral gains ($1\text{E}-12$ to 1).

D. Integration

The force-clamp system is designed to be integrated with additional components and instruments. For example, an x-y stage is used to position samples under the cantilever and to apply a controlled force to a moving animal [Fig. 1(b)]. We synchronize the force-clamp system with additional systems using three different ports: digital, analog, and ethernet. To minimize the delay between the force-clamp system and additional components, we use a trigger signal to the fast digital input to trigger the PID control loop within 2 μs (500 kHz). Using the analog ports, analog signals proportional to F and x_a are recorded with data acquisition cards in an external computer. We update control parameters using a relatively slow ethernet connection at 500 Hz.

TABLE I. Specification of piezoresistive cantilevers. All cantilevers have 30 μm width with an 8.5 μm wide piezoresistor. Sensitivity (S_c), noise (V_n), and resolution ($F_{\min}$) are calibrated at 2 V bridge bias. Noise (V_n) is calibrated over a 1-1000 Hz bandwidth, except for devices 5 and 9 (1-500 Hz) and device 10 (1-200 Hz).

Device number	t_c (μm)	l_c (μm)	l_p (μm)	k_c (N/m)	f_0 (kHz)	S_c (V/m)	V_n (nV)	$F_{\min}$ (pN)	$F_{\max}$ (μN)
1	7	300	95	11.53	93.06	2002	132.3	1087	70.94
2	7	750	111	0.6493	13.97	374.7	140.1	447.3	25.86
3	7	1000	150	0.3017	8.246	115.5	158.0	344.4	18.53
4	7	2000	153	0.04872	2.343	35.69	159.0	151.7	9.222
5	7	3000	131	0.01435	1.038	30.62	104.0	64.45	4.831
6	3	750	111	0.06939	6.975	155.8	137.0	68.93	4.750
7	3	1000	150	0.03236	4.125	76.23	150.0	51.51	3.408
8	3	2000	120	0.005159	1.165	30.52	142.2	21.31	1.690
9	3	3000	131	0.001454	0.5049	12.90	102.3	10.26	0.3803
10	3	4500	131	0.0005165	0.2457	5.239	64.82	3.928	0.1690

E. Noise minimization

Several methods were employed to isolate the force-clamp system from mechanical and electrical noise sources. We placed the force-clamp system on an optical table (Ultra clean research series with I-2000 laminar flow isolators, Newport, CA) isolated from mechanical vibration. We designed all mechanical fixtures to have a higher natural frequency than the desired 1 kHz bandwidth. We used a low pass filter in the signal conditioning circuit to reduce thermal noise. We also implemented a 60 Hz notch filter, made with UAF42 (Texas Instruments, TX), to eliminate 60 Hz noise for open-loop control. However, it is not employed for closed-loop force control operation as it distorts the step response of the system. We used a halogen lamp powered by a dc supply to avoid 60 Hz noise signal associated with ac power supply since piezoresistive cantilevers are sensitive to oscillating light due to the photoeffect of silicon.

III. SYSTEM CHARACTERIZATION

A. Cantilever characterization

The stiffness and displacement sensitivity of each cantilever was determined by a resonant frequency technique incorporating a piezoelectric shaker and laser doppler velocimetry.¹³ Excitation of the cantilever with a broad spectrum of frequencies allows the resonant frequency to be determined, from which stiffness is computed as

$$k_c = \frac{(2\pi f_0)^2}{0.24\rho_{Si}l_c w_c t_c}. \quad (5)$$

While oscillating the cantilever at its resonance, the cantilever velocity amplitude is determined using laser doppler velocimetry, from which displacement amplitude is derived as a simple derivative. Simultaneous measurement of the amplified cantilever signal allows the cantilever displacement sensitivity to be determined. Noise was characterized using an instrumentation amplifier circuit (INA103, Texas Instruments, USA), an ac bridge circuit, and an HP3562A dynamic analyzer.^{9,14} The high frequency noise was measured with a simple instrumentation amplifier circuit because the noise floor of the amplifier is lower than that of the piezoresistors.

The low frequency noise signal from the piezoresistor is modulated with 600 Hz ac to remove high frequency noise of the amplifier, and is recovered at the output of a bandpass filter (bandwidth 200 Hz, center frequency 600 Hz), a synchronous demodulator (AD630, Analog Devices, USA), and a low pass filter (cut-off frequency 100 Hz).

B. System characterization

We measured the step and sinusoidal response of the force-clamp system in both force and displacement modes, while applying the force or displacement to a hard surface (glass). We employ a 16-bit data acquisition card, (DAQCard-6036E, National Instruments, TX) sampling at 20 kHz, to record the output signal of the actuator displacement sensor and the piezoresistive cantilever.

To characterize system noise, we used a 24-bit data acquisition card (PCI-4462, National Instruments, TX) sampling at 100 kHz, to minimize quantization noise. System noise was calculated by autocorrelation. We measured the background mechanical vibration of the system by quantifying the noise of the stiffest cantilever (device 1 in Table I) while the cantilever was in contact with a glass surface. The total noise of the stiffest cantilever is smaller than the background mechanical vibration. Bode plots for the actuator alone and actuator plus cantilever were generated by measuring the actuator displacement sensor and cantilever signals while the actuator was excited at frequencies sweeping logarithmically from 1 Hz to 10 kHz over a 60 s period.

IV. RESULTS AND DISCUSSION

We tested the force and displacement-clamping modes of the system by holding scaled force or displacement at a desired magnitude (Fig. 2). The force-clamp system can operate over a nN– μN force band for a wide range of cantilever stiffnesses by implementing both the short travel and long travel actuators, as shown in Fig. 3. Here we discuss the performance and limitations of the system under a variety of experimental conditions, with an eye toward application.

FIG. 2. Force and displacement clamp: System performance in force and displacement-clamp modes demonstrating large dynamic range and fast response (<2 ms rise time). Cantilever # 1 (10 and 50 μN), # 2 (1 μN), # 6 (100 nN), # 8 (10 nN), # 9 (0.5 and 1 nN), and the short travel actuator (all force and displacement clamps) were used.

A. Piezoresistive cantilevers performance

Piezoresistive cantilevers were fabricated without visible curvature, thus having low residual stresses. Both 3 and 7 μm -thick cantilevers had stiffness values between 0.00051 and 12 N/m and resonant frequencies between 0.25 and 93 kHz. Cantilevers have high force sensitivity while maintaining low noise, as our optimization method demonstrated.^{8,9} The force resolution of the softest cantilever approaches a few pN, while that of the stiffest cantilever is a few nN from 1 Hz to 1 kHz. The cantilevers maintain linear force sensitivity up to tens of μN (Table I), as bound by structural, piezoresistive, and Wheatstone bridge nonlinearity. Structural nonlinearity occurs when large cantilever deflection results in deviation from Euler–Bernoulli beam theory. Our numerical solution of a previously derived¹⁵ differential equation results in an upper bound for applied force of

$$F_{\max st} = \xi_{st} \frac{E w_c t_c^3}{6 l_c^2}, \quad (6)$$

where ξ_{st} is a nondimensional force and 1% nonlinearity corresponds to $\xi_{st} = 0.15$. Piezoresistivity is only linear for small deformation, and prior work^{16,17} has shown that a nonlinear deviation of 1% occurs at $\xi_{pr} = 139$ MPa of stress in an n-type piezoresistor strained longitudinally. This nonlinearity implies a maximum force of

$$F_{\max pr} = \xi_{pr} \frac{w_c t_c^2}{6 l_c}. \quad (7)$$

FIG. 3. Dynamic range of the piezoresistive force clamp with (a) 7 and (b) 3 μm -thick cantilevers of varying stiffness. The upper limit is set by both cantilever nonlinearities and actuator travel length. The lower limit of applied force is governed by the resolution of both the cantilever and actuator. The force and displacement resolutions are peak to peak resolutions, which is 6.6 times the root mean square noise integrated from 1 Hz to 20 kHz (sampling rate).

FIG. 4. Step response of force-clamp system: The upper limit of applied force with a soft cantilever (device 8) is limited by actuator travel length: the maximum force is 500 nN with the long travel actuator and 50 nN with the short actuator. The response time is limited by the actuator: <20 ms with the long travel actuator and <2 ms with the short travel actuator.

Large changes in $\Delta R/R$ are associated with nonlinearity of the Wheatstone bridge voltage output. Assuming a full bridge configuration, 1% nonlinearity occurs for $\xi_{wh} = 0.02$. This corresponds to a maximum force of

$$F_{\max wh} = \xi_{wh} \frac{w_c t_c^2}{6(l_c - 0.5l_p)\pi l_{\max} \gamma \beta^*}, \quad (8)$$

where γ and β^* are a cantilever electrical interconnect geometry factor and a piezoresistor efficiency factor, respectively.⁹

B. Force and displacement clamp

The system achieves a large dynamic range (sub-nN up to few tens of μ N force and nm up to tens of μ m displacement) and a short rise time (<2 ms) in Fig. 2.

FIG. 5. Step response of force-clamp system with cantilevers of varying stiffness: (a) Very soft cantilevers can be excited at their resonant frequency due to the contact force. (b) 10 nN force profiles with soft cantilevers have lower noise due to better force resolution of piezoresistive cantilevers. In addition, the background mechanical vibration of the whole system can affect force profile with stiff cantilevers because of their higher displacement sensitivity.

The dynamic range for force-clamping is limited by properties of both the piezoresistive cantilevers and the piezoelectric actuators (Fig. 3). The upper limit of applied force with stiff cantilevers is limited by cantilever nonlinearities: the maximum force with a 3 μ m cantilever is limited by structural nonlinearity while that with a 7 μ m cantilever is limited by Wheatstone bridge nonlinearity. The upper limit of applied force with soft cantilevers is set by actuator travel length. For example, the maximum possible force with device 8 is 500 nN with the long travel actuator and 50 nN with the short actuator in Fig. 4. Very soft cantilevers with low resonant frequency generate an unstable force profile before reaching this maximum [Fig. 5(a)]. These cantilevers can be excited at their resonant frequency when contact is initiated if the rise time of the force profile is very short and the contact force is large.

Soft cantilevers have better force resolution than stiff cantilevers, because of their higher force sensitivity. Thus, the lower limit of applied force with soft cantilevers is governed by the force resolution of the cantilever. In Fig. 3, the lower limit of applied force with soft cantilevers is close to the peak to peak force resolution of the cantilever, which is 6.6 times the root-mean-square (rms) noise integrated from 1 Hz to 20 kHz (sampling rate).¹⁸ Stiff cantilevers exhibit excellent displacement resolution exceeding that of soft cantilevers and often of the actuator because of their high displacement sensitivity. This causes stiff cantilevers to be more sensitive to the background mechanical vibration. Thus, the lower limit of applied force with stiff cantilevers is governed by the displacement resolution [5 nm, Fig. 2(b)] of the actuator and the background mechanical vibration (5 nm rms) of the force-clamp system on a typical optical isolation table (Newport, CA). In Fig. 5(b), 10 nN force profiles with soft cantilevers have lower noise due to better force resolution, while stiff cantilevers have higher noise due to higher displacement resolution and the background mechanical vibration of the whole system.

C. Frequency response

The force-clamp system can generate arbitrary displacement and force profiles using the built-in function generator in the FPGA-based real-time controller. The system demonstrates 10 nN to 10 μ N oscillatory force clamping from 1 Hz up to 1 kHz frequencies in Figs. 6(a) and 6(b). We characterized the output frequency response of the system to input signals of varying frequency from 1 Hz to 10 kHz in Fig. 6(c). The displacement sensor of the short travel piezoelectric

FIG. 6. (Color online) Frequency response: (a, b) The system demonstrates 10 nN – 10 μ N force at 1 Hz – 1 kHz frequencies. (c) Amplitude and phase of actuator only (displacement sensor) and actuator plus cantilever (force sensor) roll-off at 1 kHz. The offset of the force-clamp system at low frequency is the product of the piezoresistive cantilever sensitivity (2.002 V/ μ m with 1000 gain, device 1 and the sensitivity of the actuator (1.5 μ m/V).

actuator tracks an input sinusoid up to approximately 1 kHz. The output of the force-clamp system, including a cantilever, the piezoelectric actuator, and FPGA controller, also tracked up to approximately 1 kHz. Since both the resonant frequency of the cantilever and cut-off frequency of the low pass filter in the conditioning circuit for the piezoresistor are >5 kHz, the system is limited by the actuator (1 kHz roll-off).

D. Drift

The piezoresistive cantilever force-clamp system demonstrates low thermal drift. We monitored drift in the piezoresistor output for cantilevers in contact with a glass surface and out of contact; we observed a few nN over 1 min intervals and 20 nN over 1 h (Fig. 7). The system can generate nN to μ N force without requiring frequent bridge rezeroing. Loui *et al.* has shown that the thermal drift in a piezoresistive cantilever varies with the temperature and thermal conductivity of the environment.¹⁹ Thus, we could potentially achieve even lower drift by operating the system in temperature controlled environment.

E. Operation in liquid

The performance of the piezoresistive cantilevers is not degraded in liquid. Even in ionic media suitable for physiology experiments, there is no detectable piezoresistor leakage current. In addition, there is no significant difference in noise level in water versus in air in Fig. 8(a). Because the thermal conductivity of water is better than that of air, the

FIG. 7. (Color online) Drift of piezoresistive cantilever force-clamp system (device 4). Cantilevers with and without contact on glass maintain low thermal drift.

piezoresistor temperature should decrease, thus lowering the Johnson noise and potentially the 1/f noise.^{4,20} No effect on performance was observed presumably because the heat transfer between the cantilever and the silicon base by

FIG. 8. (Color online) System performance is not degraded in liquid: (a) Noise level in air and water are the same. Cantilever # 1 was used. The data acquisition system noise was verified to be lower than force-clamp system noise. (b, c) The force-clamp system can generate step force profiles from 0.5 nN to 50 μ N and sine force profiles from 1 Hz to 1 kHz.

conduction dominates the overall heat transfer in these cantilevers.

The performance of the force-clamp system is also not degraded in liquid in Fig. 8. The system can generate step force profiles from 0.5 nN to 50 μ N and sine force profiles from 1 Hz to 1 kHz in water. Additional viscous damping in water may degrade the dynamic properties at higher frequencies, but these cantilevers with high resonant frequencies can generate sinusoidal force profiles up to 1 kHz.

F. Extensibility

A force-clamp system using 3 or 7 μ m-thick cantilevers easily demonstrates nN– μ N force profiles. However, the system resolution is limited by the piezoresistive cantilever force resolution and nonlinearities. We previously reported sub-pN theoretical force resolution for a piezoresistive cantilever with 0.5 μ m thickness and 1 kHz bandwidth. The theoretical maximum detectable force for a 25 μ m-thick cantilever and a 1 kHz bandwidth is 500 μ N.²⁰ By using different piezoresistive cantilevers with thicknesses from 0.5 to 25 μ m, one can shift the force range of the piezoresistive force-clamp system from a few pN to a few mN while maintaining a dynamic range of 1–3 orders of magnitude.

Similarly, for high frequency applications, piezoresistive cantilevers can detect from 24 pN to 7.6 μ N with 100 kHz bandwidth. Here, the bandwidth of our piezoelectric actuator is limited to approximately 1 kHz by the displacement sensor and the digital controller is limited to 100 kHz by digital to analog and analog to digital conversion. Theoretically, we could achieve higher than 100 kHz response by improving the actuator dynamics by integrating a microscale actuator on the cantilever itself²¹ and by utilizing a faster controller.

V. APPLICATIONS

A. Mechanical characterization of a motile *C. elegans* nematode

Integration of the system with an x–y stage allows the excellent dynamic response of the force clamp to be leveraged to measure the mechanical properties of a moving animal or to rapidly characterize a large field of scattered objects. In our previous measurements of *C. elegans* body mechanics, we immobilized animals and performed measurements which lasted tens of seconds to quantify body stiffness.³ This measurement provides a wealth of data but requires animals to be immobilized. To circumvent this limitation, we integrated the force clamp with an x–y stage and image processing software which allows the cantilever to be rapidly positioned over an arbitrary location on the sample. The x–y stage system then sends a digital signal to trigger the force-clamp system with less than 2 μ s latency and acquires force (F_c), and displacement (x_a) from the force-clamp system. This enables controlled force application to the animal as it moves and direct measurement of body stiffness as $k_w = F_c/x_s = F_c/(x_a - x_c - x_o)$. In an example measurement, we applied a step force to a moving animal and determined that $k_w = 0.61$ N/m, as shown in Fig. 9. This value is similar to the stiffness we measured pre-

FIG. 9. Mechanical characterization of moving *C. elegans*: (a) A computer vision-based x–y stage integrated with the force clamp allows the desired force to be applied precisely at a selected location on a moving *C. elegans*. (b) *C. elegans* experiences a time-dependent increase in indentation depth when subjected to a step constant force. Cantilever # 3 was used.

viously in immobilized animals,³ suggesting that partial immobilization has little, if any, impact on apparent body stiffness. The indentation depth increases slightly as the force is clamped, which could be due to the worm moving beneath the cantilever or due to viscoelastic properties of the animal itself.

B. Calibration of a MEMS electrostatic actuator

The force applied by an electrostatic actuator can be approximated by using beam theory to estimate the flexure stiffness and by optically measuring displacement during actuation. However, nonlinearity from nonidealities, such as fringe fields, may introduce large errors in such estimates. We used the force-clamp system to measure directly both the flexure stiffness and displacement sensitivity of an electrostatic actuator. Higgs *et al.* designed and fabricated an electrostatic actuator for direct calibration of hydrogel shear stiffness.²² The electrostatic actuator is supported by a flexure. We mounted a cantilever oriented perpendicular to the movement of the actuator. A 3 μ m-thick cantilever was inserted into the 10 μ m gap of the device [Fig. 10(a)]. For stiffness calibration, we increased the force applied to the device in displacement-clamp mode and acquired force (F_c) versus displacement (x_a). In this setup, the cantilever of the force-clamp system and the

FIG. 10. (Color online) Characterization of a MEMS electrostatic actuator with the force-clamp system: (a) Photomicrograph of the electrostatic actuator and the force-clamp system. The electrostatic actuator is supported by a flexure. We mounted a cantilever oriented perpendicular to the movement of the actuator. A 7 μm -thick cantilever (device 2) was inserted into the 20 μm gap of the device. (b, c) Suspension stiffness calibration: the force-clamp system applies force to the actuator and measures displacement of the force-clamp system and resultant indentation depth (deflection) of the actuator. Five overlaid force-indentation depth curves demonstrate both excellent linearity and repeatability. (d, e) Sensitivity calibration: the actuator applies force to the cantilever which is connected in parallel with the beam of the actuator. The theoretical and measured sensitivities agree.

device actuator beam are connected serially, so indentation depth can be calculated as $x_{\text{device}} = x_a - F_c/k_c$ in Fig. 10(b). We determined the suspension stiffness of the device to be approximately 1 N/m in Fig. 10(c). For sensitivity calibration, the electrostatic actuator applied a force to the cantilever of the force-clamp system as driving voltage of the electrostatic actuator was increased. Here the beam of the device and the cantilever are connected in parallel [Fig. 10(d)], so the force generated by the device can be calculated as $F_{\text{device,actuator}} = (k_{\text{device}} + k_c)(F_c/k_c)$. In Fig. 10(e), the calibrated result ($0.0209 \mu\text{N}/\text{V}^2$) agrees well with the theoretical estimate ($0.0199 \mu\text{N}/\text{V}^2$), but is higher at low voltages than the theoretical estimate because of nonlinearities of the electrostatic actuator.

VI. CONCLUSION

We have presented a piezoresistive cantilever force-clamp system with excellent dynamic range (0.5 nN–50 μN)

and fast response time (1 Hz–1 kHz). We characterized the system in both air and water. The effective dynamic range of the system covers one to three orders of magnitude and force and is determined by the performance of the cantilever and actuator. We used the system to measure the mechanics of a moving *C. elegans* nematode and to calibrate an electrostatic actuator, demonstrating that the system is easily integrated with other systems and is suitable for small force applications.

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