

## Single virus particle mass detection using microresonators with nanoscale thickness

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In this letter, we present the microfabrication and application of arrays of silicon cantilever beams as microresonator sensors with nanoscale thickness to detect the mass of individual virus particles. The dimensions of the fabricated cantilever beams were in the range of 4–5  $\mu\text{m}$  in length, 1–2  $\mu\text{m}$  in width and 20–30 nm in thickness. The virus particles we used in the study were vaccinia virus, which is a member of the *Poxviridae* family and forms the basis of the smallpox vaccine. The frequency spectra of the cantilever beams, due to thermal and ambient noise, were measured using a laser Doppler vibrometer under ambient conditions. The change in resonant frequency as a function of the virus particle mass binding on the cantilever beam surface forms the basis of the detection scheme. We have demonstrated the detection of a single vaccinia virus particle with an average mass of 9.5 fg. These devices can be very useful as components of biosensors for the detection of airborne virus particles. © 2004 American Institute of Physics.

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As the need for rapid, ultrasensitive, and economical methods for the detection of biochemical entities such as virus particles becomes ever more important, nanoscale fabrication technologies are increasingly being used to create nanomechanical sensors and highly sensitive lab-on-a-chip.<sup>1</sup> The applications can be very diverse and range from environmental monitoring to clinical diagnosis to combating bioterrorism. We report here the demonstration of a nanoscale cantilever beam operating as a mass detector, with a sensitivity of the mass of a single vaccinia virus particle. Vaccinia virus is a member of the *Poxviridae* family and forms the basis of the smallpox vaccine. In general, decreasing the overall dimensions of the cantilever beams results in a corresponding increase in their mass sensitivity. In previous works on the detection of virus particles, macroscale quartz crystal micro-balance devices requiring an external power supply were used and the detachment of virus particles were measured.<sup>2</sup> In our experiments, we have used micromechanical devices (with nanoscale thickness) on a chip, with the measurement setup sensitive enough to measure thermal and ambient noise induced deflections and thus not requiring an external source to excite the cantilever beams.<sup>3</sup>

P-type (100) 4 in. silicon-on-insulator (SOI) wafers were used as the starting material [see Fig. 1(a)]. The wafers had a SOI layer of 210 nm thickness and a buried oxide (BOX) thickness of around 390 nm. Wet oxidation followed by buffered hydrofluoric (BHF) etching was performed in order to thin the SOI device layer down to 30 nm. Photolithography followed by reactive ion etching using Freon 115 to etch the SOI layer and  $\text{CHF}_3/\text{O}_2$  in order to thin the BOX layer, was performed in order to pattern the cantilever beam shapes [see Fig. 1(b)]. After depositing a layer of plasma enhanced chemical vapor deposition oxide as an etch stop layer, an

etch window was photolithographically patterned using BHF oxide etch [see Fig. 1(c)]. In order to etch the underlying exposed silicon and release the cantilever beams, vapor phase etching using xenon difluoride (Xactix, Inc., Pittsburgh, PA) was used [see Fig. 1(d)]. After the cantilever beams were released, the oxide was etched in BHF, rinsed in de-ionized (DI) water, immersed in ethanol, and dried using critical point drying (CPD) [see Fig. 1(e)].

The measurement of the cantilever resonant frequency was performed using a microscope scanning laser Doppler vibrometer (MSV-300 from Polytec PI) with a laser beam spot size of around 1–2  $\mu\text{m}$ . The resonant frequencies of typical cantilever beams of length around 5  $\mu\text{m}$ , width around 1.5  $\mu\text{m}$ , and thickness around 30 nm were in the 1–2 MHz range with quality factor of around 5–7. The vaccinia virus particles were grown and purified according to the established protocols described in Zhu *et al.*<sup>4</sup>

The cantilevers beams were first cleaned in a solution of ( $\text{H}_2\text{O}_2:\text{H}_2\text{SO}_4=1:1$ ), rinsed in DI water, immersed in ethanol, and dried using CPD. The frequency spectra was then measured in order to obtain the “unloaded” resonant frequencies of the cantilever beams. Next, purified vaccinia virus particles at a concentration of  $\sim 10^9$  PFU/ml in DI water were introduced over the cantilever beams and allowed to incubate for 30 min, following which the cantilever beams were rinsed in ethanol and dried using CPD. The resonant frequencies of the cantilever beams were then measured again in order to obtain the “loaded” resonant frequencies.

Using the mechanics of a spring–mass system, we were able to determine the added mass for the corresponding change in resonant frequency. The change in mass (placed right at the free end of the cantilever beam) in relation to a change in resonant frequency can be given as<sup>5</sup>

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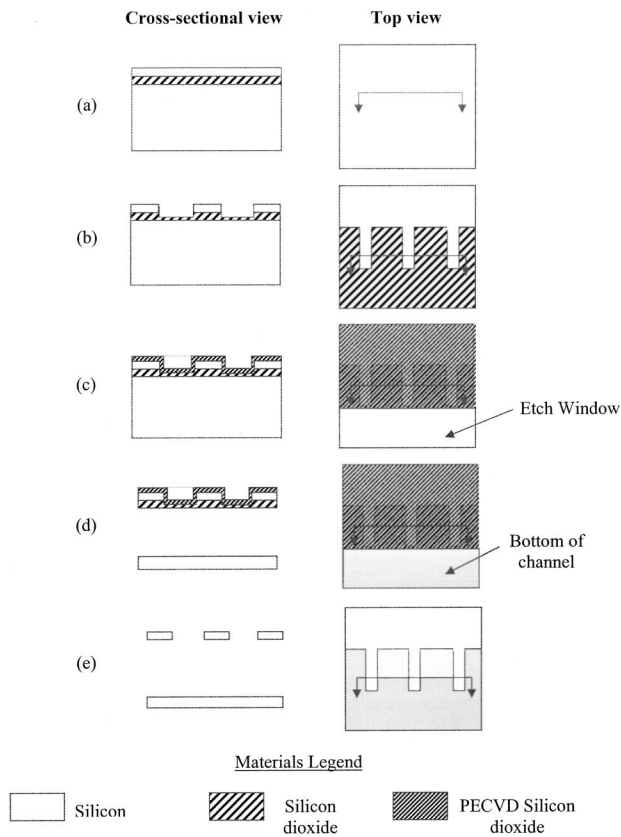


FIG. 1. Process flow used for the fabrication of an array of silicon cantilever beams.

$$\Delta m = \frac{k}{4\pi^2} \left( \frac{1}{f_1^2} - \frac{1}{f_0^2} \right), \quad (1)$$

where  $k$  is the spring constant of the cantilever beam,  $f_0$  is the initial resonant frequency, and  $f_1$  is the resonant frequency after the mass addition. The cantilever beams were calibrated by obtaining their spring constant,  $k$ , using the unloaded resonant frequency measurement  $f_0$ , quality factor  $Q$ , and the plan dimensions (length and width) of the cantilever beam.<sup>6</sup> The resonant frequency and the quality factor were obtained by fitting the vibration spectra data to the

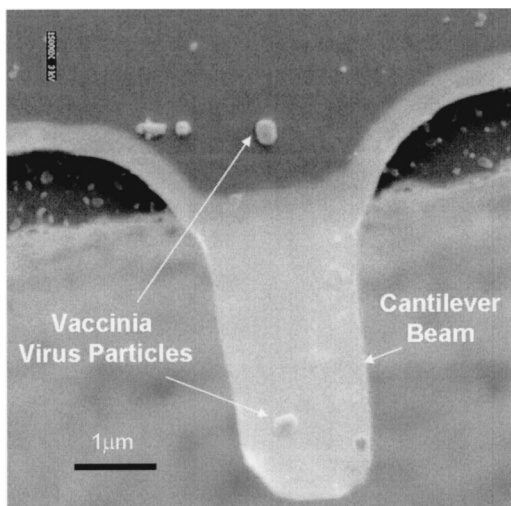


FIG. 2. Scanning electron micrograph (SEM) showing a cantilever beam with a single vaccinia virus particle. The cantilever beam has plan dimensions of length,  $L=4 \mu\text{m}$ , and width,  $W=1.8 \mu\text{m}$ .

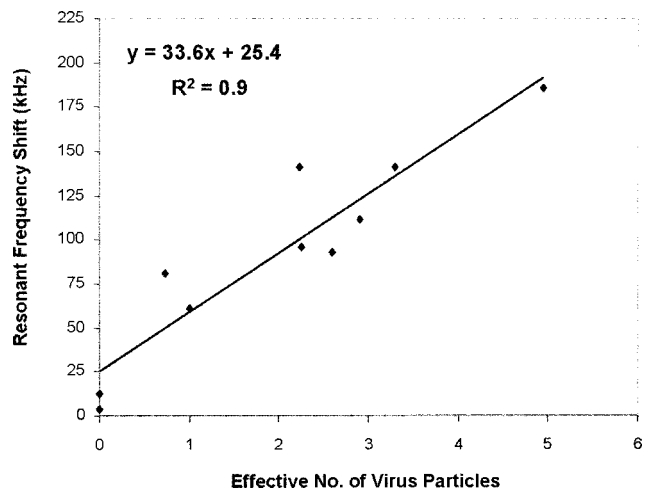


FIG. 3. Plot of measured resonant frequency shift vs the effective number of virus particles on the cantilever beams. A linear fit was performed on the data points.

amplitude response of a simple harmonic oscillator (SHO). The amplitude response of a SHO is given as<sup>7</sup>

$$A(f) = A_{dc} \frac{f_0^2}{\sqrt{(f_0^2 - f^2)^2 + \frac{f_0^2 f^2}{Q^2}}}, \quad (2)$$

where  $f$  is frequency in Hz,  $f_0$  is the resonant frequency,  $Q$  is the quality factor, and  $A_{dc}$  is the cantilever amplitude at zero frequency. The measured spring constant of the cantilever beams was around 0.005–0.01 N/m. The virus particles were counted by observing the cantilever beams and virus particles using a scanning electron micrograph (SEM), as shown in Fig. 2. The effective mass contribution of the viruses was calculated based on their relative position from the fixed end of the cantilever beams.<sup>3</sup> Using the measurements from the various cantilever beams, we plotted the resonant frequency

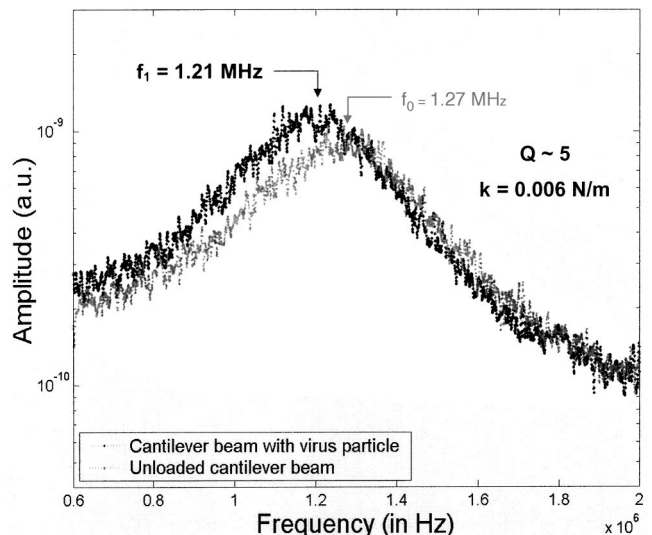


FIG. 4. Plot of resonant frequency shift after loading of a single virus particle. There is a 60 kHz decrease in the resonant frequency of the cantilever beam with plan dimension of  $L=3.6 \mu\text{m}$  and  $W=1.7 \mu\text{m}$ . The unloaded resonant frequency  $f_0=1.27 \text{ MHz}$ , quality factor  $Q=5$ , and spring constant  $k=0.006 \text{ N/m}$ . The resonant frequencies were obtained from fitting the amplitude response of a simple harmonic oscillator to the measured data.

shift (decrease) versus the effective number of virus particles that were observed on the cantilever beam, as shown in Fig. 3. The relationship was linear, as expected, clearly proving the validity of the measurements. Figure 4 shows the resonant frequency shift ( $\Delta f = 60$  kHz) after the addition of a single virus particle. We measured an average dry mass of 9.5 fg for a single vaccinia virus particle, which is in the range of the expected mass of 5–8 fg.<sup>8</sup> The measured mass sensitivity of the present cantilever beams for a 1 kHz frequency shift is 160 attogram (ag) added mass (6.3 Hz/ag). Once integrated with on-chip antibody-based recognition and sample concentrators, these microresonator devices with nanoscale thickness may prove to be viable candidates for ultrasensitive detection of airborne virus particles.

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