



HAL
open science

Improvement of Silicon Nanotweezers Sensitivity for Mechanical Characterization of Biomolecules Using Closed-Loop Control

Nicolas Lafitte, Yassine Haddab, Yann Le Gorrec, Hervé Guillou, Momoko Kumemura, Laurent Jalabert, Dominique Collard, Hiroyuki Fujita

► **To cite this version:**

Nicolas Lafitte, Yassine Haddab, Yann Le Gorrec, Hervé Guillou, Momoko Kumemura, et al.. Improvement of Silicon Nanotweezers Sensitivity for Mechanical Characterization of Biomolecules Using Closed-Loop Control. IEEE/ASME Transactions on Mechatronics, 2015, 20 (3), pp.1418-1427. 10.1109/TMECH.2014.2351415 . hal-03221857

HAL Id: hal-03221857

<https://hal.science/hal-03221857>

Submitted on 10 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Improvement of Silicon Nanotweezers Sensitivity for Mechanical Characterization of Biomolecules Using Closed-Loop Control

Nicolas Lafitte, Yassine Haddab, Yann Le Gorrec, *Member, IEEE*, Hervé Guillou, Momoko Kumemura, Laurent Jalabert, Dominique Collard, *Member, IEEE*, and Hiroyuki Fujita, *Member, IEEE*

Abstract—In this paper, we show that closed-loop control can be advantageously used for the characterization of mechanical properties of biomolecules using silicon nanotweezers (SNT). SNT have already been used in open-loop mode for the characterization of mechanical properties of DNA molecules. Up to now, such an approach allows the detection of stiffness variations equivalent to about 15 DNA molecules. Here, it is shown that this resolution is inversely proportional to the resonance frequency of the whole system and that real-time feedback control with state observer can drastically improve the performances of the tweezers used as biosensors. Such improvement is experimentally validated in the case of the manipulation of *fibronectin* molecules. The results are promising for the accurate characterization of biopolymers such as DNA molecules.

Index Terms—Biosensor, MEMS tweezers, parameter detection, sensitivity improvement, state feedback.

I. INTRODUCTION

RECENT developments in micronano manipulation tools have revealed crucial information on the mechanical behavior of biomolecules [1]–[3]. These manipulations generally performed on a single molecule have given the quantitative data needed to elucidate fundamental biological processes as DNA wrapping [4] and replication [5], or cell cytoskeleton dynamics through actin filament [6], and microtubule mechanical responses [7].

Several methods are already available for performing biological experiments at the molecular level: magnetic tweezers [8], [9], optical tweezers [10], AFM cantilevers [11], and microfibers [2], [12]. A single filamentary macromolecule, such as DNA, is first attached to a surface at one end, and to a bead

Manuscript received October 8, 2012; revised April 9, 2014; accepted June 10, 2014. Recommended by Technical Editor S. O. R. Moheimani. The work of Y. Haddab and Y. Le Gorrec was supported in part by the LABEX ACTION under Grant ANR-11-LABX-01-01 and the NANOROBUST Project (ANR-2011 NANO 006).

N. Lafitte, H. Guillou, M. Kumemura, L. Jalabert, and D. Collard are with the LIMMS/CNRS-IIS (UMI 2820), Institute of Industrial Science, The University of Tokyo, Tokyo 153-8505, Japan (e-mail: lafitte@iis.u-tokyo.ac.jp; herveg@iis.u-tokyo.ac.jp; momo@iis.u-tokyo.ac.jp; jalabert@iis.u-tokyo.ac.jp; collard@iis.u-tokyo.ac.jp).

Y. Haddab and Y. Le Gorrec are with the FEMTO-ST/UFC-ENSMM-UTBM-CNRS, 25044 Besançon, France (e-mail: yassine.haddab@ens2m.fr; Yann.Le.Gorrec@ens2m.fr).

H. Fujita is with the Center for International Research on Micromechanics, Institute of Industrial Science, The University of Tokyo, Tokyo 153-8505, Japan (e-mail: fujita@iis.u-tokyo.ac.jp).

Color versions of one or more of the figures in this paper are available online at <http://ieeexplore.ieee.org>.

Digital Object Identifier 10.1109/TMECH.2014.2351415

or a flexible cantilever at the other end. Forces or displacements are sensed by optical measurements. Nevertheless, the real-time operation and the routine implementation of these techniques remain difficult to achieve, as they require complex experimental procedures. In this respect, microelectromechanical systems (MEMS) offer an advantage for systematic analysis since accurate molecular level tools (actuator, end effectors, and sensor) can be integrated on a MEMS platform. Furthermore, these devices can be produced at low cost with batch fabrication, as have the microgrippers developed to grab micro-sized objects [13] or cells [14]. However, up to now, the performances of such tools are not sufficient to deal with single molecule characterization. In previous reports, silicon nanotweezers (SNT) were designed and fabricated to trap DNA molecules [15], [16] and characterize DNA bundles [17] and reactions on DNA [18]. The stiffness of DNA bundles was derived from the open-loop measurement of the mechanical resonance frequency shift. The current sensing resolution is in the range of $1 \text{ mN} \cdot \text{m}^{-1}$, which corresponds to approximately 15 λ -DNA molecules¹ [20]. This resolution is limited by the design of the tweezers and the noise of the electronic instrumentation. To improve the overall system sensitivity towards lower stiffness, the primary route consists of reducing the tweezers stiffness to bring the sensor and measured object characteristics close to the same range. Unfortunately, such a design would result in a system that would be too fragile to be processed and utilized.

Our approach consists of scaling down the resonance frequency of the closed-loop system to enhance the sensitivity to SNT stiffness change. After a proper identification of the SNT model, the feedback control has been simulated, optimized, and then implemented. This control strategy has been evaluated with thin *fibronectin* molecular bundles trapped between SNT tips, and we propose to discuss the advantages and the limits of such closed-loop operations.

This paper is organized as follows. Section II describes the tweezers, the sensing principle, and provides an example of monitoring the DNA trapping. The dynamic model of the tweezers is then detailed in Section III-A. The closed-loop control strategy and its potential to improve the system sensitivity are discussed in Sections III-B and III-C. Section IV details the experimental implementation and provides information on *fibronectin* molecules measurement with different

¹ λ -DNA is DNA of the bacteriophage λ having a contour length of $16.4 \mu\text{m}$ and a stiffness in elastic regime of $66 \mu\text{N} \cdot \text{m}^{-1}$ in low ionic solution [19].

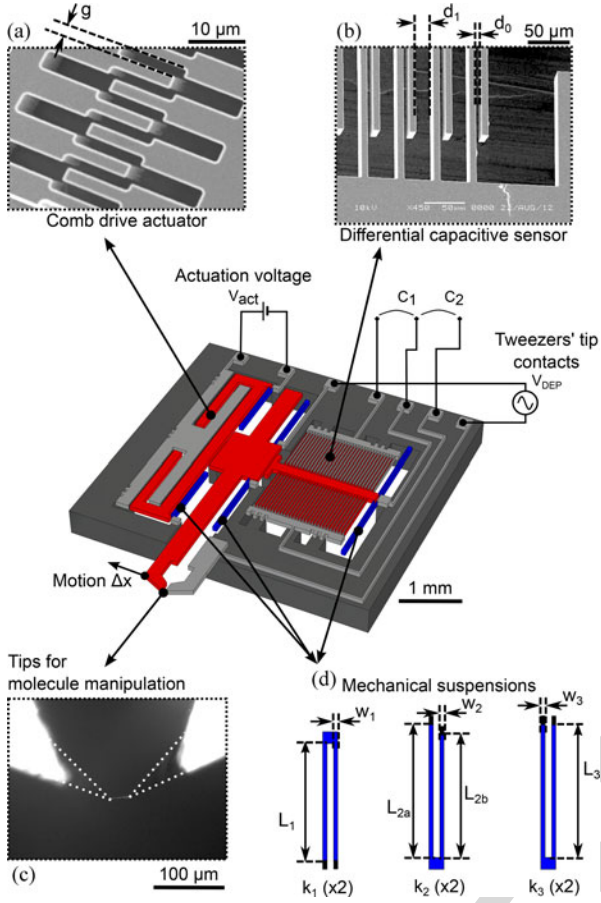


Fig. 1. SNT description. 3-D view of the device whose overall dimensions are $4.5 \times 4.5 \times 0.5$ mm. The mobile parts are shown in red and the mechanical suspensions in blue. (a) Scanning electronic microscopic (SEM) image of the actuator consisting of 880 pairs of interdigitated combs separated by a $2\text{-}\mu\text{m}$ gap, and organized in two series. (b) SEM image of the integrated sensor of two opposing series of 30 combs. (c) Microscope image of the tweezers tips with a DNA bundle in between. (d) Design of the three sets of suspensions, k_1 for the actuator polarization, k_2 for the mobile arm, and k_3 for central sensor plate.

78 closed-loop settings. Closing remarks and perspectives are given
79 in Section V.

80 II. DESCRIPTION OF THE DEVICE AND ITS USE FOR THE 81 MECHANICAL CHARACTERIZATION 82 OF BIOMOLECULES BUNDLES

83 A. Device Description

84 1) *Design*: Fig. 1 shows a three-dimensional illustration of
85 the SNT device. It consists of two arms terminated in sharp tips
86 that act as electrodes to trap biomolecules by dielectrophoresis
87 (DEP) forces [15]. One tip is fixed; the other one is moved by
88 an electrostatic actuator. The motion of the mobile electrode
89 is measured by capacitances whose value linearly varies with
90 electrode displacement; two variable capacitances are mounted
91 in differential mode [21].

92 2) *Electrostatic Actuation*: The mobile arm is actuated by
93 attractive electrostatic forces generated in an interdigitated comb
94 drive [see Fig. 1(a)], one of the most widely used architecture

in MEMS design [22], [23]. The maximal stroke is limited
95 compared to that achievable by a parallel plate actuator but the
96 generated force only depends on the actuation voltage and not on
97 the electrode position, thus, enabling a simpler actuation control.
98 The force is given by (1) where ϵ_0 is the vacuum permittivity (we
99 consider the relative permittivity of air = 1), N_a is the number
100 of comb fingers, t is the device thickness, g is the lateral gap
101 between fingers, and V_{act} is the actuation voltage. Due to its
102 capacitive structure, the comb drive generates attractive forces
103 between its two electrodes
104

$$F_{es} = \frac{1}{2} \frac{\epsilon_0 \times N_a \times t}{g} V_{act}^2 = \alpha_{es} \times V_{act}^2. \quad (1)$$

3) *Mechanical Structure*: The mobile part of the tweezers
105 is linked to the silicon frame by flexible beams [see Fig. 1(d)].
106 Commonly integrated in mechanical microsystems [23], [24],
107 folded beam springs are designed to minimize beam areas,
108 decrease their mechanical stiffness and enhance displacement
109 ranges [25]. In the SNT design of Fig. 1, three sets of folded
110 beam suspensions support the mobile part of the system (the
111 comb-drive actuator, the mobile tip, and the capacitive sensor)
112 and provide the electrical connections for the actuation and the
113 sensing. The springs are arranged symmetrically along the
114 actuation and sensing axis to minimize any rotation, and their
115 sum gives the total stiffness k of the device.
116

A highly compliant system is required to sense the mechani-
117 cal characteristic of trapped molecules on the tweezers response.
118 On the other hand, a minimum stiffness is mandatory, 1) to en-
119 dure the fabrication processes and manipulations, 2) to support
120 the mobile system weight, and 3) to prevent sticking due to at-
121 tractive surface forces between the comb-drive actuator and the
122 capacitive sensor electrodes.
123

4) *Displacement/Velocity Sensing*: The tip position is mea-
124 sured by a capacitive sensor designed in a triplate configuration
125 with transverse combs [see Fig. 1(b)] [26]. The central elec-
126 trode, linked to the mobile arm, moves in between two fixed
127 electrodes creating the two differential capacitances C_1 and C_2
128 whose difference, ΔC , is related to the displacement x . For
129 small displacements (i.e., x is much smaller than the gaps be-
130 tween electrodes d_0 and d_1), ΔC is proportional to x [17], N_b
131 is the number of capacitance electrodes, and L the length of the
132 electrodes
133

$$\Delta C = C_1 - C_2 \quad (2)$$

$$\simeq 2\epsilon_0 N_b L t \left(\frac{1}{d_0^2} - \frac{1}{d_1^2} \right) x = \beta_C \times x \quad (3)$$

5) *Electronic Read-Out*: The tweezers motion is sensed by
134 the measurement of the differential capacitance ΔC (2) through
135 current sensing. In dynamic mode, a dc voltage (V_{polar}) is ap-
136 plied on the mobile central electrode, whose motion generates
137 dynamic currents i_1 and i_2 flowing through the capacitances C_1
138 and C_2 , respectively, [27], [28]. The resulting currents related
139 to the motion velocity are converted into voltages V_1 and V_2 by
140 two low-noise current-to-voltage (A/V) preamplifiers (Signal
141 Recovery, model 5182). The low input impedance of the pream-
142 plifier (virtual ground) ensures an accurate current conversion
143 [17], [29].
144

TABLE I
NUMERICAL VALUES OF DIMENSIONS AND PARAMETERS OF THE SNT

Silicon thickness	
t (μm)	30
Comb drive actuator	
N_a	440
g (μm)	2
Mechanical suspensions	
L_1, L_{2a} (μm)	900
L_{2b}, L_3 (μm)	1000
w_1, w_2, w_3 (μm)	15
Capacitive sensor	
N_b	30
L (μm)	585
d_0 (μm)	5
d_1 (μm)	20

The device dimensions are defined in Fig. 1.

145 Finally, in harmonic mode, when the tweezers is moved by
146 a sinusoidal actuation, a lock-in amplifier (NF, model LI 5640)
147 performs the low-noise detection of the differential signal (amplitude and phase of $V_1 - V_2$) at the actuation frequency. The
148 reference signal is the motion frequency imposed by the actuation voltage.
149

151 All dimensions and parameters that has been considered for
152 a proper model of the SNT are summed up in the TableI. Furthermore, Young's modulus and the density of the silicon are,
153 respectively, 165 GPa and $2329 \text{ kg} \cdot \text{m}^{-3}$, and the permittivity
154 ϵ_0 is $8.85 \times 10^{-12} \text{ kg}^{-1} \cdot \text{m}^{-3} \cdot \text{A}^2 \cdot \text{s}^4$.
155

156 B. Monitoring Biomolecules Manipulation With SNT

157 This Section presents the way that the SNT are used as biosensors and points out the measurement sensitivity requirements.
158 This example is illustrated with DNA but the same technique
159 has been applied to gelatin [30] and microtubules [31].
160

161 1) *DNA Bundle Trapping by DEP*: The trapping is achieved
162 by applying an ac electric field ($E = 1 \text{ MV} \cdot \text{m}^{-1}$) between
163 the two opposing tips of the tweezers [32] once partially
164 immersed in the solution. The retrieving of the DNA bundle
165 is routinely performed from a droplet solution [15], [17], and
166 single molecule trapping has even been demonstrated with a
167 pulsed DEP signal [16].

168 In [20], an improved method is proposed in which the tips
169 are introduced into an open fluidic cavity optimized to guaranty
170 a stable meniscus. The SNT position is fixed and the cavity
171 is mounted on a *XYZ* stage controlled by programmable robot
172 for repeatable and controlled tip immersion, as illustrated in
173 Fig. 2(a)–(c).

174 2) *Real-Time Monitoring*: The SNT characterization allows
175 monitoring of the DNA trapping in real time. The characterization
176 of the SNT in real time allows the monitoring of the DNA
177 trapping. The number of trapped DNA molecules during the
178 DEP is controlled by the evolution of the resonance frequency
179 and quality factor of the system (Tweezers+DNA bundle). The
180 SNT are modeled by a linear second-order model as depicted in
181 Fig. 2(d). The resonance frequency, f_R , and the quality factor

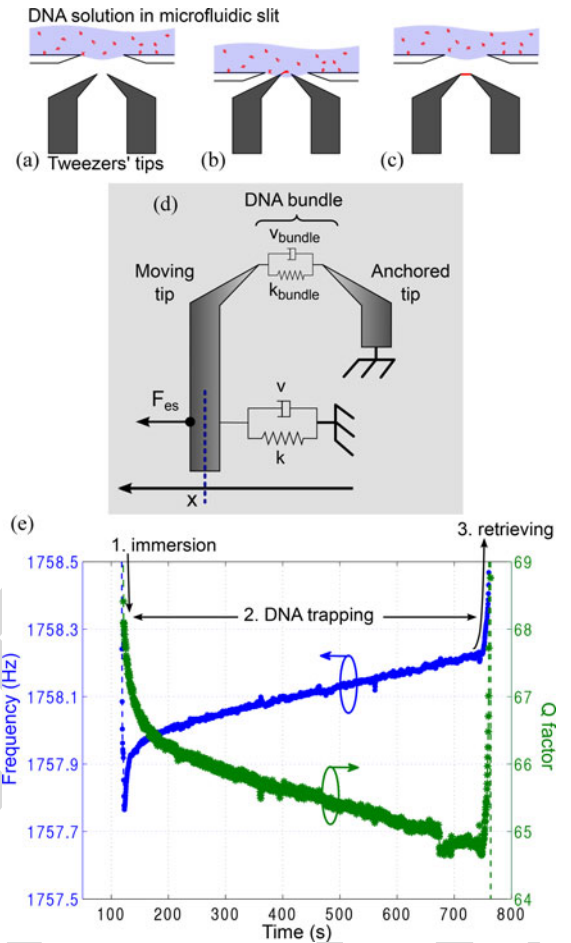


Fig. 2. λ -DNA molecules trapping with SNT. The first three sketches illustrate the key phases of the experiment: (a) the immersion of the tips in the DNA solution, (b) the DNA trapping by DEP, and (c) the retrieving of DNA bundle between the tips. (d) Equivalent dynamic model of the SNT with bundle. (e) Evolution of the *SNT+bundle* resonance frequency and *Q* factor during the trapping.

Q of the *SNT+bundle* damped oscillator are defined by 182

$$f_R(t) = \frac{1}{2\pi} \sqrt{\frac{(k + k_{\text{bundle}}(t))}{M}} \quad (4)$$

$$Q(t) = \frac{\sqrt{(k + k_{\text{bundle}}(t)) \times M}}{(\nu + \nu_{\text{bundle}}(t))} \quad (5)$$

where M is the mass of the movable tip, k is the stiffness of the suspensions, and ν is the equivalent viscous losses. k_{bundle} and ν_{bundle} are the time varying bundle stiffness and viscosity; the mass of the bundle is neglected compared to M . 183 184 185 186

As shown in Fig. 2(e), as the DEP trapping progresses, the system moves to higher resonance frequencies indicating an increase in the bundle stiffness according to (4). At the same time, the signal amplitude at resonance, proportional to the quality factor Q , is decreasing, revealing higher viscous losses ν_{bundle} as the bundle forms, in accordance with (5). 187 188 189 190 191 192

In this experiment, the resonance frequency experiences a shift of 0.4 Hz that corresponds to an increase in the stiffness of $12 \text{ mN} \cdot \text{m}^{-1}$, i.e., a bundle containing $\sim 200 \lambda$ -DNA molecules 193 194 195

[19]. (For the sake of clarity, it should be noted that the SNT used in this trapping experiment has different characteristics than those used in the feedback approach of Sections III and IV)

3) *Sensor Resolution*: Accurate biosensing by SNT requires high-resolution measurements. Thus, special care in signal conditioning (detection of small currents < 10 pA), noise reduction, and control of the experimental conditions (e.g., the stability of the meniscus air/biological liquid/tweezers) have allowed to sense resonance frequency shifts of 25 mHz and quality factor changes of 0.2. Based on this resolution, the minimum change that can be sensed due to the stiffness variation is in the range of 15 molecules of λ -DNA [20].

After trapping, the bundle of molecules is mechanically stimulated (in air or in a biological solution) with a sinusoidal signal at the resonance frequency of the system. Slight changes in the frequency response permit a fine characterization of the evolution of the mechanical properties of the molecule bundle revealing biological interactions.

III. DYNAMIC MODELING AND IMPROVEMENT OF THE PARAMETRIC SENSITIVITY BY A FEEDBACK APPROACH

In this Section, we describe how to reduce the closed-loop resonance frequency of the tweezers in order to improve its sensitivity to molecule stiffness variations.

A. Dynamic Modeling and Open-Loop Sensitivity to Stiffness Variations

1) *Modeling*: Newton's second law applied to the damped oscillator formed by the *SNT+bundle* of Fig. 2(d) can be formulated by

$$\dot{X} = \underbrace{\begin{bmatrix} 0 & 1 \\ -(k + k_{\text{bundle}}(t)) & -(\nu + \nu_{\text{bundle}}(t)) \end{bmatrix}}_A \frac{1}{M} X + \underbrace{\begin{bmatrix} 0 \\ 1 \\ M \end{bmatrix}}_B F_{\text{es}} \quad (6)$$

and

$$y = \underbrace{\begin{bmatrix} 0 & 1 \end{bmatrix}}_C X \quad (7)$$

where $A \in \mathbb{R}^2$, $B \in \mathbb{R}^{(2,1)}$, $C \in \mathbb{R}^{(1,2)}$, X is the state vector (displacement & velocity, $X = \begin{pmatrix} x \\ \dot{x} \end{pmatrix}$), and y is the output vector or the measurement.

2) *Identification*: Prior to the development of an accurate control strategy, the model parameters [M , k , ν , actuation, and sensor gains of (1)–(5)] need to be extracted from device measurements. These parameters are identified through standard recursive approach with least squares method from SNT responses to small signal actuation with a 20 V offset. Identified parameters are summarized in Table II.

TABLE II
THEORETICAL AND IDENTIFIED MODEL PARAMETER VALUES OF THE SNT

Parameters	Theoretical	Identified
Mechanical parameters		
M (kg)	360×10^{-9}	360×10^{-9}
k ($\text{N} \cdot \text{m}^{-1}$)	49.3 ^a	24.9
ν ($\text{Ns} \cdot \text{m}^{-1}$)	-	100×10^{-6}
Comb-drive actuator		
α_{es} ($\text{N} \cdot \text{V}^{-2}$)	29.2×10^{-9a}	35.5×10^{-9}
Capacitive sensor		
β_C ($\text{F} \cdot \text{m}^{-1}$)	412.2×10^{-9a}	227.5×10^{-9}

^a These values are calculated from the designed springs and electrodes widths and does not take into account the significant beam narrowing during etching processes.

3) *Open-Loop Sensitivity to Stiffness Variation*: The minimum number of molecules that can be discerned by resonance frequency shifts due to the molecular stiffness variation is linked to the SNT sensitivity to stiffness variation Δk , i.e.,

$$\frac{\partial f_R}{\partial k} = \frac{1}{4\pi\sqrt{kM}} \text{ or } \frac{1}{8\pi^2 M f_R}. \quad (8)$$

This sensitivity is equal to 26.4 Hz/($\text{N} \cdot \text{m}^{-1}$) with the identified parameters of Table II. It appears that for a given molecular stiffness change, Δk , the effect on the resonance frequency variation Δf_R is enhanced with low SNT stiffness, k , and, therefore, for low resonance frequency f_R . In relation to Fig. 2, the current experimental resolution in the resonant frequency shift measurement is close to 25 mHz, a value that corresponds to $1 \text{ mN} \cdot \text{m}^{-1}$ or 15 λ -DNA molecules. From (8), a reduction of f_R by one order of magnitude; i.e. 2 orders of magnitude for k , would improve the detection threshold down to the single molecule level.

The development of a new MEMS device having a very low stiffness ($< 0.2 \text{ N} \cdot \text{m}^{-1}$) remains extremely challenging in terms of fabrication and operation. Thus, we propose to reduce the overall stiffness by closed-loop control. In such a control strategy, the physical system (i.e., the SNT) remains the same but the closed-loop system *SNT combined with the feedback law* will have the desired designed dynamic behavior.

B. Improvement of the Parametric Sensitivity by a Feedback Approach

1) *Control Strategy*: The control design strategy is depicted in the flowchart of Fig. 3. The aim of the feedback is to reduce the resonance frequency of the closed-loop system. To this end, we use a state feedback eigenstructure assignment in order to assign both the closed-loop resonance frequency and damping factor. This state feedback is then implemented using a Luenberger-kind observer. The originality of the observer we use is that it has to be designed in order to neither deteriorate the closed-loop sensitivity nor amplify the noise.

2) *Eigenstructure Assignment Using State Feedback*: The system is under its controllable canonical state-space representation (A, B, C) given by (6) and (7). The state feedback gain vector $L = (l_1, l_2) \in \mathbb{R}^{(1,2)}$ is designed to assign the desired

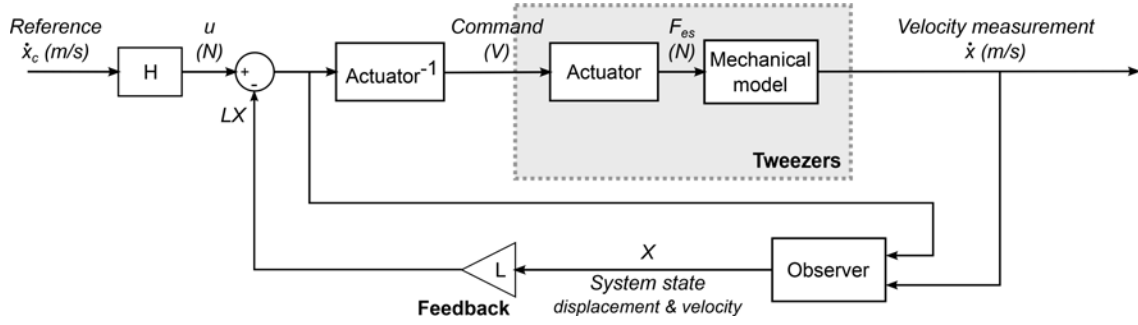


Fig. 3. Feedback control scheme. Gray box, called *Tweezers*, comprises the mechanical and the actuator models. Actuator square law needs to be inverted in order to linearize the control signal. The state feedback gain vector L and the feedforward gain H are designed to assign the desired closed-loop poles and to ensure unitary static gain. The observer is a Luenberger observer reconstructing the state of the system X for the state feedback. The sensor model is not shown here. The device output voltage is converted into the tip velocity by dividing by the sensor gain $1/\beta_C$.

TABLE III
POLE PLACEMENT OF THE CLOSED-LOOP SYSTEM AND STATE FEEDBACK GAIN L ACCORDING TO THE RESONANCE FREQUENCY REDUCTION n

Freq.	L ($[N \cdot m^{-1}], [Ns \cdot m^{-1}]$)	Poles
f_R	(0, 0)	$-138.9 \pm 8.3 \times 10^3 i$
$f_R/1.2$	$(-7.6, -16.7 \times 10^{-6})$	$-115.7 \pm 6.9 \times 10^3 i$
$f_R/2.0$	$(-18.7, -50.0 \times 10^{-6})$	$-69.4 \pm 4.2 \times 10^3 i$
$f_R/3.2$	$(-22.5, -68.8 \times 10^{-6})$	$-43.4 \pm 2.6 \times 10^3 i$
$f_R/5.0$	$(-23.9, -80.0 \times 10^{-6})$	$-27.8 \pm 1.7 \times 10^3 i$
$f_R/10$	$(-24.7, -90.0 \times 10^{-6})$	$-13.9 \pm 0.8 \times 10^3 i$

272 closed-loop poles. The actuation of the system F_{es} is equal to
273 $u - LX$, and the closed-loop system model becomes

$$\dot{X} = \begin{bmatrix} 0 & 1 \\ -\frac{k+l_1}{M} & -\frac{\nu+l_2}{M} \end{bmatrix} X + \begin{bmatrix} 0 \\ \frac{1}{M} \end{bmatrix} u. \quad (9)$$

274 Thus, the closed-loop resonance frequency and sensitivity are
275 given by

$$f_{R-cl} = \frac{1}{2\pi} \sqrt{\frac{(k+l_1)}{M}} \quad (10)$$

$$\frac{\partial f_{R-cl}}{\partial k} = \frac{1}{4\pi \sqrt{(k+l_1)M}} = \frac{1}{8\pi^2 M f_{R-cl}}. \quad (11)$$

276 The state feedback gain L is, then, determined in order to
277 assign the closed-loop poles in such a way that the system
278 resonates at a lower frequency than the natural frequency of
279 the tweezers (see Table III). L is computed to scale down this
280 resonant frequency (initially 1323.6 Hz), by a given factor de-
281 pending on the desired sensitivity enhancement while keeping
282 the damping factor unchanged.

283 When the closed-loop resonance frequency is scaled down by
284 a factor n compared to the SNT natural resonance frequency,
285 the sensitivity to small stiffness variation is improved with the
286 same ratio according to (11). Fig. 4 shows the computed fre-
287 quency shift of the closed-loop system caused by a signifi-
288 cant stiffness variation for different frequency scaling down
289 factor, n .

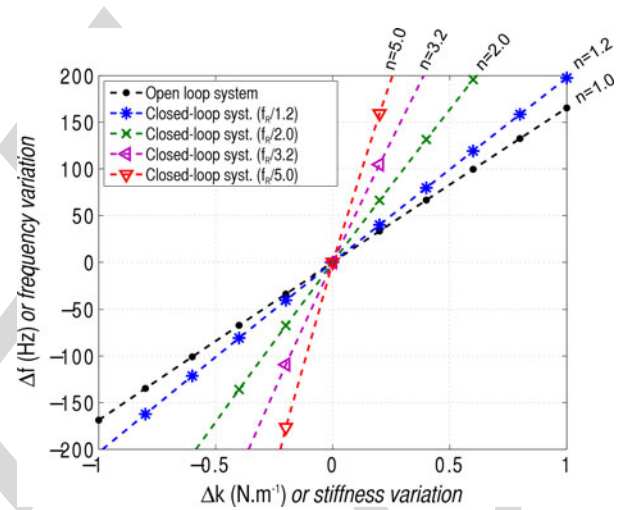


Fig. 4. Evolution of the resonance frequency shift Δf_R and Δf_{R-cl} with the added stiffness Δk variation for different frequency reduction ratio n .

C. Observer Design

290 The implementation of the aforementioned state feedback is
291 done through the use of a Luenberger observer. The observer is
292 designed such that it preserves the good closed-loop sensitivity
293 to parametric variations and it does not amplify too much noise.
294

295 The poles of the observer have then to be carefully chosen.
296 We used the following guidelines.

- 297 (1) The observer is designed to be faster than the original
298 device—with poles at least two times faster than the poles
299 of the system in open loop (cf., Table III).
- 300 (2) The observer poles are chosen not too fast in order to
301 avoid excessive noise amplification.
- 302 (3) The final location of the observer poles is chosen in order
303 to preserve the sensitivity of the system to stiffness
304 variations (by using root locus).

305 In the following section, simulations illustrate 1) the relevance
306 of the method and 2) the importance of the design of the observer
307 towards the enhancement of the sensitivity.

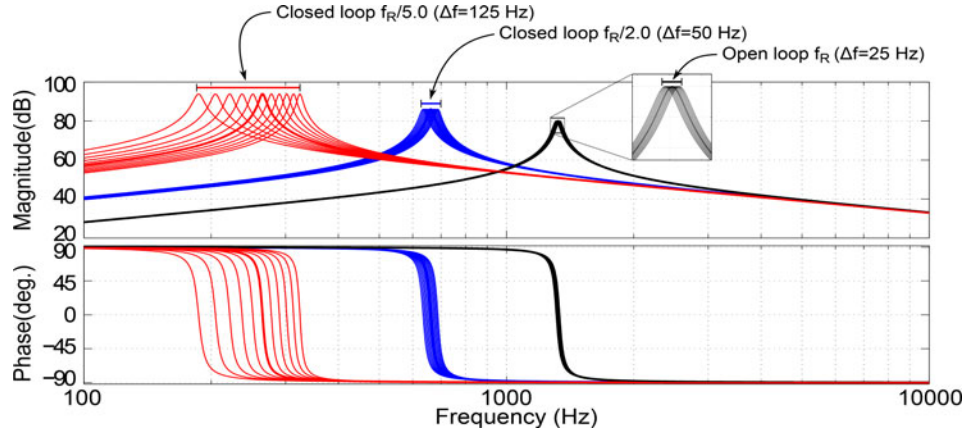


Fig. 5. Simulated frequency results for open-loop driven system and two different closed-loop systems. The three systems undergo a variation of k the system stiffness. k vary from -0.5 to 0.5 N/m by 0.1 N/m step. In black line, the tweezer transfer function, i.e., the open-loop system. In blue line, the implemented ($n = 2$) closed-loop system transfer function. The system resonates at a frequency twice lower. In red line, the implemented ($n = 5$) closed-loop system transfer function. The system resonates at a frequency five times lower.

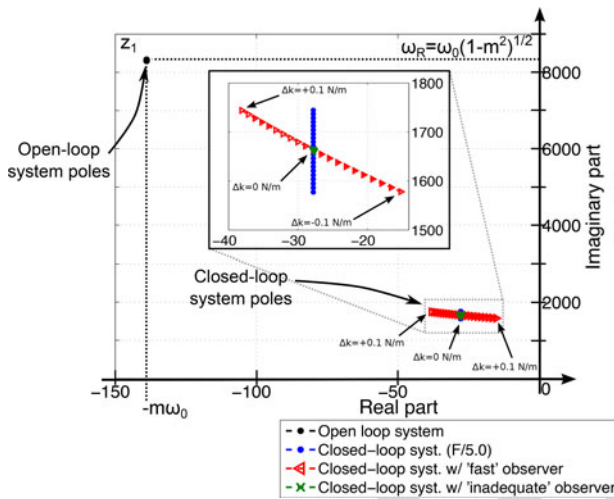


Fig. 6. Root locus of the closed-loop systems with $n = 5$ and with different observers. Complex conjugate poles are plotted in the positive imaginary half s plan. The parameter k varies from -0.1 to 0.1 N/m by 0.01 N/m steps. The black dots show the poles of the open-loop driven system. The ideal pole path of the closed-loop system due to the k variations are plotted with blue dots (i.e., no observer). For different observers designs, poles dependencies (and equivalent resonance frequency $\omega_R = 2\pi f_R$ and damping m) on the tweezers stiffness k are shown by red triangle and green cross plots. NB: Green cross dots path are imperceptible since they are at this scale all concentrate in the middle.

308 D. Simulation Results

309 From parameters identified in Table II, the feedback scheme
310 (see Fig. 3) has been first programmed and tested under MAT-
311 LAB/Simulink without implementing the observer (i.e., all the
312 state variables are supposed to be measured). Fig. 5 compares
313 the resonance frequency shift between the open-loop driven sys-
314 tem and the closed-loop systems computed for two resonance
315 frequency reduction factors set to $n = 2$ and 5 . As expected, the
316 sensitivity of the closed-loop system is improved proportionally
317 to n .

318 In a second instance, we implemented two different kinds of
319 observers (with different dynamics); Fig. 6 illustrates two cases.

TABLE IV
POLE PLACEMENT OF THE OBSERVER FOR STATE FEEDBACK IMPLEMENTATION

Open-loop system (Tweezers)	$z_{1,2} = -138.9 \pm 8.3 \times 10^3 i$
closed-loop system ($n = 5$)	$z_{1,2} = -27.8 \pm 1.7 \times 10^3 i$
Observer 1 (fast)	$z_{1,2} = -277.8 \pm 0.2 \times 10^3 i$
Observer 2 (slow)	$z_{1,2} = -27.8 \pm 0.2 \times 10^3 i$
Observer 3 (inadequate)	$z_{1,2} = -277.8 \pm 16.7 \times 10^3 i$

This Figure shows that the root locus (due to k parameter varia- 320
tions) is deformed by the observer dynamics. The sensitivity of 321
the resonance frequency to stiffness variations is given by the 322
amplitude of the imaginary axis variations. Table IV reports the 323
poles of the observers for which simulation results are presented. 324

In the insert of Fig. 6, the poles of the system implemented 325
with the observer named *inadequate* do not move compared to 326
the ideal root locus (ideal state feedback case). If the poles of 327
the observer are inappropriately chosen, the sensitivity of the 328
poles to variations of k is significantly reduced. Observer poles 329
close to the real axis, i.e., with an imaginary part close to 0, 330
are the more appropriate to reconstruct the displacement state 331
and to preserve the sensitivity of the system to variations of k . 332
The so-called *fast* observer allows us to increase the resonance 333
frequency shift ($\omega_R = 2\pi f_R$ in Fig. 6) close to the performances 334
theoretically expected. 335

IV. EXPERIMENTAL APPLICATION FOR EXTRA-STIFFNESS CHARACTERIZATION

The previous method has been experimentally tested with the 338
mechanical characterization of biomolecules of *fibronectin*. The 339
physical sensor is the SNT, while the feedback controller and 340
the observer are implemented in a dSPACE prototyping board. 341

A. Experimental Protocol

Fibronectin is a protein, 100 nm in length, of the extracel- 343
lular matrix which plays a major role in cell adhesion, growth, 344

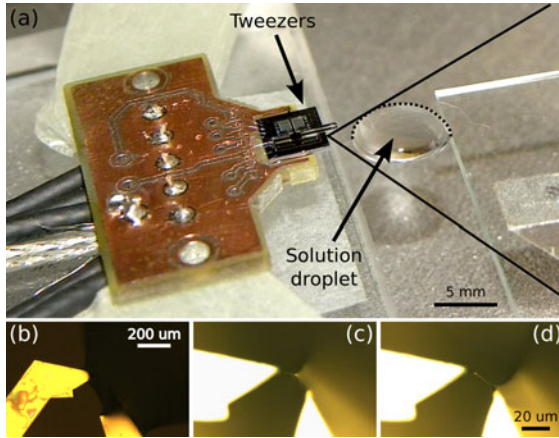


Fig. 7. Experimental setup for the trapping of *fibronectin* molecules. (a) The SNT are fixed. A droplet of *fibronectin* solution is dropped on a glass slice which is mounted on the XYZ stage. (b)–(d) Sequences of the molecule combing. Under optical microscopy, the tweezers' tips are retrieved from the solution meniscus. A bundle of *fibronectin* is released between the tips.

345 migration, and differentiation [33]. These molecules are used to
 346 polymerize the fibrillar matrix at the surface of the cell. We have
 347 used this property to trap by combing a bundle of *fibronectin*.
 348 After immersion of the tips in the solution, they are retrieved by
 349 the side forming a polymerized bundle of molecules (see Fig. 7).

350 The closed-loop systems are evaluated with *fibronectin* bundle
 351 in air, in dry condition. This stable condition was preferred
 352 to the immersed one as we can avoid any effect of liquid surface
 353 tension stability that can interfere with the sole system response.
 354 For each case, a sinusoidal reference of $0.02 V_{AC}$ with an offset
 355 of $20 V_{DC}$ is applied to the system and the oscillation frequen-
 356 cies are spanned from 1000 to 2000 Hz with a step of 2 Hz.
 357 Then, the measurement is performed with the lock-in amplifier.

358 Two sets of experiments have been performed with two differ-
 359 ent bundles. From the resonance frequency shifts in open loop,
 360 the stiffness of the first bundle is $11.8 N \cdot m^{-1}$, and $4.3 N \cdot m^{-1}$
 361 for the second one. In total, 22 resonance responses are recorded
 362 and compared.

363 B. Results and Discussions

364 1) *Experimental Results*: Fig. 8 shows the frequency re-
 365 sponses of four different systems with and without a bundle
 366 of *fibronectin*.

367 In Fig. 8(a), the responses of the open-loop driven SNT are
 368 given. The dashed-line curve is the reference curve, which cor-
 369 responds to the frequency response of the SNT alone driven in
 370 open loop. The solid line is the response of the tweezers with
 371 the bundle trapped in between the tips. The resonance frequency
 372 increases and the amplitude decreases due to the additional stiff-
 373 ness and loss contributions of the molecules. In Fig. 8(b)–(d),
 374 the results of the closed-loop systems with desired reduction
 375 ratios are of 1.1, 1.2 and 1.5.

376 Resonance responses are fitted with a second-order resonator
 377 model and characteristics such as the resonance frequency are
 378 extracted. The Table V sums up the resonance frequencies for
 379 the experiment with/without the first bundle for five different

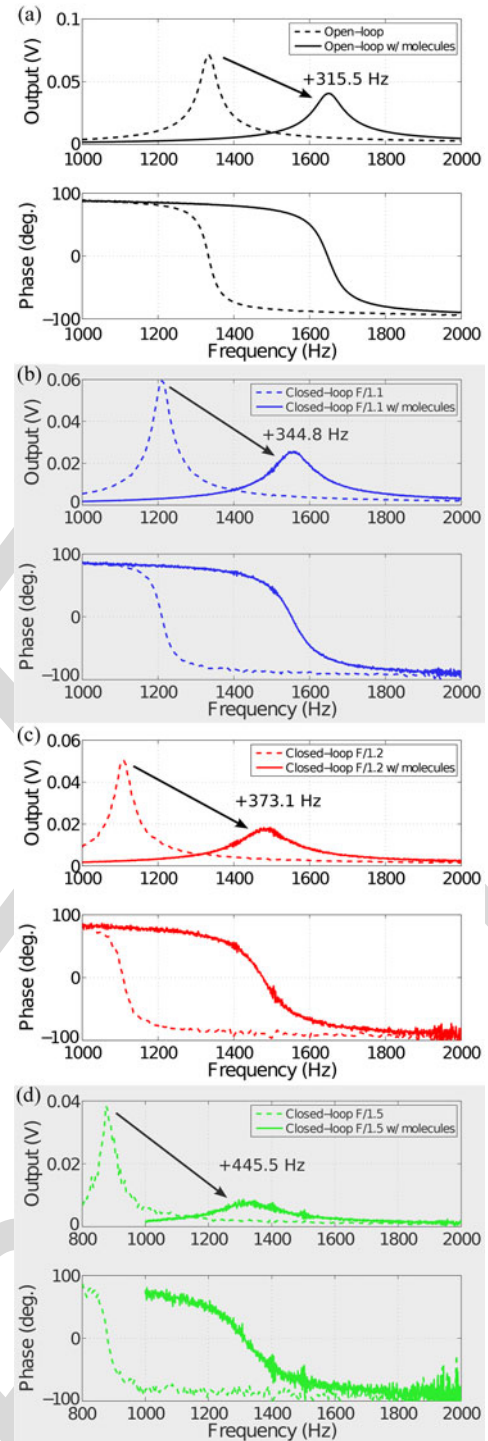


Fig. 8. Frequency responses of different implemented closed-loop systems for $0.02 V$ sinusoidal signal with an offset of $20 V$. In dotted lines, the responses of the systems with the bundle of molecules. In plain lines, the responses of the systems without molecules. (a) Open-loop driven systems. (b) Closed-loop systems implemented with $f_R/1.1$. (c) Closed-loop systems implemented with $f_R/1.2$. (d) Closed-loop systems with $f_R/1.5$.

380 systems. Through reducing the resonance frequency of the sys-
 381 tem, the measured results prove the increase of the frequency
 382 shift due to the extra-stiffness of the bundle.

383 Moreover Fig. 9 tends to 1) confirm with second set of exper-
 384 iments, the enhancement of the sensitivity through the proposed

TABLE V
EXPERIMENTAL RESULTS FOR THE BUNDLE 1 (RELATED TO FIG. 8)

System (n)	Freq. w/o bundle (Hz)	Freq. w/ bundle (Hz)	Freq. shift (Hz)
Open loop (1.0)	1333.6	1649.1	+315.5
Closed-loop (1.1)	1211.1	1555.9	+344.8
Closed-loop (1.2)	1109.1	1482.2	+373.1
Closed-loop (1.5)	881.9	1327.4	+445.5
Closed-loop (2.0)	653.9	1175.6	+521.7

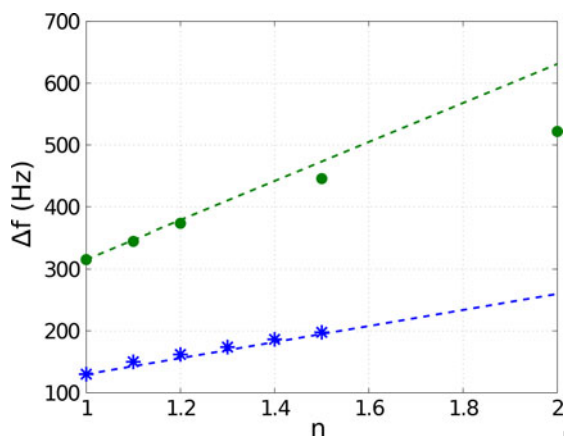


Fig. 9. Synthesis of the experimental results. In abscissa, is informed the reduction factor (n) applied to the resonance frequency for the closed-loop system. The shift Δf is the resonance frequency difference due to the presence of the bundle of molecules in between the tips. The dotted lines represent the theoretical improvement expected. The two sets of results obtained with two different bundles are plotted. The green dots correspond to the experiment of Fig 8 and TableV (bundle of $11.8 \text{ N} \cdot \text{m}^{-1}$ stiffness). The blue stars corresponds to the second bundle of $4.3 \text{ N} \cdot \text{m}^{-1}$ stiffness.

385 feedback strategy and 2) show a good agreement with the theory.
386 Small differences are discussed in the following section.

387 2) *Discussion*: The synthesis of Fig. 9 demonstrates the en-
388 hancement of the frequency shifts in agreement with the theory.
389 However, the enhancement for closed-loop system with a reduc-
390 tion factor of 2.0 is lower than expected, and the implementation
391 for a higher reduction factor has not been achieved. In this case,
392 the system tends to become unstable. We mainly associated this
393 phenomenon to the observer implementation and to the presence
394 of some delays in the feedback loop.

395 We have demonstrated previously that observer's dynamic
396 changes the root locus of system's poles, the behavior of the
397 closed-loop system under parameter variations and impacts the
398 performances of the method. Furthermore, delays have been
399 characterized due to D/A and A/D converters dead times and to
400 the phase shift brought by the current amplifiers. Current works
401 are devoted to the precise characterization of such delays and to
402 their integration in the control design process.

V. CONCLUSION AND FUTURE WORK

404 The present study investigates the relevance of a feedback
405 approach in the improvement of the performances of MEMS
406 tweezers for the detection and the mechanical characterization of
407 biological molecules. An improvement of 50% on the sensitivity

to mechanical stiffness variations has been reached theoretically
and experimentally on *fibronectin* bundles through the emulation
of a more compliant system. The stiffness have been brought
down to 10.5 N/m instead of originally 24.5 N/m .

The main drawback of this approach is the sensitivity of
the observer to the stiffness variations. Unusually, the poles of
the observer are uncontrollable resulting from the separation
principle. During the sensing of the molecules stiffness, the
separation principle is not satisfied anymore and the observer
poles can become unstable. We are actually working on self-
scheduled observers aiming at adapting the observer dynamics
with respect to sensed parametric variations. It should solve the
main drawback of this promising approach and allows us to go
one step further into the theoretical closed-loop performances.

ACKNOWLEDGMENT

The authors would like to thank A. W. Coleman from the
University of Lyon, Lyon, France, for the discussion and proof-
reading of the text. The photolithography masks were fabricated
with the 8-inch EB writer F5112+VD01 donated by Advantest
Corporation to the VLSI Design and Education Center (the Uni-
versity of Tokyo).

REFERENCES

- [1] C. Bustamante, J. Marko, E. Siggia, and S. Smith, "Entropic elasticity of lambda-phage DNA," *Science*, vol. 265, no. 5178, pp. 1599–1600, 1994.
- [2] P. Cluzel, A. Lebrun, C. Heller, R. Lavery, J. L. Viovy, D. Chate-
nay, and F. Caron, "DNA: An extensible molecule," *Science*, vol. 271,
no. 5250, pp. 792–794, Feb. 1996.
- [3] G. Bao, "Mechanics of biomolecules," *J. Mech. Phys. Solids*, vol. 50, no.
11, pp. 2237–2274, 2002.
- [4] A. Bancaud, N. Conde E Silva, M. Barbi, G. Wagner, J.-F. Allemand,
J. Mozziconacci, C. Lavelle, V. Croquette, J.-M. Victor, A. Prunell, and
J.-L. Viovy, "Structural plasticity of single chromatin fibers revealed by
torsional manipulation," *Nat. Struct. Mol. Biol.*, vol. 13, no. 5, pp. 444–450,
May. 2006.
- [5] J. Gore, Z. Bryant, M. D. Stone, M. Nöllmann, N. R. Cozzarelli, and
C. Bustamante, "Mechanochemical analysis of DNA gyrase using rotor
bead tracking," *Nature*, vol. 439, no. 7072, pp. 100–104, Jan. 2006.
- [6] O. Chaudhuri, S. H. Parekh, and D. A. Fletcher, "Reversible stress soft-
ening of actin networks," *Nature*, vol. 445, no. 7125, pp. 295–298, Jan.
2007.
- [7] T. Hawkins, M. Mirigian, M. S. Yasar, and J. L. Ross, "Mechanics of
microtubules," *J. Biomech.*, vol. 43, no. 1, pp. 23–30, Jan. 2010.
- [8] F. Amblard, B. Yurke, A. Pargellis, and S. Leibler, "A magnetic ma-
nipulator for studying local rheology and micromechanical properties of
biological systems," *Rev. Sci. Instrum.*, vol. 67, no. 3, pp. 818–827, 1996.
- [9] C. Gosse and V. Croquette, "Magnetic tweezers: Micromanipulation and
force measurement at the molecular level," *Biophys. J.*, vol. 82, no. 6,
pp. 3314–3329, 2002.
- [10] R. Simmons, J. Finer, S. Chu, and J. Spudich, "Quantitative measurements
of force and displacement using an optical trap," *Biophys. J.*, vol. 70,
no. 4, pp. 1813–1822, 1996.
- [11] E. L. Florin, V. T. Moy, and H. E. Gaub, "Adhesion forces between
individual ligand-receptor pairs," *Science*, vol. 264, no. 5157, pp. 415–
417, Apr. 1994.
- [12] A. Ishijima, T. Doi, K. Sakurada, and T. Yanagida, "Sub-piconewton force
fluctuations of actomyosin in vitro," *Nature*, vol. 352, pp. 301–306, 1991.
- [13] F. Beyeler, A. Neild, S. Oberti, D. Bell, Y. Sun, J. Dual, and B. Nelson,
"Monolithically fabricated microgripper with integrated force sensor for
manipulating microobjects and biological cells aligned in an ultrasonic
field," *J. Microelectromech. Syst.*, vol. 16, no. 1, pp. 7–15, 2007.
- [14] K. Kim, X. Liu, Y. Zhang, and Y. Sun, "Nanonewton force-controlled
manipulation of biological cells using a monolithic MEMS microgripper
with two-axis force feedback," *J. Micromech. Microeng.*, vol. 18, no. 5,
p. 055013, Apr. 2008.
- [15] G. Hashiguchi, T. Goda, M. Hosogi, K. Hirano, N. Kaji, Y. Baba,
K. Kakushima, and H. Fujita, "DNA manipulation and retrieval from

- an aqueous solution with micromachined nanotweezers," *Anal. Chem.*, vol. 75, no. 17, pp. 4347–4350, 2003.
- [16] M. Kumemura, D. Collard, N. Sakaki, C. Yamahata, M. Hosogi, G. Hashiguchi, and H. Fujita, "Single-DNA-molecule trapping with silicon nanotweezers using pulsed dielectrophoresis," *J. Micromech. Microeng.*, vol. 21, p. 054020, 2011.
- [17] C. Yamahata, D. Collard, B. Legrand, T. Takekawa, M. Kumemura, G. Hashiguchi, and H. Fujita, "Silicon nanotweezers with subnanometer resolution for the micromanipulation of biomolecules," *J. Microelectromech. Syst.*, vol. 17, no. 3, pp. 623–631, 2008.
- [18] M. Kumemura, D. Collard, S. Yoshizawa, D. Fourmy, N. Lafitte, L. Jalabert, S. Takeuchi, T. Fujii, and H. Fujita, "Direct bio-mechanical sensing of enzymatic reaction on DNA by silicon nanotweezers," in *Proc. IEEE 23rd Int. Conf. Microelectromech. Syst.*, Hong Kong, 2010, pp. 915–918.
- [19] S. Smith, Y. Cui, and C. Bustamante, "Overstretching B-DNA: The elastic response of individual double-stranded and single-stranded DNA molecules," *Science*, vol. 271, no. 5250, pp. 795–799, 1996.
- [20] N. Lafitte, M. Kumemura, L. Jalabert, D. Collard, and H. Fujita, "Real-Time sensing of molecule binding on DNA with silicon nanotweezers," in *Proc. 15th Int. Conf. Miniaturized Syst. Chem. Life Sci.*, Seattle, WA, USA, 2011, pp. 389–372.
- [21] Y. Sun, B. J. Nelson, D. P. Potasek, and E. Enikov, "A bulk microfabricated multi-axis capacitive cellular force sensor using transverse comb drives," *J. Micromech. Microeng.*, vol. 12, no. 6, p. 832, 2002.
- [22] W. Tang, T.-C. H. Nguyen, and R. Howe, "Laterally driven resonant microstructures," *Sens. Actuators*, vol. 20, pp. 25–32, 1989.
- [23] G. Zhou and P. Dowd, "Tilted folded-beam suspension for extending the stable travel range of comb-drive actuators," *J. Micromech. Microeng.*, vol. 13, no. 2, pp. 178–183, 2003.
- [24] T. Clark and R. Howe, "An integrated CMOS micromechanical resonator high-Q oscillator," *IEEE J. Solid-State Circuits*, vol. 34, no. 4, pp. 440–455, Apr. 1999.
- [25] R. Legtenberg, A. Groeneveld, and M. Elwenspoek, "Comb-drive actuators for large displacements," *J. Micromech. Microeng.*, vol. 6, no. 3, pp. 320–329, 1996.
- [26] Y. Sun, S. N. Fry, D. P. Potasek, D. J. Bell, and B. J. Nelson, "Characterizing fruit fly flight behavior using a microforce sensor with a new comb-drive configuration," *J. Micromech. Syst.*, vol. 14, pp. 4–11, 2005.
- [27] M. Bao, *Analysis and Design Principles of MEMS Devices*. New York, NY, USA: Elsevier, 2005.
- [28] N. Lafitte, "Modeling and control of MEMS tweezers for the characterizations of enzymatic reactions on DNA molecules," Ph. D. dissertation, Université de Franche-Comté, Besançon, France, Apr. 2012.
- [29] D. Collard, C. Yamahata, B. Legrand, T. Takekawa, M. Kumemura, N. Sakaki, G. Hashiguchi, and H. Fujita, "Towards mechanical characterization of biomolecules by MNEMS tools," *IEEJ Trans. Electr. Electron. Eng.*, vol. 2, no. 3, pp. 262–271, Apr. 2007.
- [30] D. Collard, "Silicon nanotweezers for biomechanical and bioelectrical assays," *Front. Biosci.*, vol. E5, pp. 955–965, Jun. 2013.
- [31] M. C. Tarhan, D. Collard, L. Jalabert, M. Kumemura, N. Lafitte, Q. Delouée, S. L. Karsten, and H. Fujita, "Continuous real-time monitoring of molecular detection by silicon nanotweezers-integrated microfluidic device," in *Proc. 16th Int. Conf. Miniaturized Syst. Chem. Life Sci.*, Okinawa, Japan, Oct. 2012, pp. 1897–1899.
- [32] M. Washizu and O. Kurosawa, "Electrostatic manipulation of DNA in microfabricated structures," *IEEE Trans. Ind. Appl.*, vol. 26, no. 6, pp. 1165–1172, Nov/Dec. 1990.
- [33] R. Pankov, "Fibronectin at a glance," *J. Cell Sci.*, vol. 115, no. 20, pp. 3861–3863, Oct. 2002.

533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548



Nicolas Lafitte was born in Bordeaux, France, in 1983. He received the M.S. degree in electrical engineering speciality microelectronics from the University of Bordeaux I, Bordeaux, and the graduate school ENSEIRB, Talence, France, in 2008, and the Ph.D. degree from the University of Franche-Comté, Besançon, France, for his work on the modeling and the control of MEMS tweezers for characterizations of bioreactions on DNA molecules, in 2012. This work was conducted in LIMMS (CNRS/University of Tokyo joint laboratory) and in FEMTO-ST (CNRS/University of Franche-Comté). This publication deals with this work.

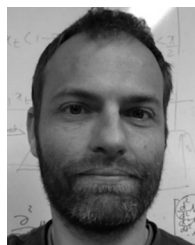
His current research interests include the MEMS integration for biological applications at the University of Tokyo, Japan.



Yassine Haddab received the Engineering degree in electrical engineering from the University of Tizi-Ouzou, Tizi-Ouzou, Algeria, the M.S. degree from ENSMM, Besançon, France, and the Ph.D. degree from the University of Franche-Comté, Besançon, France, in 2000. In 2012, he received the Habilitation a Diriger de Recherches degree from the University of Franche-Comté, Besançon.

Since 2002, he is an Associate Professor with ENSMM, Besançon, where he teaches control, micro-robotics and embedded real-time systems. His research interests include the design, modeling, and control of high precision microrobots and microsystems. He also contributes to the development of microfactory concepts and new microrobots architectures.

549
550
551
552
553
554
555
556
557
558
559
560
561
562
563



Yann Le Gorrec (M'XX) was graduated as engineer in "control, electronics, computer engineering" from the National Institute of Applied Sciences, Toulouse, France, in 1995, and received the Ph.D. degree from the National Higher School of Aeronautics and Aerospace, Supaero, Toulouse, in 1998.

From 1999 to 2008, he was an Associate Professor in Automatic Control at the Laboratory of Control and Chemical Engineering of Lyon Claude Bernard University, Villeurbanne, France. He worked on port Hamiltonian systems and their use for modeling and control of irreversible and distributed parameter systems with an application to physico-chemical processes. Since september 2008, he has been a Professor with the National Engineering Institute in Mechanics and Microtechnologies. He is currently the Head of the COnTrol DEsign research group of FEMTO-ST AS2M department. His research interests include robust control and self scheduled controller synthesis, the control of nonlinear systems, irreversible and infinite dimensional systems with an application to smart material-based actuators, and microactuators.

564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583



Hervé Guillou was born in Grenoble, France, in 1973. He studied fundamental physics from the University of Grenoble Alpes, Grenoble, France, the University of Karlsruhe, Karlsruhe, Germany, and at New York University, New York, NY, USA. He received the Master of Sciences degree from New York University and then a Ph.D. degree from the university of Grenoble Alpes, in 1998 and 2001, respectively.

Since 2004, he has been a Lecturer in physics with the University of Grenoble Alpes. His first research interests include low temperature physics and superconductivity. He moved, then, to the field of biophysics where his interests include cell architecture, cell and molecular force generation, and development of methods, instruments, and microsystems for applications in biophysics and medicine.

584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599



Momoko Kumemura was born in Kagoshima, Japan, in 1975. She received the M.S. degree in engineering from Tokushima University, Tokushima, Japan, and the Ph.D. degree in chemistry from Tokyo Metropolitan University, Tokyo, Japan, in 2002 and 2005, respectively.

She was a Postdoctoral Researcher in the Center for International Research on Micromechanics, Institute of Industrial Science, The University of Tokyo (2005–2008 and 2010–2013) and in the Centre National de la Recherche Scientifique (2008–2010). Since 2014, she has been a Research Associate in Institute of Industrial Science, The University of Tokyo. She is currently researching microsystems for biological applications.

600
601
602
603
604
605
606
607
608
609
610
611
612
613
614

615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632



Laurent Jalabert was born in L'Union, France, in 1974. He received the Ph.D. degree from the Université Paul Sabatier, Toulouse, France, in 2001 dealing with CMOS gate engineering and reliability of ultrathin oxides.

From 2001 to 2003, he joined LIMMS (CNRS-University of Tokyo joint laboratory), Tokyo, Japan, as a Postdoctoral Researcher granted from the Japanese Society for the Promotion of Science and he developed piezoresistive MEMS cantilever for metrology in high aspect ratio microholes. In 2004, he joined Institut National Polytechnique de Toulouse as a Research Engineer and worked at Laboratoire d'Analyse et d'Architecture des Systèmes on ICP-RIE plasma and alternative technologies (nanoimprint). Since 2007 till now, he is with the LIMMS as a CNRS Research Engineer hosted in Pr. Hiroyuki Fujita laboratory. His current research interests include atomic heat transfer in UHV-TEM and MEMS fabrication (silicon nanotweezers, liquid-cells, etc.).

633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656



Dominique Collard (M'01) was born in Cambrai, France in 1958. He received the Engineering Degree from the Institut Supérieur d'Electronique et du Numérique and the Ph.D. degree from the University of Lille, Lille, France, in 1980 and 1984, respectively.

From 1985 to 1986, he was with the Toshiba VLSI Research Center, Kawasaki, Japan, as a Visiting Scientist. Since 1988, he has been with the Centre National de la Recherche Scientifique (CNRS), being alternatively with the Institut d'Electronique, de Microélectronique et de Nanotechnologie, Lille, and with the Laboratory for Integrated MicroMechatronic Systems (LIMMS/CNRS-IIS), Tokyo. From August 2005, he joint a second time LIMMS where he was appointed as a Director in September 2007 and got a title of Project Professor of IIS, the University of Tokyo, Tokyo, Japan. In December 2011, he became Coordinator of EC/FP7 INCOLAB: EUJO-LIMMS aiming to open LIMMS to European partners and first EC laboratory in Japan. His current scientific interests include micro- and nano-systems for applications in biology and nanotechnology. He is the Author or Coauthor of more than 250 international publications.

Dr. Collard received the IBM price on intensive numerical calculation in 1990, the CNRS bronze medal in 1992, and was in 2004 Laureate of the French academic palms from Ministry of Higher Education and Research.



Hiroyuki Fujita (S'76-M'80) received B.S., M.S., and Ph.D. degrees in electrical engineering from the University of Tokyo, Tokyo, Japan, in 1975, 1977, and 1980, respectively.

Since 2000, he is the Director of the Center for International Research on MicroMechatronics. Also, since 1993, he has been a Professor, an Associate Professor (1981-1993), and a Lecturer (1980-1981) with the Institute of Industrial Science, the University of Tokyo. He is currently involved in the investigation of microelectromechanical systems fabricated by IC-based processes and applications to optics, hard disk drives, and bio/nanotechnology. His research interest includes autonomous distributed microsystems.

Dr. Fujita is a Fellow of the Institute of Electrical Engineers of Japan.

657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672

QUERIES

- Q1. Author: Please provide the full page range in Refs. [14], [16], and [21].
Q2. Author: Please provide the department name in Ref. [28].
Q3. Author: Please provide the year in which the author “Yann Le Gorrec” became a Member of the IEEE.

673

674

675

676

IEEE
Proof

Improvement of Silicon Nanotweezers Sensitivity for Mechanical Characterization of Biomolecules Using Closed-Loop Control

Nicolas Lafitte, Yassine Haddab, Yann Le Gorrec, *Member, IEEE*, Hervé Guillou, Momoko Kumemura, Laurent Jalabert, Dominique Collard, *Member, IEEE*, and Hiroyuki Fujita, *Member, IEEE*

Abstract—In this paper, we show that closed-loop control can be advantageously used for the characterization of mechanical properties of biomolecules using silicon nanotweezers (SNT). SNT have already been used in open-loop mode for the characterization of mechanical properties of DNA molecules. Up to now, such an approach allows the detection of stiffness variations equivalent to about 15 DNA molecules. Here, it is shown that this resolution is inversely proportional to the resonance frequency of the whole system and that real-time feedback control with state observer can drastically improve the performances of the tweezers used as biosensors. Such improvement is experimentally validated in the case of the manipulation of *fibronectin* molecules. The results are promising for the accurate characterization of biopolymers such as DNA molecules.

Index Terms—Biosensor, MEMS tweezers, parameter detection, sensitivity improvement, state feedback.

I. INTRODUCTION

RECENT developments in micronano manipulation tools have revealed crucial information on the mechanical behavior of biomolecules [1]–[3]. These manipulations generally performed on a single molecule have given the quantitative data needed to elucidate fundamental biological processes as DNA wrapping [4] and replication [5], or cell cytoskeleton dynamics through actin filament [6], and microtubule mechanical responses [7].

Several methods are already available for performing biological experiments at the molecular level: magnetic tweezers [8], [9], optical tweezers [10], AFM cantilevers [11], and microfibers [2], [12]. A single filamentary macromolecule, such as DNA, is first attached to a surface at one end, and to a bead

Manuscript received October 8, 2012; revised April 9, 2014; accepted June 10, 2014. Recommended by Technical Editor S. O. R. Moheimani. The work of Y. Haddab and Y. Le Gorrec was supported in part by the LABEX ACTION under Grant ANR-11-LABX-01-01 and the NANOROBUST Project (ANR-2011 NANO 006).

N. Lafitte, H. Guillou, M. Kumemura, L. Jalabert, and D. Collard are with the LIMMS/CNRS-IIS (UMI 2820), Institute of Industrial Science, The University of Tokyo, Tokyo 153-8505, Japan (e-mail: lafitte@iis.u-tokyo.ac.jp; herveg@iis.u-tokyo.ac.jp; momo@iis.u-tokyo.ac.jp; jalabert@iis.u-tokyo.ac.jp; collard@iis.u-tokyo.ac.jp).

Y. Haddab and Y. Le Gorrec are with the FEMTO-ST/UFC-ENSMM-UTBM-CNRS, 25044 Besançon, France (e-mail: yassine.haddab@ens2m.fr; Yann.Le.Gorrec@ens2m.fr).

H. Fujita is with the Center for International Research on Micromechanics, Institute of Industrial Science, The University of Tokyo, Tokyo 153-8505, Japan (e-mail: fujita@iis.u-tokyo.ac.jp).

Color versions of one or more of the figures in this paper are available online at <http://ieeexplore.ieee.org>.

Digital Object Identifier 10.1109/TMECH.2014.2351415

or a flexible cantilever at the other end. Forces or displacements are sensed by optical measurements. Nevertheless, the real-time operation and the routine implementation of these techniques remain difficult to achieve, as they require complex experimental procedures. In this respect, microelectromechanical systems (MEMS) offer an advantage for systematic analysis since accurate molecular level tools (actuator, end effectors, and sensor) can be integrated on a MEMS platform. Furthermore, these devices can be produced at low cost with batch fabrication, as have the microgrippers developed to grab micro-sized objects [13] or cells [14]. However, up to now, the performances of such tools are not sufficient to deal with single molecule characterization. In previous reports, silicon nanotweezers (SNT) were designed and fabricated to trap DNA molecules [15], [16] and characterize DNA bundles [17] and reactions on DNA [18]. The stiffness of DNA bundles was derived from the open-loop measurement of the mechanical resonance frequency shift. The current sensing resolution is in the range of $1 \text{ mN} \cdot \text{m}^{-1}$, which corresponds to approximately 15 λ -DNA molecules¹ [20]. This resolution is limited by the design of the tweezers and the noise of the electronic instrumentation. To improve the overall system sensitivity towards lower stiffness, the primary route consists of reducing the tweezers stiffness to bring the sensor and measured object characteristics close to the same range. Unfortunately, such a design would result in a system that would be too fragile to be processed and utilized.

Our approach consists of scaling down the resonance frequency of the closed-loop system to enhance the sensitivity to SNT stiffness change. After a proper identification of the SNT model, the feedback control has been simulated, optimized, and then implemented. This control strategy has been evaluated with thin *fibronectin* molecular bundles trapped between SNT tips, and we propose to discuss the advantages and the limits of such closed-loop operations.

This paper is organized as follows. Section II describes the tweezers, the sensing principle, and provides an example of monitoring the DNA trapping. The dynamic model of the tweezers is then detailed in Section III-A. The closed-loop control strategy and its potential to improve the system sensitivity are discussed in Sections III-B and III-C. Section IV details the experimental implementation and provides information on *fibronectin* molecules measurement with different

¹ λ -DNA is DNA of the bacteriophage λ having a contour length of $16.4 \mu\text{m}$ and a stiffness in elastic regime of $66 \mu\text{N} \cdot \text{m}^{-1}$ in low ionic solution [19].

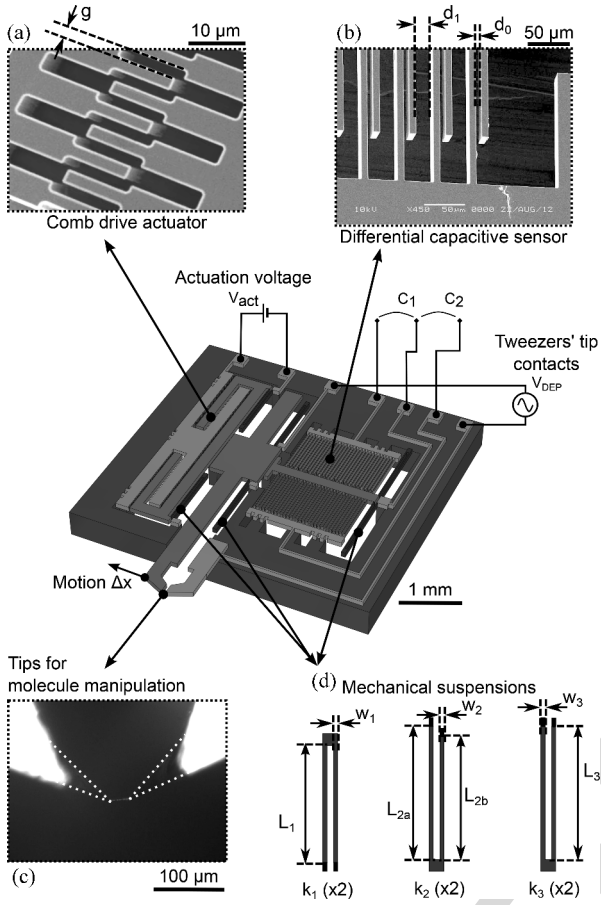


Fig. 1. SNT description. 3-D view of the device whose overall dimensions are $4.5 \times 4.5 \times 0.5$ mm. The mobile parts are shown in red and the mechanical suspensions in blue. (a) Scanning electronic microscopic (SEM) image of the actuator consisting of 880 pairs of interdigitated combs separated by a $2\text{-}\mu\text{m}$ gap, and organized in two series. (b) SEM image of the integrated sensor of two opposing series of 30 combs. (c) Microscope image of the tweezers tips with a DNA bundle in between. (d) Design of the three sets of suspensions, k_1 for the actuator polarization, k_2 for the mobile arm, and k_3 for central sensor plate.

78 closed-loop settings. Closing remarks and perspectives are given
79 in Section V.

80 II. DESCRIPTION OF THE DEVICE AND ITS USE FOR THE 81 MECHANICAL CHARACTERIZATION 82 OF BIOMOLECULES BUNDLES

83 A. Device Description

84 1) *Design*: Fig. 1 shows a three-dimensional illustration of
85 the SNT device. It consists of two arms terminated in sharp tips
86 that act as electrodes to trap biomolecules by dielectrophoresis
87 (DEP) forces [15]. One tip is fixed; the other one is moved by
88 an electrostatic actuator. The motion of the mobile electrode
89 is measured by capacitances whose value linearly varies with
90 electrode displacement; two variable capacitances are mounted
91 in differential mode [21].

92 2) *Electrostatic Actuation*: The mobile arm is actuated by
93 attractive electrostatic forces generated in an interdigitated comb
94 drive [see Fig. 1(a)], one of the most widely used architecture

in MEMS design [22], [23]. The maximal stroke is limited
95 compared to that achievable by a parallel plate actuator but the
96 generated force only depends on the actuation voltage and not on
97 the electrode position, thus, enabling a simpler actuation control.
98 The force is given by (1) where ϵ_0 is the vacuum permittivity (we
99 consider the relative permittivity of air = 1), N_a is the number
100 of comb fingers, t is the device thickness, g is the lateral gap
101 between fingers, and V_{act} is the actuation voltage. Due to its
102 capacitive structure, the comb drive generates attractive forces
103 between its two electrodes
104

$$F_{es} = \frac{1}{2} \frac{\epsilon_0 \times N_a \times t}{g} V_{act}^2 = \alpha_{es} \times V_{act}^2. \quad (1)$$

3) *Mechanical Structure*: The mobile part of the tweezers
105 is linked to the silicon frame by flexible beams [see Fig. 1(d)].
106 Commonly integrated in mechanical microsystems [23], [24],
107 folded beam springs are designed to minimize beam areas,
108 decrease their mechanical stiffness and enhance displacement
109 ranges [25]. In the SNT design of Fig. 1, three sets of folded
110 beam suspensions support the mobile part of the system (the
111 comb-drive actuator, the mobile tip, and the capacitive sensor)
112 and provide the electrical connections for the actuation and the
113 sensing. The springs are arranged symmetrically along the
114 actuation and sensing axis to minimize any rotation, and their
115 sum gives the total stiffness k of the device.
116

A highly compliant system is required to sense the mechani-
117 cal characteristic of trapped molecules on the tweezers response.
118 On the other hand, a minimum stiffness is mandatory, 1) to en-
119 dure the fabrication processes and manipulations, 2) to support
120 the mobile system weight, and 3) to prevent sticking due to at-
121 tractive surface forces between the comb-drive actuator and the
122 capacitive sensor electrodes.
123

4) *Displacement/Velocity Sensing*: The tip position is mea-
124 sured by a capacitive sensor designed in a triplate configuration
125 with transverse combs [see Fig. 1(b)] [26]. The central elec-
126 trode, linked to the mobile arm, moves in between two fixed
127 electrodes creating the two differential capacitances C_1 and C_2
128 whose difference, ΔC , is related to the displacement x . For
129 small displacements (i.e., x is much smaller than the gaps be-
130 tween electrodes d_0 and d_1), ΔC is proportional to x [17], N_b
131 is the number of capacitance electrodes, and L the length of the
132 electrodes
133

$$\Delta C = C_1 - C_2 \quad (2)$$

$$\simeq 2\epsilon_0 N_b L t \left(\frac{1}{d_0^2} - \frac{1}{d_1^2} \right) x = \beta_C \times x \quad (3)$$

5) *Electronic Read-Out*: The tweezers motion is sensed by
134 the measurement of the differential capacitance ΔC (2) through
135 current sensing. In dynamic mode, a dc voltage (V_{polar}) is
136 applied on the mobile central electrode, whose motion generates
137 dynamic currents i_1 and i_2 flowing through the capacitances C_1
138 and C_2 , respectively, [27], [28]. The resulting currents related
139 to the motion velocity are converted into voltages V_1 and V_2 by
140 two low-noise current-to-voltage (A/V) preamplifiers (Signal
141 Recovery, model 5182). The low input impedance of the pream-
142 plifier (virtual ground) ensures an accurate current conversion
143 [17], [29].
144

TABLE I
NUMERICAL VALUES OF DIMENSIONS AND PARAMETERS OF THE SNT

Silicon thickness	
t (μm)	30
Comb drive actuator	
N_a	440
g (μm)	2
Mechanical suspensions	
L_1, L_{2a} (μm)	900
L_{2b}, L_3 (μm)	1000
w_1, w_2, w_3 (μm)	15
Capacitive sensor	
N_b	30
L (μm)	585
d_0 (μm)	5
d_1 (μm)	20

The device dimensions are defined in Fig. 1.

145 Finally, in harmonic mode, when the tweezers is moved by
146 a sinusoidal actuation, a lock-in amplifier (NF, model LI 5640)
147 performs the low-noise detection of the differential signal (amplitude and phase of $V_1 - V_2$) at the actuation frequency. The
148 reference signal is the motion frequency imposed by the actuation voltage.
149 The reference signal is the motion frequency imposed by the actuation
150 voltage.

151 All dimensions and parameters that has been considered for
152 a proper model of the SNT are summed up in the Table. Furthermore, Young's modulus and the density of the silicon are,
153 respectively, 165 GPa and $2329 \text{ kg} \cdot \text{m}^{-3}$, and the permittivity
154 ϵ_0 is $8.85 \times 10^{-12} \text{ kg}^{-1} \cdot \text{m}^{-3} \cdot \text{A}^2 \cdot \text{s}^4$.
155

156 B. Monitoring Biomolecules Manipulation With SNT

157 This Section presents the way that the SNT are used as biosensors and points out the measurement sensitivity requirements.
158 This example is illustrated with DNA but the same technique
159 has been applied to gelatin [30] and microtubules [31].
160

161 1) *DNA Bundle Trapping by DEP*: The trapping is achieved
162 by applying an ac electric field ($E = 1 \text{ MV} \cdot \text{m}^{-1}$) between
163 the two opposing tips of the tweezers [32] once partially
164 immersed in the solution. The retrieving of the DNA bundle
165 is routinely performed from a droplet solution [15], [17], and
166 single molecule trapping has even been demonstrated with a pulsed
167 DEP signal [16].

168 In [20], an improved method is proposed in which the tips
169 are introduced into an open fluidic cavity optimized to guaranty
170 a stable meniscus. The SNT position is fixed and the cavity
171 is mounted on a XYZ stage controlled by programmable robot
172 for repeatable and controlled tip immersion, as illustrated in
173 Fig. 2(a)–(c).

174 2) *Real-Time Monitoring*: The SNT characterization allows
175 monitoring of the DNA trapping in real time. The characterization
176 of the SNT in real time allows the monitoring of the DNA
177 trapping. The number of trapped DNA molecules during the
178 DEP is controlled by the evolution of the resonance frequency
179 and quality factor of the system (Tweezers+DNA bundle). The
180 SNT are modeled by a linear second-order model as depicted in
181 Fig. 2(d). The resonance frequency, f_R , and the quality factor

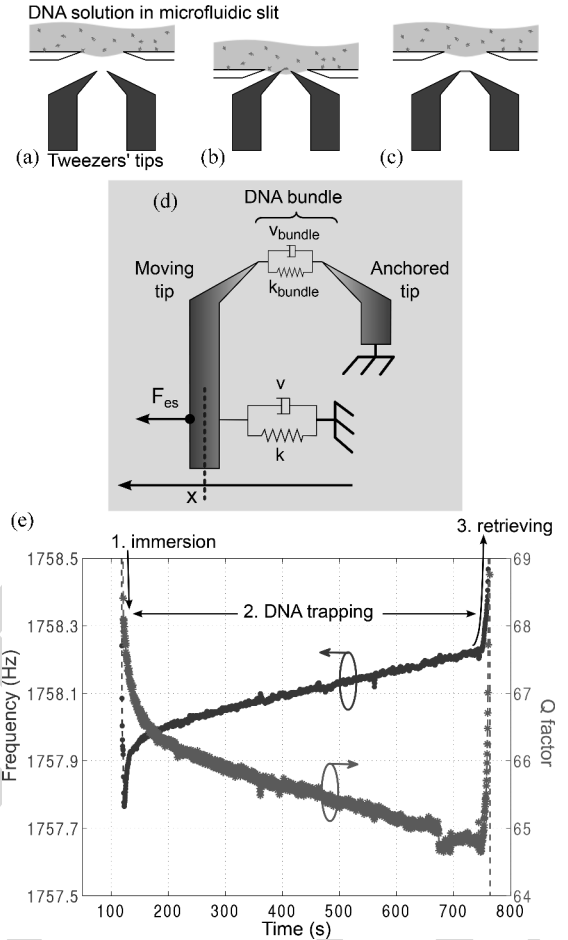


Fig. 2. λ -DNA molecules trapping with SNT. The first three sketches illustrate the key phases of the experiment: (a) the immersion of the tips in the DNA solution, (b) the DNA trapping by DEP, and (c) the retrieving of DNA bundle between the tips. (d) Equivalent dynamic model of the SNT with bundle. (e) Evolution of the SNT+bundle resonance frequency and Q factor during the trapping.

Q of the SNT+bundle damped oscillator are defined by 182

$$f_R(t) = \frac{1}{2\pi} \sqrt{\frac{(k + k_{\text{bundle}}(t))}{M}} \quad (4)$$

$$Q(t) = \frac{\sqrt{(k + k_{\text{bundle}}(t)) \times M}}{(\nu + \nu_{\text{bundle}}(t))} \quad (5)$$

where M is the mass of the movable tip, k is the stiffness of the suspensions, and ν is the equivalent viscous losses. k_{bundle} and ν_{bundle} are the time varying bundle stiffness and viscosity; the mass of the bundle is neglected compared to M . 183 184 185 186

As shown in Fig. 2(e), as the DEP trapping progresses, the system moves to higher resonance frequencies indicating an increase in the bundle stiffness according to (4). At the same time, the signal amplitude at resonance, proportional to the quality factor Q , is decreasing, revealing higher viscous losses ν_{bundle} as the bundle forms, in accordance with (5). 187 188 189 190 191 192

In this experiment, the resonance frequency experiences a shift of 0.4 Hz that corresponds to an increase in the stiffness of $12 \text{ mN} \cdot \text{m}^{-1}$, i.e., a bundle containing $\sim 200 \lambda$ -DNA molecules 193 194 195

[19]. (For the sake of clarity, it should be noted that the SNT used in this trapping experiment has different characteristics than those used in the feedback approach of Sections III and IV)

3) *Sensor Resolution*: Accurate biosensing by SNT requires high-resolution measurements. Thus, special care in signal conditioning (detection of small currents < 10 pA), noise reduction, and control of the experimental conditions (e.g., the stability of the meniscus air/biological liquid/tweezers) have allowed to sense resonance frequency shifts of 25 mHz and quality factor changes of 0.2. Based on this resolution, the minimum change that can be sensed due to the stiffness variation is in the range of 15 molecules of λ -DNA [20].

After trapping, the bundle of molecules is mechanically stimulated (in air or in a biological solution) with a sinusoidal signal at the resonance frequency of the system. Slight changes in the frequency response permit a fine characterization of the evolution of the mechanical properties of the molecule bundle revealing biological interactions.

III. DYNAMIC MODELING AND IMPROVEMENT OF THE PARAMETRIC SENSITIVITY BY A FEEDBACK APPROACH

In this Section, we describe how to reduce the closed-loop resonance frequency of the tweezers in order to improve its sensitivity to molecule stiffness variations.

A. Dynamic Modeling and Open-Loop Sensitivity to Stiffness Variations

1) *Modeling*: Newton's second law applied to the damped oscillator formed by the *SNT+bundle* of Fig. 2(d) can be formulated by

$$\dot{X} = \underbrace{\begin{bmatrix} 0 & 1 \\ -(k + k_{\text{bundle}}(t)) & -(\nu + \nu_{\text{bundle}}(t)) \end{bmatrix}}_A \frac{1}{M} X + \underbrace{\begin{bmatrix} 0 \\ 1 \\ M \end{bmatrix}}_B F_{\text{es}} \quad (6)$$

and

$$y = \underbrace{\begin{bmatrix} 0 & 1 \end{bmatrix}}_C X \quad (7)$$

where $A \in \mathbb{R}^2$, $B \in \mathbb{R}^{(2,1)}$, $C \in \mathbb{R}^{(1,2)}$, X is the state vector (displacement & velocity, $X = \begin{pmatrix} x \\ \dot{x} \end{pmatrix}$), and y is the output vector or the measurement.

2) *Identification*: Prior to the development of an accurate control strategy, the model parameters [M , k , ν , actuation, and sensor gains of (1)–(5)] need to be extracted from device measurements. These parameters are identified through standard recursive approach with least squares method from SNT responses to small signal actuation with a 20 V offset. Identified parameters are summarized in Table II.

TABLE II
THEORETICAL AND IDENTIFIED MODEL PARAMETER VALUES OF THE SNT

Parameters	Theoretical	Identified
Mechanical parameters		
M (kg)	360×10^{-9}	360×10^{-9}
k ($\text{N} \cdot \text{m}^{-1}$)	49.3 ^a	24.9
ν ($\text{Ns} \cdot \text{m}^{-1}$)	-	100×10^{-6}
Comb-drive actuator		
α_{es} ($\text{N} \cdot \text{V}^{-2}$)	29.2×10^{-9a}	35.5×10^{-9}
Capacitive sensor		
β_C ($\text{F} \cdot \text{m}^{-1}$)	412.2×10^{-9a}	227.5×10^{-9}

^a These values are calculated from the designed springs and electrodes widths and does not take into account the significant beam narrowing during etching processes.

3) *Open-Loop Sensitivity to Stiffness Variation*: The minimum number of molecules that can be discerned by resonance frequency shifts due to the molecular stiffness variation is linked to the SNT sensitivity to stiffness variation Δk , i.e.,

$$\frac{\partial f_R}{\partial k} = \frac{1}{4\pi\sqrt{kM}} \text{ or } \frac{1}{8\pi^2 M f_R}. \quad (8)$$

This sensitivity is equal to 26.4 Hz/($\text{N} \cdot \text{m}^{-1}$) with the identified parameters of Table II. It appears that for a given molecular stiffness change, Δk , the effect on the resonance frequency variation Δf_R is enhanced with low SNT stiffness, k , and, therefore, for low resonance frequency f_R . In relation to Fig. 2, the current experimental resolution in the resonant frequency shift measurement is close to 25 mHz, a value that corresponds to $1 \text{ mN} \cdot \text{m}^{-1}$ or 15 λ -DNA molecules. From (8), a reduction of f_R by one order of magnitude; i.e. 2 orders of magnitude for k , would improve the detection threshold down to the single molecule level.

The development of a new MEMS device having a very low stiffness ($< 0.2 \text{ N} \cdot \text{m}^{-1}$) remains extremely challenging in terms of fabrication and operation. Thus, we propose to reduce the overall stiffness by closed-loop control. In such a control strategy, the physical system (i.e., the SNT) remains the same but the closed-loop system *SNT combined with the feedback law* will have the desired designed dynamic behavior.

B. Improvement of the Parametric Sensitivity by a Feedback Approach

1) *Control Strategy*: The control design strategy is depicted in the flowchart of Fig. 3. The aim of the feedback is to reduce the resonance frequency of the closed-loop system. To this end, we use a state feedback eigenstructure assignment in order to assign both the closed-loop resonance frequency and damping factor. This state feedback is then implemented using a Luenberger-kind observer. The originality of the observer we use is that it has to be designed in order to neither deteriorate the closed-loop sensitivity nor amplify the noise.

2) *Eigenstructure Assignment Using State Feedback*: The system is under its controllable canonical state-space representation (A, B, C) given by (6) and (7). The state feedback gain vector $L = (l_1, l_2) \in \mathbb{R}^{(1,2)}$ is designed to assign the desired

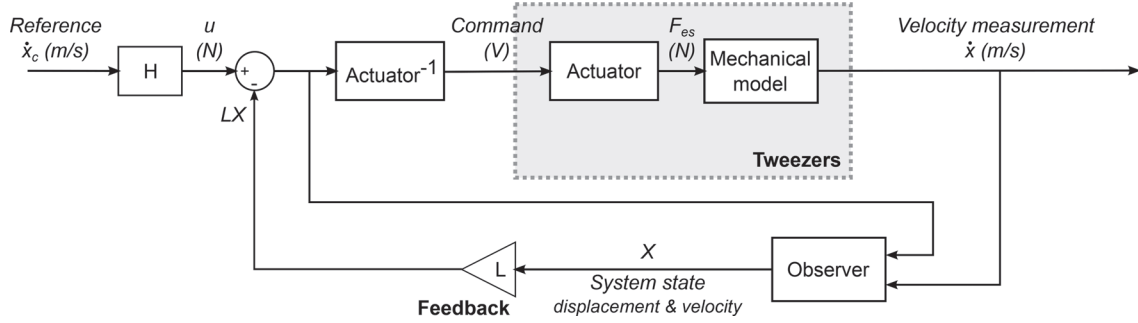


Fig. 3. Feedback control scheme. Gray box, called *Tweezers*, comprises the mechanical and the actuator models. Actuator square law needs to be inverted in order to linearize the control signal. The state feedback gain vector L and the feedforward gain H are designed to assign the desired closed-loop poles and to ensure unitary static gain. The observer is a Luenberger observer reconstructing the state of the system X for the state feedback. The sensor model is not shown here. The device output voltage is converted into the tip velocity by dividing by the sensor gain $1/\beta_C$.

TABLE III
POLE PLACEMENT OF THE CLOSED-LOOP SYSTEM AND STATE FEEDBACK GAIN L ACCORDING TO THE RESONANCE FREQUENCY REDUCTION n

Freq.	L ($[N \cdot m^{-1}], [Ns \cdot m^{-1}]$)	Poles
f_R	(0, 0)	$-138.9 \pm 8.3 \times 10^3 i$
$f_R/1.2$	$(-7.6, -16.7 \times 10^{-6})$	$-115.7 \pm 6.9 \times 10^3 i$
$f_R/2.0$	$(-18.7, -50.0 \times 10^{-6})$	$-69.4 \pm 4.2 \times 10^3 i$
$f_R/3.2$	$(-22.5, -68.8 \times 10^{-6})$	$-43.4 \pm 2.6 \times 10^3 i$
$f_R/5.0$	$(-23.9, -80.0 \times 10^{-6})$	$-27.8 \pm 1.7 \times 10^3 i$
$f_R/10$	$(-24.7, -90.0 \times 10^{-6})$	$-13.9 \pm 0.8 \times 10^3 i$

272 closed-loop poles. The actuation of the system F_{es} is equal to
273 $u - LX$, and the closed-loop system model becomes

$$\dot{X} = \begin{bmatrix} 0 & 1 \\ -\frac{k+l_1}{M} & -\frac{\nu+l_2}{M} \end{bmatrix} X + \begin{bmatrix} 0 \\ \frac{1}{M} \end{bmatrix} u. \quad (9)$$

274 Thus, the closed-loop resonance frequency and sensitivity are
275 given by

$$f_{R-cl} = \frac{1}{2\pi} \sqrt{\frac{(k+l_1)}{M}} \quad (10)$$

$$\frac{\partial f_{R-cl}}{\partial k} = \frac{1}{4\pi \sqrt{(k+l_1)M}} = \frac{1}{8\pi^2 M f_{R-cl}}. \quad (11)$$

276 The state feedback gain L is, then, determined in order to
277 assign the closed-loop poles in such a way that the system
278 resonates at a lower frequency than the natural frequency of
279 the tweezers (see Table III). L is computed to scale down this
280 resonant frequency (initially 1323.6 Hz), by a given factor de-
281 pending on the desired sensitivity enhancement while keeping
282 the damping factor unchanged.

283 When the closed-loop resonance frequency is scaled down by
284 a factor n compared to the SNT natural resonance frequency,
285 the sensitivity to small stiffness variation is improved with the
286 same ratio according to (11). Fig. 4 shows the computed fre-
287 quency shift of the closed-loop system caused by a signifi-
288 cant stiffness variation for different frequency scaling down
289 factor, n .

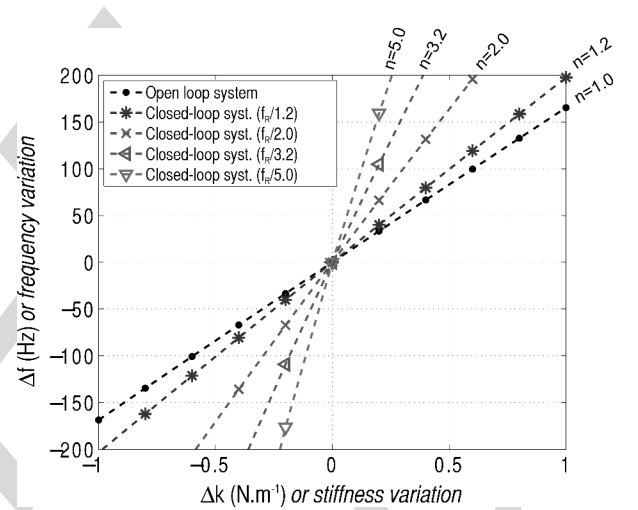


Fig. 4. Evolution of the resonance frequency shift Δf_R and Δf_{R-cl} with the added stiffness Δk variation for different frequency reduction ratio n .

C. Observer Design

290 The implementation of the aforementioned state feedback is
291 done through the use of a Luenberger observer. The observer is
292 designed such that it preserves the good closed-loop sensitivity
293 to parametric variations and it does not amplify too much noise.
294

295 The poles of the observer have then to be carefully chosen.
296 We used the following guidelines.

- 297 (1) The observer is designed to be faster than the original
298 device—with poles at least two times faster than the poles
299 of the system in open loop (cf., Table III).
- 300 (2) The observer poles are chosen not too fast in order to
301 avoid excessive noise amplification.
- 302 (3) The final location of the observer poles is chosen in or-
303 der to preserve the sensitivity of the system to stiffness
304 variations (by using root locus).

305 In the following section, simulations illustrate 1) the relevance
306 of the method and 2) the importance of the design of the observer
307 towards the enhancement of the sensitivity.

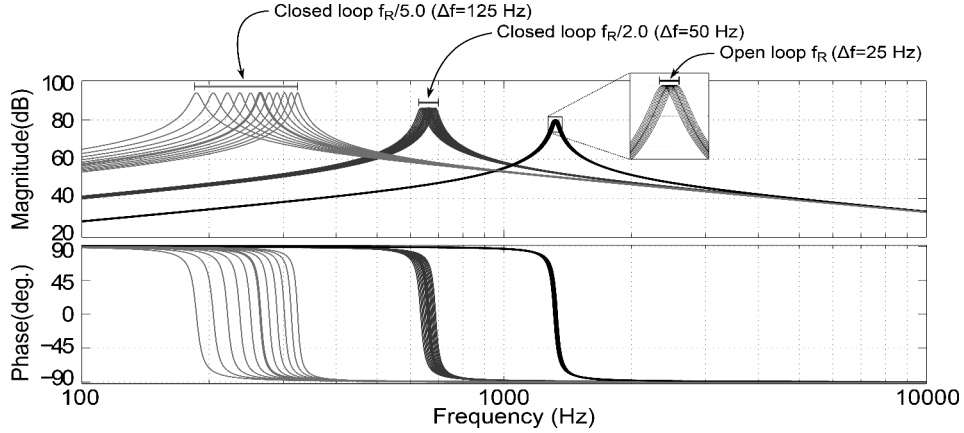


Fig. 5. Simulated frequency results for open-loop driven system and two different closed-loop systems. The three systems undergo a variation of k the system stiffness. k vary from -0.5 to 0.5 N/m by 0.1 N/m step. In black line, the tweezer transfer function, i.e., the open-loop system. In blue line, the implemented ($n = 2$) closed-loop system transfer function. The system resonates at a frequency twice lower. In red line, the implemented ($n = 5$) closed-loop system transfer function. The system resonates at a frequency five times lower.

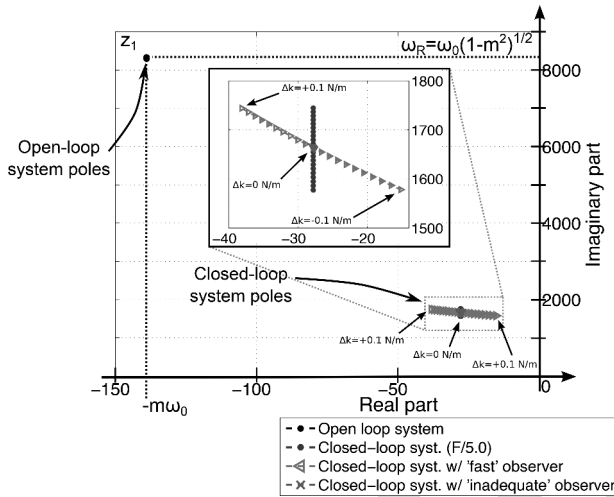


Fig. 6. Root locus of the closed-loop systems with $n = 5$ and with different observers. Complex conjugate poles are plotted in the positive imaginary half s plan. The parameter k varies from -0.1 to 0.1 N/m by 0.01 N/m steps. The black dots show the poles of the open-loop driven system. The ideal pole path of the closed-loop system due to the k variations are plotted with blue dots (i.e., no observer). For different observers designs, poles dependencies (and equivalent resonance frequency $\omega_R = 2\pi f_R$ and damping m) on the tweezers stiffness k are shown by red triangle and green cross plots. NB: Green cross dots path are imperceptible since they are at this scale all concentrate in the middle.

308 D. Simulation Results

309 From parameters identified in Table II, the feedback scheme
310 (see Fig. 3) has been first programmed and tested under MAT-
311 LAB/Simulink without implementing the observer (i.e., all the
312 state variables are supposed to be measured). Fig. 5 compares
313 the resonance frequency shift between the open-loop driven sys-
314 tem and the closed-loop systems computed for two resonance
315 frequency reduction factors set to $n = 2$ and 5 . As expected, the
316 sensitivity of the closed-loop system is improved proportionally
317 to n .

318 In a second instance, we implemented two different kinds of
319 observers (with different dynamics); Fig. 6 illustrates two cases.

TABLE IV
POLE PLACEMENT OF THE OBSERVER FOR STATE FEEDBACK IMPLEMENTATION

Open-loop system (Tweezers)	$z_{1,2} = -138.9 \pm 8.3 \times 10^3 i$
closed-loop system ($n = 5$)	$z_{1,2} = -27.8 \pm 1.7 \times 10^3 i$
Observer 1 (<i>fast</i>)	$z_{1,2} = -277.8 \pm 0.2 \times 10^3 i$
Observer 2 (<i>slow</i>)	$z_{1,2} = -27.8 \pm 0.2 \times 10^3 i$
Observer 3 (<i>inadequate</i>)	$z_{1,2} = -277.8 \pm 16.7 \times 10^3 i$

This Figure shows that the root locus (due to k parameter varia- 320
tions) is deformed by the observer dynamics. The sensitivity of 321
the resonance frequency to stiffness variations is given by the 322
amplitude of the imaginary axis variations. Table IV reports the 323
poles of the observers for which simulation results are presented. 324

In the insert of Fig. 6, the poles of the system implemented 325
with the observer named *inadequate* do not move compared to 326
the ideal root locus (ideal state feedback case). If the poles of 327
the observer are inappropriately chosen, the sensitivity of the 328
poles to variations of k is significantly reduced. Observer poles 329
close to the real axis, i.e., with an imaginary part close to 0, 330
are the more appropriate to reconstruct the displacement state 331
and to preserve the sensitivity of the system to variations of k . 332
The so-called *fast* observer allows us to increase the resonance 333
frequency shift ($\omega_R = 2\pi f_R$ in Fig. 6) close to the performances 334
theoretically expected. 335

IV. EXPERIMENTAL APPLICATION FOR EXTRA-STIFFNESS CHARACTERIZATION

The previous method has been experimentally tested with the 338
mechanical characterization of biomolecules of *fibronectin*. The 339
physical sensor is the SNT, while the feedback controller and 340
the observer are implemented in a dSPACE prototyping board. 341

A. Experimental Protocol

Fibronectin is a protein, 100 nm in length, of the extracel- 343
lular matrix which plays a major role in cell adhesion, growth, 344

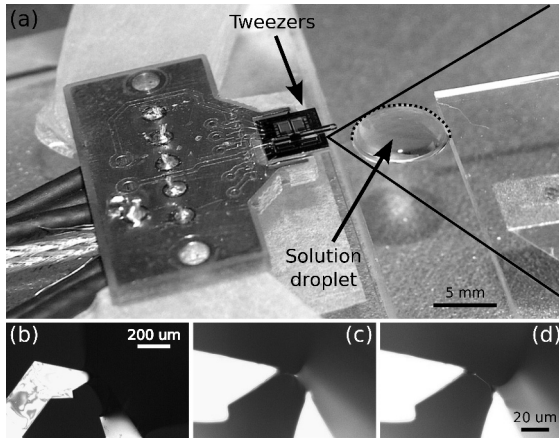


Fig. 7. Experimental setup for the trapping of *fibronectin* molecules. (a) The SNT are fixed. A droplet of *fibronectin* solution is dropped on a glass slice which is mounted on the XYZ stage. (b)–(d) Sequences of the molecule combing. Under optical microscopy, the tweezers' tips are retrieved from the solution meniscus. A bundle of *fibronectin* is released between the tips.

345 migration, and differentiation [33]. These molecules are used to
 346 polymerize the fibrillar matrix at the surface of the cell. We have
 347 used this property to trap by combing a bundle of *fibronectin*.
 348 After immersion of the tips in the solution, they are retrieved by
 349 the side forming a polymerized bundle of molecules (see Fig. 7).

350 The closed-loop systems are evaluated with *fibronectin* bundle
 351 in air, in dry condition. This stable condition was preferred
 352 to the immersed one as we can avoid any effect of liquid surface
 353 tension stability that can interfere with the sole system response.
 354 For each case, a sinusoidal reference of $0.02 V_{AC}$ with an offset
 355 of $20 V_{DC}$ is applied to the system and the oscillation frequen-
 356 cies are spanned from 1000 to 2000 Hz with a step of 2 Hz.
 357 Then, the measurement is performed with the lock-in amplifier.

358 Two sets of experiments have been performed with two differ-
 359 ent bundles. From the resonance frequency shifts in open loop,
 360 the stiffness of the first bundle is $11.8 N \cdot m^{-1}$, and $4.3 N \cdot m^{-1}$
 361 for the second one. In total, 22 resonance responses are recorded
 362 and compared.

363 B. Results and Discussions

364 1) *Experimental Results*: Fig. 8 shows the frequency re-
 365 sponses of four different systems with and without a bundle
 366 of *fibronectin*.

367 In Fig. 8(a), the responses of the open-loop driven SNT are
 368 given. The dashed-line curve is the reference curve, which cor-
 369 responds to the frequency response of the SNT alone driven in
 370 open loop. The solid line is the response of the tweezers with
 371 the bundle trapped in between the tips. The resonance frequency
 372 increases and the amplitude decreases due to the additional stiff-
 373 ness and loss contributions of the molecules. In Fig. 8(b)–(d),
 374 the results of the closed-loop systems with desired reduction
 375 ratios are of 1.1, 1.2 and 1.5.

376 Resonance responses are fitted with a second-order resonator
 377 model and characteristics such as the resonance frequency are
 378 extracted. The Table V sums up the resonance frequencies for
 379 the experiment with/without the first bundle for five different

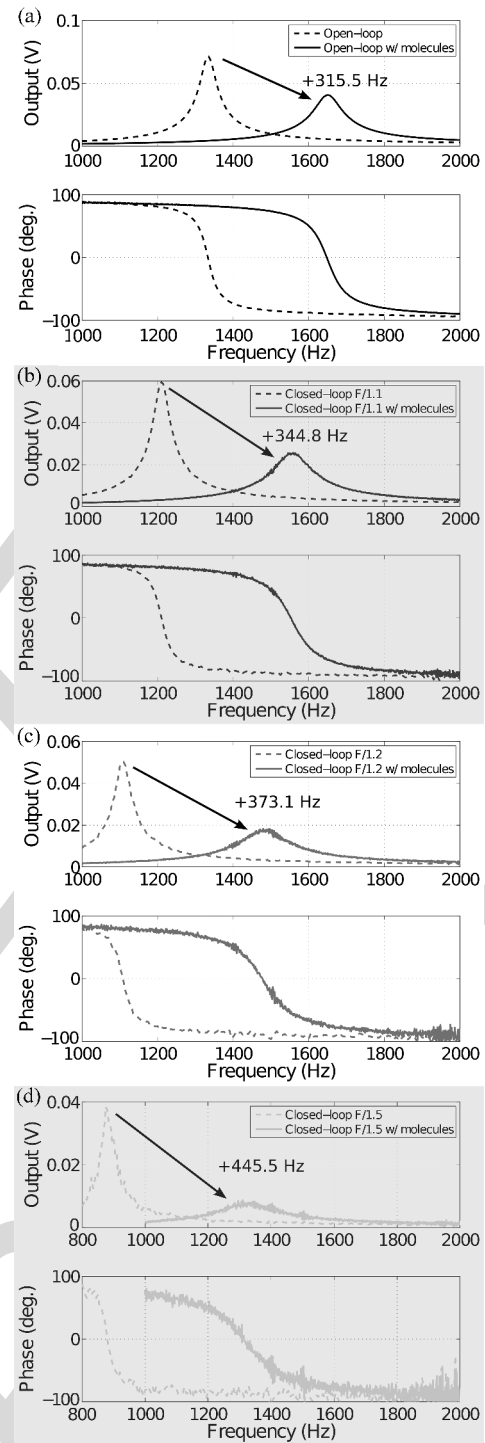


Fig. 8. Frequency responses of different implemented closed-loop systems for $0.02 V$ sinusoidal signal with an offset of $20 V$. In dotted lines, the responses of the systems with the bundle of molecules. In plain lines, the responses of the systems without molecules. (a) Open-loop driven systems. (b) Closed-loop systems implemented with $f_R/1.1$. (c) Closed-loop systems implemented with $f_R/1.2$. (d) Closed-loop systems with $f_R/1.5$.

380 systems. Through reducing the resonance frequency of the sys-
 381 tem, the measured results prove the increase of the frequency
 382 shift due to the extra-stiffness of the bundle.

383 Moreover Fig. 9 tends to 1) confirm with second set of exper-
 384 iments, the enhancement of the sensitivity through the proposed

TABLE V
EXPERIMENTAL RESULTS FOR THE BUNDLE 1 (RELATED TO FIG. 8)

System (n)	Freq. w/o bundle (Hz)	Freq. w/ bundle (Hz)	Freq. shift (Hz)
Open loop (1.0)	1333.6	1649.1	+315.5
Closed-loop (1.1)	1211.1	1555.9	+344.8
Closed-loop (1.2)	1109.1	1482.2	+373.1
Closed-loop (1.5)	881.9	1327.4	+445.5
Closed-loop (2.0)	653.9	1175.6	+521.7

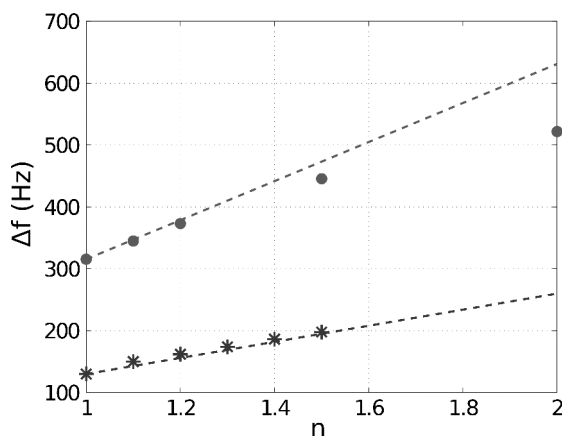


Fig. 9. Synthesis of the experimental results. In abscissa, is informed the reduction factor (n) applied to the resonance frequency for the closed-loop system. The shift Δf is the resonance frequency difference due to the presence of the bundle of molecules in between the tips. The dotted lines represent the theoretical improvement expected. The two sets of results obtained with two different bundles are plotted. The green dots correspond to the experiment of Fig 8 and Table V (bundle of $11.8 \text{ N} \cdot \text{m}^{-1}$ stiffness). The blue stars corresponds to the second bundle of $4.3 \text{ N} \cdot \text{m}^{-1}$ stiffness.

385 feedback strategy and 2) show a good agreement with the theory.
386 Small differences are discussed in the following section.

387 2) *Discussion*: The synthesis of Fig. 9 demonstrates the en-
388 hancement of the frequency shifts in agreement with the theory.
389 However, the enhancement for closed-loop system with a reduc-
390 tion factor of 2.0 is lower than expected, and the implementation
391 for a higher reduction factor has not been achieved. In this case,
392 the system tends to become unstable. We mainly associated this
393 phenomenon to the observer implementation and to the presence
394 of some delays in the feedback loop.

395 We have demonstrated previously that observer's dynamic
396 changes the root locus of system's poles, the behavior of the
397 closed-loop system under parameter variations and impacts the
398 performances of the method. Furthermore, delays have been
399 characterized due to D/A and A/D converters dead times and to
400 the phase shift brought by the current amplifiers. Current works
401 are devoted to the precise characterization of such delays and to
402 their integration in the control design process.

V. CONCLUSION AND FUTURE WORK

404 The present study investigates the relevance of a feedback
405 approach in the improvement of the performances of MEMS
406 tweezers for the detection and the mechanical characterization of
407 biological molecules. An improvement of 50% on the sensitivity

to mechanical stiffness variations has been reached theoretically
and experimentally on *fibronectin* bundles through the emulation
of a more compliant system. The stiffness have been brought
down to 10.5 N/m instead of originally 24.5 N/m .

The main drawback of this approach is the sensitivity of
the observer to the stiffness variations. Unusually, the poles of
the observer are uncontrollable resulting from the separation
principle. During the sensing of the molecules stiffness, the
separation principle is not satisfied anymore and the observer
poles can become unstable. We are actually working on self-
scheduled observers aiming at adapting the observer dynamics
with respect to sensed parametric variations. It should solve the
main drawback of this promising approach and allows us to go
one step further into the theoretical closed-loop performances.

ACKNOWLEDGMENT


The authors would like to thank A. W. Coleman from the
University of Lyon, Lyon, France, for the discussion and proof-
reading of the text. The photolithography masks were fabricated
with the 8-inch EB writer F5112+VD01 donated by Advantest
Corporation to the VLSI Design and Education Center (the Uni-
versity of Tokyo).

REFERENCES

- [1] C. Bustamante, J. Marko, E. Siggia, and S. Smith, "Entropic elasticity of lambda-phage DNA," *Science*, vol. 265, no. 5178, pp. 1599–1600, 1994.
- [2] P. Cluzel, A. Lebrun, C. Heller, R. Lavery, J. L. Viovy, D. Chate-
nay, and F. Caron, "DNA: An extensible molecule," *Science*, vol. 271,
no. 5250, pp. 792–794, Feb. 1996.
- [3] G. Bao, "Mechanics of biomolecules," *J. Mech. Phys. Solids*, vol. 50, no.
11, pp. 2237–2274, 2002.
- [4] A. Bancaud, N. Conde E Silva, M. Barbi, G. Wagner, J.-F. Allemand,
J. Mozziconacci, C. Lavelle, V. Croquette, J.-M. Victor, A. Prunell, and
J.-L. Viovy, "Structural plasticity of single chromatin fibers revealed by
torsional manipulation," *Nat. Struct. Mol. Biol.*, vol. 13, no. 5, pp. 444–450,
May. 2006.
- [5] J. Gore, Z. Bryant, M. D. Stone, M. Nöllmann, N. R. Cozzarelli, and
C. Bustamante, "Mechanochemical analysis of DNA gyrase using rotor
bead tracking," *Nature*, vol. 439, no. 7072, pp. 100–104, Jan. 2006.
- [6] O. Chaudhuri, S. H. Parekh, and D. A. Fletcher, "Reversible stress soft-
ening of actin networks," *Nature*, vol. 445, no. 7125, pp. 295–298, Jan.
2007.
- [7] T. Hawkins, M. Mirigian, M. S. Yasar, and J. L. Ross, "Mechanics of
microtubules," *J. Biomech.*, vol. 43, no. 1, pp. 23–30, Jan. 2010.
- [8] F. Amblard, B. Yurke, A. Pargellis, and S. Leibler, "A magnetic ma-
nipulator for studying local rheology and micromechanical properties of
biological systems," *Rev. Sci. Instrum.*, vol. 67, no. 3, pp. 818–827, 1996.
- [9] C. Gosse and V. Croquette, "Magnetic tweezers: Micromanipulation and
force measurement at the molecular level," *Biophys. J.*, vol. 82, no. 6,
pp. 3314–3329, 2002.
- [10] R. Simmons, J. Finer, S. Chu, and J. Spudich, "Quantitative measurements
of force and displacement using an optical trap," *Biophys. J.*, vol. 70,
no. 4, pp. 1813–1822, 1996.
- [11] E. L. Florin, V. T. Moy, and H. E. Gaub, "Adhesion forces between
individual ligand-receptor pairs," *Science*, vol. 264, no. 5157, pp. 415–
417, Apr. 1994.
- [12] A. Ishijima, T. Doi, K. Sakurada, and T. Yanagida, "Sub-piconewton force
fluctuations of actomyosin in vitro," *Nature*, vol. 352, pp. 301–306, 1991.
- [13] F. Beyeler, A. Neild, S. Oberti, D. Bell, Y. Sun, J. Dual, and B. Nelson,
"Monolithically fabricated microgripper with integrated force sensor for
manipulating microobjects and biological cells aligned in an ultrasonic
field," *J. Microelectromech. Syst.*, vol. 16, no. 1, pp. 7–15, 2007.
- [14] K. Kim, X. Liu, Y. Zhang, and Y. Sun, "Nanonewton force-controlled
manipulation of biological cells using a monolithic MEMS microgripper
with two-axis force feedback," *J. Micromech. Microeng.*, vol. 18, no. 5,
p. 055013, Apr. 2008.
- [15] G. Hashiguchi, T. Goda, M. Hosogi, K. Hirano, N. Kaji, Y. Baba,
K. Kakushima, and H. Fujita, "DNA manipulation and retrieval from

474 an aqueous solution with micromachined nanotweezers," *Anal. Chem.*,
475 vol. 75, no. 17, pp. 4347–4350, 2003.

- 476 [16] M. Kumemura, D. Collard, N. Sakaki, C. Yamahata, M. Hosogi,
477 G. Hashiguchi, and H. Fujita, "Single-DNA-molecule trapping with silicon
478 nanotweezers using pulsed dielectrophoresis," *J. Micromech. Microeng.*,
479 vol. 21, p. 054020, 2011.
- 480 [17] C. Yamahata, D. Collard, B. Legrand, T. Takekawa, M. Kumemura,
481 G. Hashiguchi, and H. Fujita, "Silicon nanotweezers with subnanometer
482 resolution for the micromanipulation of biomolecules," *J. Microelectromech. Syst.*,
483 vol. 17, no. 3, pp. 623–631, 2008.
- 484 [18] M. Kumemura, D. Collard, S. Yoshizawa, D. Fourmy, N. Lafitte, L. Jalabert,
485 S. Takeuchi, T. Fujii, and H. Fujita, "Direct bio-mechanical sensing of
486 enzymatic reaction on DNA by silicon nanotweezers," in *Proc. IEEE 23rd Int. Conf. Microelectromech. Syst.*, Hong Kong, 2010, pp. 915–918.
- 487 [19] S. Smith, Y. Cui, and C. Bustamante, "Overstretching B-DNA: The
488 elastic response of individual double-stranded and single-stranded DNA
489 molecules," *Science*, vol. 271, no. 5250, pp. 795–799, 1996.
- 490 [20] N. Lafitte, M. Kumemura, L. Jalabert, D. Collard, and H. Fujita, "Real-
491 Time sensing of molecule binding on DNA with silicon nanotweezers,"
492 in *Proc. 15th Int. Conf. Miniaturized Syst. Chem. Life Sci.*, Seattle, WA,
493 USA, 2011, pp. 389–372.
- 494 [21] Y. Sun, B. J. Nelson, D. P. Potasek, and E. Enikov, "A bulk microfabricated
495 multi-axis capacitive cellular force sensor using transverse comb drives,"
496 *J. Micromech. Microeng.*, vol. 12, no. 6, p. 832, 2002.
- 497 [22] W. Tang, T.-C. H. Nguyen, and R. Howe, "Laterally driven resonant micro-
498 structures," *Sens. Actuators*, vol. 20, pp. 25–32, 1989.
- 499 [23] G. Zhou and P. Dowd, "Tilted folded-beam suspension for extending the
500 stable travel range of comb-drive actuators," *J. Micromech. Microeng.*,
501 vol. 13, no. 2, pp. 178–183, 2003.
- 502 [24] T. Clark and R. Howe, "An integrated CMOS micromechanical resonator
503 high-Q oscillator," *IEEE J. Solid-State Circuits*, vol. 34, no. 4, pp. 440–
504 455, Apr. 1999.
- 505 [25] R. Legtenberg, A. Groeneveld, and M. Elwenspoek, "Comb-drive actuators
506 for large displacements," *J. Micromech. Microeng.*, vol. 6, no. 3, pp.
507 320–329, 1996.
- 508 [26] Y. Sun, S. N. Fry, D. P. Potasek, D. J. Bell, and B. J. Nelson, "Char-
509 acterizing fruit fly flight behavior using a microforce sensor with a new
510 comb-drive configuration," *J. Micromech. Syst.*, vol. 14, pp. 4–11, 2005.
- 511 [27] M. Bao, *Analysis and Design Principles of MEMS Devices*. New York,
512 NY, USA: Elsevier, 2005.
- 513 [28] N. Lafitte, "Modeling and control of MEMS tweezers for the character-
514 izations of enzymatic reactions on DNA molecules," Ph. D. dissertation,
515 Université de Franche-Comté, Besançon, France, Apr. 2012.
- 516 [29] D. Collard, C. Yamahata, B. Legrand, T. Takekawa, M. Kumemura, N.
517 Sakaki, G. Hashiguchi, and H. Fujita, "Towards mechanical characteri-
518 zation of biomolecules by MNEMS tools," *IEEJ Trans. Electr. Electron.
519 Eng.*, vol. 2, no. 3, pp. 262–271, Apr. 2007.
- 520 [30] D. Collard, "Silicon nanotweezers for biomechanical and bioelectrical
521 assays," *Front. Biosci.*, vol. E5, pp. 955–965, Jun. 2013.
- 522 [31] M. C. Tarhan, D. Collard, L. Jalabert, M. Kumemura, N. Lafitte, Q. De-
523 loupée, S. L. Karsten, and H. Fujita, "Continuous real-time monitoring of
524 molecular detection by silicon nanotweezers-integrated microfluidic de-
525 vice," in *Proc. 16th Int. Conf. Miniaturized Syst. Chem. Life Sci.*, Okinawa,
526 Japan, Oct. 2012, pp. 1897–1899.
- 527 [32] M. Washizu and O. Kurosawa, "Electrostatic manipulation of DNA
528 in microfabricated structures," *IEEE Trans. Ind. Appl.*, vol. 26, no. 6,
529 pp. 1165–1172, Nov/Dec. 1990.
- 530 [33] R. Pankov, "Fibronectin at a glance," *J. Cell Sci.*, vol. 115, no. 20,
531 pp. 3861–3863, Oct. 2002.

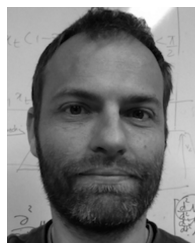
533  **Nicolas Lafitte** was born in Bordeaux, France, in
534 1983. He received the M.S. degree in electrical en-
535 gineering speciality microelectronics from the Uni-
536 versity of Bordeaux I, Bordeaux, and the gradu-
537 ate school ENSEIRB, Talence, France, in 2008, and
538 the Ph.D. degree from the University of Franche-
539 Comté, Besançon, France, for his work on the mod-
540 eling and the control of MEMS tweezers for char-
541 acterizations of bioreactions on DNA molecules,
542 in 2012. This work was conducted in LIMMS
543 (CNRS/University of Tokyo joint laboratory) and in
544 FEMTO-ST (CNRS/University of Franche-Comté). This publication deals with
545 this work.

546 His current research interests include the MEMS integration for biological
547 applications at the University of Tokyo, Japan.
548



Yassine Haddab received the Engineering degree
549 in electrical engineering from the University of
550 Tizi-Ouzou, Tizi-Ouzou, Algeria, the M.S. degree
551 from ENSMM, Besançon, France, and the Ph.D.
552 degree from the University of Franche-Comté,
553 Besançon, France, in 2000. In 2012, he received the
554 Habilitation a Diriger de Recherches degree from the
555 University of Franche-Comté, Besançon.

556 Since 2002, he is an Associate Professor with
557 ENSMM, Besançon, where he teaches control, mi-
558 cro-robotics and embedded real-time systems. His re-
559 search interests include the design, modeling, and control of high precision
560 microrobots and microsystems. He also contributes to the development of mi-
561 crofactory concepts and new microrobots architectures.
562
563



Yann Le Gorrec (M'XX) was graduated as en-
564 gineer in "control, electronics, computer engineer-
565 ing" from the National Institute of Applied Sciences,
566 Toulouse, France, in 1995, and received the Ph.D. de-
567 gree from the National Higher School of Aeronautics
568 and Aerospace, Supaero, Toulouse, in 1998.

569 From 1999 to 2008, he was an Associate
570 Professor in Automatic Control at the Laboratory of
571 Control and Chemical Engineering of Lyon Claude
572 Bernard University, Villeurbanne, France. He worked
573 on port Hamiltonian systems and their use for the
574 modeling and control of irreversible and distributed parameter systems with
575 an application to physico-chemical processes. Since september 2008, he has
576 been a Professor with the National Engineering Institute in Mechanics and
577 Microtechnologies. He is currently the Head of the COntrol DEsign research
578 group of FEMTO-ST AS2M department. His research interests include robust
579 control and self scheduled controller synthesis, the control of nonlinear sys-
580 tems, irreversible and infinite dimensional systems with an application to smart
581 material-based actuators, and microactuators.
582
583



Hervé Guillou was born in Grenoble, France, in
584 1973. He studied fundamental physics from the
585 University of Grenoble Alpes, Grenoble, France, the
586 University of Karlsruhe, Karlsruhe, Germany, and
587 at New York University, New York, NY, USA. He
588 received the Master of Sciences degree from New
589 York University and then a Ph.D. degree from the
590 university of Grenoble Alpes, in 1998 and 2001,
591 respectively.

592 Since 2004, he has been a Lecturer in physics with
593 the University of Grenoble Alpes. His first research
594 interests include low temperature physics and superconductivity. He moved,
595 then, to the field of biophysics where his interests include cell architecture, cell
596 and molecular force generation, and development of methods, instruments, and
597 microsystems for applications in biophysics and medicine.
598
599



Momoko Kumemura was born in Kagoshima,
600 Japan, in 1975. She received the M.S. degree in
601 engineering from Tokushima University, Tokushima,
602 Japan, and the Ph.D. degree in chemistry from Tokyo
603 Metropolitan University, Tokyo, Japan, in 2002 and
604 2005, respectively.

605 She was a Postdoctoral Researcher in the Cen-
606 ter for International Research on Micromechatronics,
607 Institute of Industrial Science, The University of
608 Tokyo (2005–2008 and 2010–2013) and in the
609 Centre National de la Recherche Scientifique (2008–
610 2010). Since 2014, she has been a Research Associate in Institute of Industrial
611 Science, The University of Tokyo. She is currently researching microsystems
612 for biological applications.
613
614

615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632



Laurent Jalabert was born in L'Union, France, in 1974. He received the Ph.D. degree from the Université Paul Sabatier, Toulouse, France, in 2001 dealing with CMOS gate engineering and reliability of ultrathin oxides.

From 2001 to 2003, he joined LIMMS (CNRS-University of Tokyo joint laboratory), Tokyo, Japan, as a Postdoctoral Researcher granted from the Japanese Society for the Promotion of Science and he developed piezoresistive MEMS cantilever for metrology in high aspect ratio microholes. In 2004, he joined Institut National Polytechnique de Toulouse as a Research Engineer and worked at Laboratoire d'Analyse et d'Architecture des Systèmes on ICP-RIE plasma and alternative technologies (nanoimprint). Since 2007 till now, he is with the LIMMS as a CNRS Research Engineer hosted in Pr. Hiroyuki Fujita laboratory. His current research interests include atomic heat transfer in UHV-TEM and MEMS fabrication (silicon nanotweezers, liquid-cells, etc.).

633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656



Dominique Collard (M'01) was born in Cambrai, France in 1958. He received the Engineering Degree from the Institut Supérieur d'Electronique et du Numérique and the Ph.D. degree from the University of Lille, Lille, France, in 1980 and 1984, respectively.

From 1985 to 1986, he was with the Toshiba VLSI Research Center, Kawasaki, Japan, as a Visiting Scientist. Since 1988, he has been with the Centre National de la Recherche Scientifique (CNRS), being alternatively with the Institut d'Electronique, de Microélectronique et de Nanotechnologie, Lille, and with the Laboratory for Integrated MicroMechatronic Systems (LIMMS/CNRS-IIS), Tokyo. From August 2005, he joint a second time LIMMS where he was appointed as a Director in September 2007 and got a title of Project Professor of IIS, the University of Tokyo, Tokyo, Japan. In December 2011, he became Coordinator of EC/FP7 INCOLAB: EUJO-LIMMS aiming to open LIMMS to European partners and first EC laboratory in Japan. His current scientific interests include micro- and nano-systems for applications in biology and nanotechnology. He is the Author or Coauthor of more than 250 international publications.

Dr. Collard received the IBM price on intensive numerical calculation in 1990, the CNRS bronze medal in 1992, and was in 2004 Laureate of the French academic palms from Ministry of Higher Education and Research.



Hiroyuki Fujita (S'76-M'80) received B.S., M.S., and Ph.D. degrees in electrical engineering from the University of Tokyo, Tokyo, Japan, in 1975, 1977, and 1980, respectively.

Since 2000, he is the Director of the Center for International Research on MicroMechatronics. Also, since 1993, he has been a Professor, an Associate Professor (1981-1993), and a Lecturer (1980-1981) with the Institute of Industrial Science, the University of Tokyo. He is currently involved in the investigation of microelectromechanical systems fabricated by IC-based processes and applications to optics, hard disk drives, and bio/nanotechnology. His research interest includes autonomous distributed microsystems.

Dr. Fujita is a Fellow of the Institute of Electrical Engineers of Japan.

657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672

QUERIES

- Q1. Author: Please provide the full page range in Refs. [14], [16], and [21].
Q2. Author: Please provide the department name in Ref. [28].
Q3. Author: Please provide the year in which the author “Yann Le Gorrec” became a Member of the IEEE.

673

674

675

676

IEEE
Proof