CONTINUOUS REAL-TIME MONITORING OF MOLECULAR DETECTION BY SILICON NANOTWEEZERS-INTEGRATED MICROFLUIDIC DEVICE

Mehmet C. Tarhan,[1] Dominique Collard,[1,2] Laurent Jalabert,[2] Momoko Kumemura,[2] Nicolas Lafitte,[2] Quentin Delouvee,[1] Stanislav L. Karsten[3] and Hiroyuki Fujita[1]

[1]CIRMM, IIS, The University of Tokyo, JAPAN,
[2]LIMMS/CNRS-IIS, The University of Tokyo, JAPAN
[3]NeuroInDx, Inc., USA

ABSTRACT

Molecular detection devices for early-diagnosis require high-sensitivity and reliability. However, different detection methods have limitations preventing them to achieve continuous, real-time detection with standardized results especially when working in liquid environment. Here, we have demonstrated continuous, real-time monitoring of microtubule (MT) capturing in liquid by integrating silicon nanotweezers (SNTs) with microfluidics. These results pave the way to molecular detection by outputting electrical signals as a reliable indicator to standardize the detection.

KEYWORDS

Silicon nano tweezers, molecular detection, microtubules, tau

INTRODUCTION

There have been numerous successful attempts to build functional-molecule-based microfluidic devices. However, issues on robustness, standardization and reliability of the detection mechanism are not cleared. Fluorescence imaging damages molecules preventing from real-time long-period monitoring while immunoassays suffers standardization problem at low concentrations and electrical measurements have performance limitation in buffer environment.

Molecular biomechanical detection with the integrated silicon nanotweezers (SNTs) is a promising approach as already demonstrated in air [1] and in liquid [2]. Yet, performing all steps of the experiment in liquid is still a great challenge, which requires very stable and reliable microfluidic integration. In-liquid experiments show lower sensitivity when compared to those in air. However, high sensitivity is crucial to detect biomarkers at very low concentrations for diagnostic purposes.

Here, we proposed a method for easy-integration of SNTs with microfluidics while maximizing the sensitivity. A PDMS channel was placed on the edge of a cover slip, with a lateral opening for SNT tips to enter (Figure 1a). Due to the design, the area of the SNT tips exposed to liquid was minimized for achieving higher sensitivity. After capturing necessary molecules, input solution could be exchanged (with an integrated syringe pump) for detection. The result was monitored in real time and continuously as electrical signals (Figure 1b) showing the change in resonance frequency and quality factor (Q-factor).

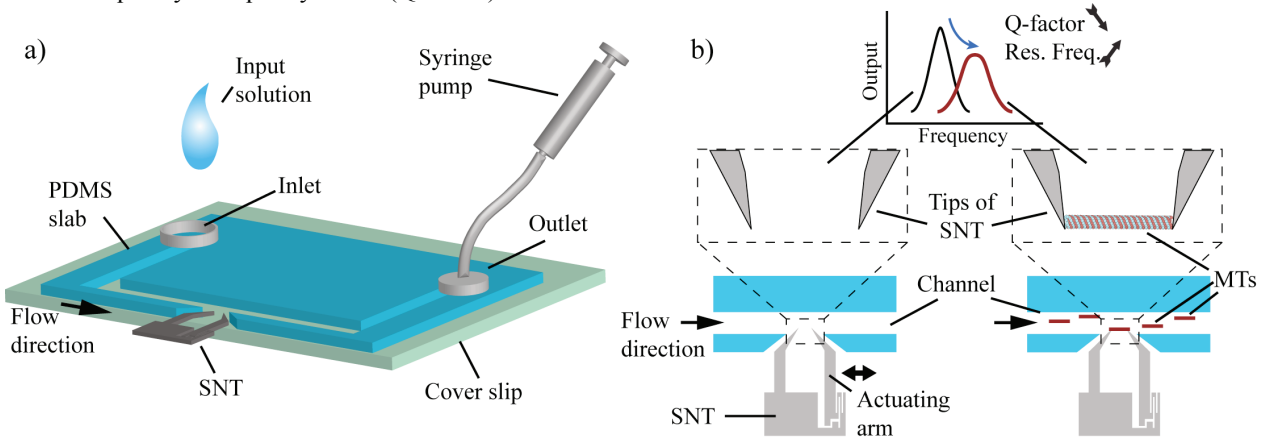

Figure 1. Schematic view of the developed system. a) SNT was inserted into the channel via the lateral opening of the syringe pump integrated PDMS device. b) The liquid inside the channel was changed with syringe pump. Changes in the resonance frequency and Q-factor monitor MT capturing. The device can then be used for monitoring molecular attachment on MTs

EXPERIMENTAL

Experiments were performed using two devices: SNTs and microfluidic device (Figure 2). The fabrication process and working principles of SNTs have already been reported [1]. The microfluidic device consisted of a PDMS slab placed on a cover slip. A 500-μm-wide channel was buried in the PDMS channel with a lateral opening for SNT entrance (Figure 2b). A syringe pump was connected to the outlet of the channel for exchanging the

solutions inside (Figure 2c). High flow rates (>10 µl/min) caused air bubbles to enter the channel while low flow rates (<0.1 µl/min) let the liquid to move out of the lateral opening. As a result of this, the liquid could move up to the SNT actuating arms caused a higher load on the arms and thus, lower sensitivity of the system. 1µl/min of flow was used in the experiments keeping the air-liquid interface constant while allowing the liquid exchange.

The PDMS device was designed for SNT-tips to enter the channel without touching the walls. This was crucial because the arms of the SNTs were actuating at certain frequencies to monitor the resonance frequency and quality factor (Q-factor). The measurements were performed using capacitance changes in the on-chip differential capacitive sensor. A phase-lock-loop device was crucial to amplify the signal for high-sensitivity. Due to the electrical integration of the SNTs with the amplifier system and the fluidic integration of the PDMS device with the syringe pump (Figure 2), real time and continuous monitoring of resonance frequency and the Q-factor was successfully performed.

Sharp, protruding SNT entered the channel at the very front side of the tips through the lateral opening of the PDMS slab (Figure 3a). Integrated syringe pump provided a flow (1µl/min) to keep the liquid interface constant and to allow liquid exchange throughout the experiment (Figure 3b). Low level of load and losses (due to liquid) provided higher sensitivity for the detection (Figure 3c). At first, 0.1% poly-L-lysine (a polycation that MTs can bind on) solution was injected. After 3 minutes for poly-L-lysine coating, water and buffer were injected respectively to wash away the unbound poly-L-lysine. Finally, 0.1mg/ml MT solution was injected in the channel. After 8 minutes, unbound MTs were washed away with buffer solution.

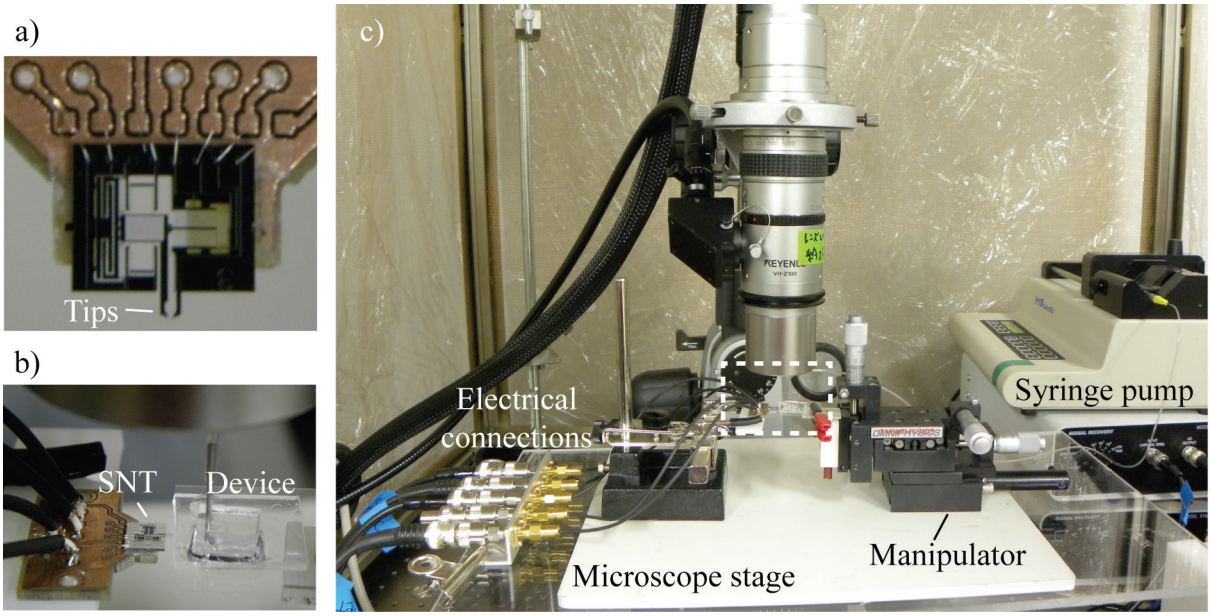

Figure 2. Setup of the system: a) Top view of the SNT, b) Tips of SNTs were inserted into the microfluidic channel via lateral opening. c) SNT was connected to the electrical setup (including amplifiers and a function generator) while the microfluidic device was integrated with the syringe pump. (b) is the enlarged view of the white-dashed line in (c).

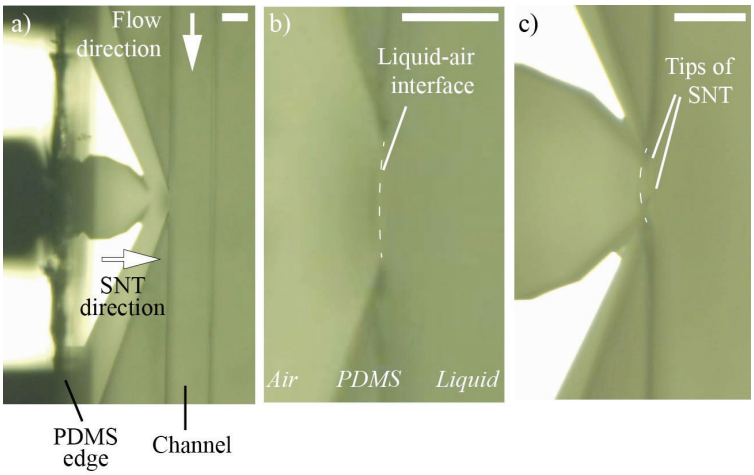

Figure 3. Microscope image showing a) SNT tips inserted in the lateral opening b) the liquid-air interface at the lateral opening and c) the very front of the SNT tips inside the liquid. Scale bars correspond to 30 µm.

RESULTS AND DISCUSSION

In air measurements of the SNTs was very stable and robust. The SNTs used in the experiments had a resonance frequency of 1862 Hz with a Q-factor of 84 (Figure 4). Inserting the tips into the liquid changed the characteristics of the SNTs due to the load applied by the liquid. A 5 Hz-increase was detected in the resonance frequency (reaching 1867 Hz) and a 7 au-decrease was detected in the Q-factor (reaching 77 au). Changing the liquid to BRB80 did not affect the resonance frequency (or the Q-factor) showing an excellent stability within 0.1 Hz range. As this was the first demonstration of the proposed system, we used very high concentration of MTs (0.1 mg/ml) to demonstrate the success of the detection process. As a result, quite high increase was detected in the resonance frequency. After the MT capturing, the detected resonance frequency increased up to ~1892 Hz (an increase of 25 Hz, black line in Figure 5). The captured MTs act as an additional spring constant to the SNTs system. Therefore, higher number of captured MTs results in higher shift in the resonance frequency. Considering that the system can easily detect sub-Hz levels of change in the resonance frequency, much lower number of MTs can be captured while monitoring in real time. The captured MTs, on the other hand, increase the losses in the system. These losses result in a decrease in the Q-factor. The results (red line in Figure 5) show a 30 au decrease in the Q-factor. Both of the changes in the resonance frequency and the Q-factor were successfully monitored as continuous, real-time capturing of MTs (Figure 5).

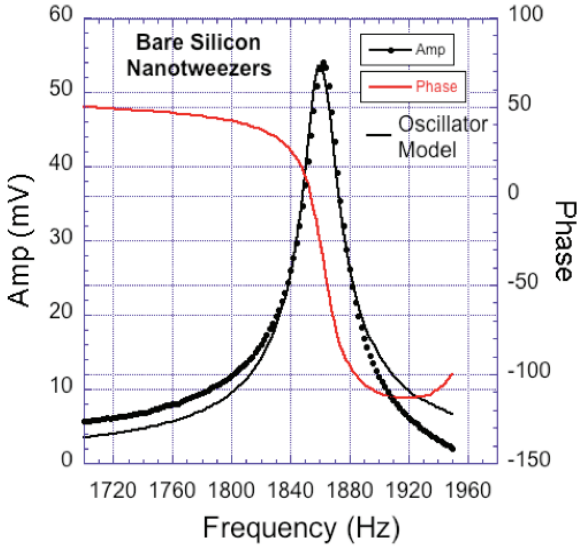

Figure 4. In-air characterization of SNT. The resonance frequency was measured as 1862 Hz and the Q-factor was 84.

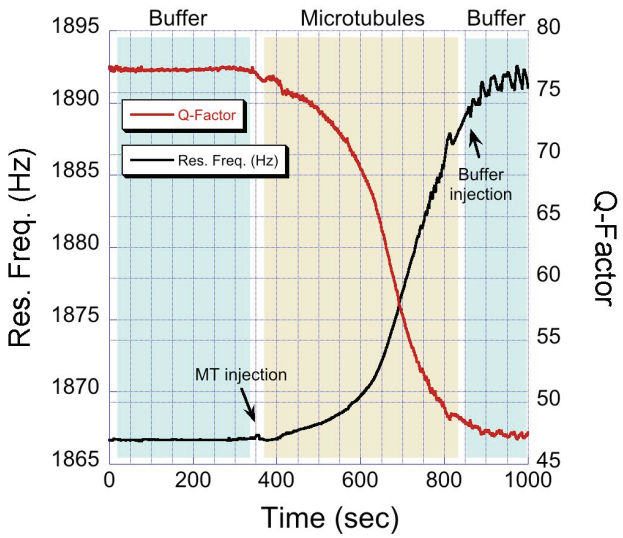

Figure 5. Real-time monitoring of the MT capturing. After buffer flow, high concentration of MT solution was injected resulted in a significant increase in resonance frequency (stiffening) and a decrease in Q-factor (more losses), Both effects attest of the capture of MT between the SNT tips.

CONCLUSION

We have successfully integrated SNTs with microfluidics to monitor real time and continuous molecular detection. Minimizing the area of the SNT tips exposed to liquid provided high sensitivity. Obtained system could easily detect sub-Hz levels of changes. For demonstration purposes, a big bundle of MTs were captured and the real time capturing process was monitored continuously.

Capturing MTs is a critical step to use the proposed system for several diagnostic methods because MTs are directly related with neurodegenerative diseases. Several biochemical disease markers, e.g. tau, can be detected with MT-based systems [3]. The proposed detection system can be used to monitor the existence of tau based on the increasing stiffness due to attachment to MTs [4]. Consequently, the proposed method has supreme potential to use as a diagnostic or drug-discovery method for several neurodegenerative diseases.

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CONTACT

M. C. Tarhan, mctarhan@iis.u-tokyo.ac.jp