High-speed XYZ-nanopositioner for scanning ion conductance microscopy

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We describe a tip-scan-type high-speed XYZ-nanopositioner designed for scanning ion conductance microscopy (SICM). The nanopipette probe is mounted in the center of a hollow piezoactuator, both ends of which are attached to identical diaphragm flexures, for Z-positioning. This design minimizes the generation of undesirable mechanical vibrations. Mechanical amplification is used to increase the XY-travel range of the nanopositioner. The first resonance frequencies of the nanopositioner are measured as \( \sim 100 \) and \( \sim 2.3 \) kHz for the Z- and XY-displacements, respectively. The travel ranges are \( \sim 6 \) and \( \sim 34 \) \( \mu \)m for Z and XY, respectively. When this nanopositioner is used for hopping mode imaging of SICM with a \( \sim 20 \)-nm diameter tip, the vertical tip velocity can be increased to 400 nm/ms; hence, the one-pixel acquisition time can be minimized to \( \sim 1 \) ms.

In biological research, atomic force microscopy (AFM)\textsuperscript{1} has been widely used to visualize the topographic structure of biological specimens under physiological liquid environments\textsuperscript{2}. Nevertheless, the cantilever tip must make contact with the sample in the solution, and even exerted forces below 10 pN considerably deform extremely soft surfaces\textsuperscript{3,4} such as the plasma membranes of live eukaryotic cells, which prohibits high-resolution surface imaging\textsuperscript{5}. Scanning ion conductance microscopy (SICM) is an alternative imaging method based on an entirely different working principle for capturing a topographic image\textsuperscript{6} (Fig. 1). SICM uses an electrolyte-filled glass pipette (nanopipette) as a probe and relies on an ion current flowing between an electrode inside the nanopipette and another in an external bath solution. The ion current passing through the opening of the nanopipette is sensitive to the tip-sample surface separation\textsuperscript{7,8}; therefore, SICM can capture topographic images without any tip-sample contact.

However, the temporal resolution of SICM is much lower than that of AFM, especially when SICM is operated in the hopping mode\textsuperscript{9}, where the tip is moved up and down to avoid lateral tip-sample contact. This low temporal resolution, due to the time delay in the vertical tip-position control, limits the fall velocity of the tip, \( v_f \); namely, the speed at which the tip approaches the sample surface. Since glass nanopipettes are very fragile, they are easily damaged when they contact surfaces. To avoid surface contact, \( v_f \) must be restricted. When the bandwidth of the ion current detection is sufficiently high, the low mechanical resonance frequency of the Z-nanopositioner is the dominant contributor to the delay\textsuperscript{10}. The difficulty to decrease the delay is how to assemble the nanopipette to the Z-nanopositioner since the nanopipette is very massive and large compared to AFM cantilever tip. For instance, commercial SICM systems use a long-travel-range (\( \sim 25 \) \( \mu \)m) Z-nanopositioner whose resonance frequency is less than 1 kHz when the nanopipette is assembled, producing a long delay (longer than 1 ms) for vertical tip positioning. As a result, it typically takes 10–100 ms for one-pixel acquisition when imaging samples showing very rough surfaces\textsuperscript{9}. Thus, in SICM, it is difficult to visualize morphological changes in samples that occur in under a minute, which significantly limits the applicability of SICM in biological studies.

In this letter, we describe the design of a tip-scan-type high-speed XYZ-nanopositioner for SICM. One of the distinctive features of the developed nanopositioner is the large product of “travel range \( \times \) resonance frequency” for the Z-positioner, which represents the positioner performance because the travel range and resonance frequency are tradeoffs. The product of our Z-positioner, \( 6 \) \( \mu \)m \( \times \) 100 kHz , is more than ten-fold larger than those of conventional SICM systems and 2–3 times larger than that of the Z-scanner employed in high-speed AFM\textsuperscript{11}. The performance can significantly

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increase $v_f$ to 400–600 nm/ms and hence minimize the one-pixel acquisition time to $\sim 1$ ms. Here, we show the detailed design and performance of our nanopositioner and demonstrate the stable high-speed imaging of test samples at 3.5 s per frame over a scan area of $\sim 25 \times 25 \mu m^2$ with $50 \times 50$ pixels.

The Z-nanopositioner mounted on the XY-nanopositioner, as illustrated in Fig. 2(a), is designed to achieve a high mechanical resonance frequency and hence a large $v_f$. The frame body of the XYZ-nanopositioner was fabricated from alloy A7075. Before constructing the actual device, we conducted a finite-element analysis (FEA) to estimate the mechanical properties of designed structures, using a commercially available finite-element package, COMSOL Multiphysics 5.0 (COMSOL AB). The following mechanical parameters for the alloy A7075/piezoactuator were used in the FEA simulation: Young’s modulus, 72/33 GPa; Poisson’s ratio, 0.3/0.3; density, 2810/7800 kg/m$^3$. The central idea for suppressing unwanted mechanical vibrations in the Z-nanopositioner is the use of momentum cancellation$^{12}$. That is, the hollow Z-piezoactuator (AE0505D08D-H0F, NEC/Tokin) is sandwiched with a pair of identical diaphragm-like flexures$^{13}$ so that the center of mass of the Z-piezoactuator shows negligible change during fast displacement. The flexures were designed to have a stiffness of $\sim 18.2$ N/µm, which provides a suitable preload to the actuator ($\sim 10–20$ N). The magnitude of the preload is adjustable with screws.

This design can mechanically cancel dynamic forces exerted onto the Z-support frame when the Z-piezoactuator is quickly displaced. Moreover, it permits the Z-positioner to have a resonance frequency comparable to that of the Z-piezoactuator under free oscillation. Injecting epoxy glue between the diaphragm flexures and the Z-piezoactuator improved the mechanical stability of the Z-positioner and enabled its robust long-term actuation. The $\sim 12–14$-mm-long nanopipette is mechanically connected to the top flexure by gluing the nanopipette only to the top clamp [Fig. 2(b)] so that the nanopipette can move with the z-movement of the top flexure. In order to avoid unintentional mechanical vibrations of the nanopipette, the nanopipette length was shortened as much as possible and the nanopipette was supported by the spacers made of an elastic material at the top and bottom clamps. A polyimide tube inserted between the nanopipette at the glue point enhances the fixing strength between the nanopipette to the clamp. This assembly only decreases the resonance frequency of the Z-piezoactuator by 10% since the total mass of the nanopipette and clamp are much smaller than that of the Z-piezoactuator. Importantly, undesirable vibrations were suppressed in the Z-positioner below the first resonance frequency at 100 kHz [Fig. 3(a)]. The measured first resonance frequency agreed reasonably well with the result of the FEA simulation [Fig. 3(b)].

The travel range of Z-nanopositioner was $\sim 6$ µm, as measured with a laser vibrometer (NLV-2500, Polytech), approximately half the original maximum displacement of the Z-piezoactuator, as expected. Since the travel range is inversely proportional to the resonance frequency$^{14}$, the performance of our Z-nanopositioner can be evaluated from the product of travel range $\times$ resonance frequency, which at $6 \mu m \times 100$ kHz significantly exceeds (by more than 10-fold) the $25 \mu m \times 1–2$ kHz shown in conventional SICM nanopositioners.

For XY-displacements, we used mechanical amplification$^{15}$ to magnify the original travel range (9 µm at 150 V) of XY-piezoactuators (AE0505D08DF, NEC/Tokin).
In our XY-nanopositioner design, the XY-travel range can be controlled by varying the thickness of the beam flexures that connect the Z-nanopositioner and the surrounding base frame, indicated by the broken circle in Fig. 4(c), without changing the overall dimensions of the nanopositioner. The measured travel range increased from 16 [Fig. 4(a)] to 34 [Fig. 4(b)] with decreasing beam-flexure thickness from 300 to 200 µm, which is the practical limit for fabrication by wire electrical discharge machining. The mechanical amplification factors, 1.4 and 2.7, were consistent with the results of the FEA simulation (not shown). The measured dominant resonance frequency of the XY-nanopositioner assembled with the full components of the Z-nanopositioner decreased from 4.5 to 2.3 kHz, adequate for achieving high-speed SICM, with decreasing beam-flexure thickness (not shown).

We first captured hopping-mode SICM images of Grating 1 samples immersed in a solution containing 0.15 M KCl. The \( v_1 \) and hopping amplitude were set at 400 nm/ms and 300 nm, respectively. Figure 5(a) shows a topographic image captured at 3.5 s/frame for a scan range of \( \sim 25 \times 25 \mu m^2 \) with 50 \( \times \) 50 pixels. The image shows a grating pitch with variations of 20% in the X- and Y-directions, mainly due to the hysteresis effect of the XY-piezoactuators and partially due to the cross-coupling between them. Since it is possible to compensate for these effects by exploiting previously reported methods\(^{10, 16}\), we do not focus on this issue in this study. In Fig. 5(a), no notable undesirable vibrations appeared even at the left edge region of the image, where the scanning direction was inverted and hence the X-positioner was driven at high frequencies. The \( v_1 \) value, 400 nm/ms, used in this imaging is significantly larger than the typical \( v_1 \) values used in conventional SICM systems (\( \sim 20 \) nm/ms). Although the comparable \( v_1 \) value \( \sim 500 \) nm/ms was reported for a large nanopore diameter \( \sim 100 \) nm\(^3\), attainable \( v_1 \) value will decrease to \( \sim 100 \) nm/ms when the previous report uses an identical nanopore diameter (\( \sim 20 \) nm) used in this study. Therefore, our positioner significantly improved the \( v_1 \).

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FIG. 5. (Color online) SICM images of Gratings (a) 1 and (b) 2 captured at 3.5 and 26 s/frame, respectively. (c) Height profiles of images (a) and (b) along the light blue broken lines shown in (a) and (b). (d) Five images of Grating 2 clipped from successive images captured at ~26 s/frame for longer than 5 min. Scale bars, 5 µm.

Effect of the XY-piezoactuators and partially due to the cross-coupling between them. Since it is possible to compensate for these effects by exploiting previously reported methods, we do not focus on this issue in this study. In Fig. 5(a), no notable undesirable vibrations appeared even at the left edge region of the image, where the scanning direction was inverted and hence the X-positioner was driven at high frequencies. The $v_f$ value, 400 nm/ms, used in this imaging is significantly larger than the typical $v_f$ values used in conventional SICM systems (~20 nm/ms). Although the comparable $v_f$ value ~500 nm/ms was reported for a large nanopore diameter ~ 100 nm, the $v_f$ value will decrease to ~100 nm/ms when the previous report uses an identical nanopore diameter (~20 nm) used in this study. Therefore, our positioner significantly improved the $v_f$.

To further evaluate the performance of our XYZ-nanopositioner, we captured a topographic image of Grating 2 with rougher surfaces than Grating 1, in which wire-like objects were seen on the surface, as shown in Fig. 5(b). This image was captured at ~26 s/frame for a scan range of ~25 × 25 µm$^2$ with 100 × 100 pixels, using a hopping amplitude of 600 nm and $v_f$ of 400 nm/ms. By increasing the hopping amplitude to 600 nm, wire-like objects likely to be partially suspended between grids [Fig. 5(c)] were captured without their disruption, although the time required for one-pixel acquisition increased from ~750 to 1500 µs. Figure 5(d) shows successive images of Grating 2 captured for longer than 5 min. Since touching the surface manifests as ‘tail like patterns’ in observed images regardless with the stiffness of wire-like objects, no noticeable changes in these images demonstrate the robustness of our XYZ-positioning.

In summary, we developed a tip-scan-type high-speed XYZ-nanopositioner for SICM. The installation of the nanopipette mount in the center of a hollow piezoactuator and the use of a momentum cancellation mechanism for the Z-positioner resulted in a high resonance frequency of 100 kHz even with a relatively long travel range, 6 µm. The improved time delay of the Z-nanopositioner increases the $v_f$ by more than 20 times that of conventional SICM nanopositioners. These excellent features allowed the high-speed imaging of Grating 1 at 3.5 s/frame for a scan area of 25 × 25 µm$^2$ with 50 × 50 pixels without generating undesirable vibrations. Moreover, a rougher surface could be captured without decreasing $v_f$. This study is the first step toward achieving high-speed SICM that can capture biological samples in dynamic action in real time.

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