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Reduction of the image acquisition time in scanning ion conductance microscopy in the „hopping“ mode

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An adaptive algorithm has been developed and experimentally validated for significantly reducing image acquisition time and enhancing the operational stability of scanning ion conductance microscopy (SICM) in the .hopping. mode. The implementation of an analog feedback loop markedly improved performance. Experimental results demonstrate a fourfold reduction in scanning time while maintaining image stability and quality.

Keywords: scanning ion conductance microscope, nanopipette, tracking system, „hopping“ mode, piezoscanner.

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Scanning ion conductance microscopy (SICM), also known as scanning capillary microscopy (SCM), is a variation of scanning probe microscopy (SPM) that employs a glass nanopipette (NP) with an inner aperture radius of approximately 50−100 nm through which ion current flows [1]. The primary application of SCM is the investigation of soft biological objects in liquid environments. Three primary scanning modes of SCM operation include direct current (DC) mode [2], alternating current (AC) mode [3], and the „hopping“ mode [4]. The AC and DC modes are known to produce less stable images compared to the approach/retract mode. This instability occurs because, in AC and DC modes, after the NP approaches the surface under study and captures the required ion current, it moves from point to point close to the surface along a trajectory that follows the surface relief. Due to the finite thickness of the NP wall and the possibility of tracking errors, collisions with the test object surface can occur, rendering the images unstable.

In contrast, in the „hopping“ mode, transitions between points occur far from the surface. After the transition, the NP, controlled by the tracking system (TS), is brought to the sample surface to capture the required ion current. This scanning technique minimizes uncontrollable NP collisions with the test sample and enhances SCM stability. However, the approach/retract cycle in the „hopping“ mode requires more time, significantly increasing the overall scanning duration.

This paper describes an SCM algorithm designed to minimize scanning time in the „hopping“ mode and presents its application results using an analog feedback (FB) loop. Figure 1, *a* illustrates a TS circuit with an analog feedback loop. In the conventional approach/retract mode, the NP, secured on the piezoscanner, approaches the test surface (switch SW2 is open) due to the gradual extension of the piezoscanner controlled by the FB loop integrator until the ion current signal stabilizes at the reference signal level. After determining the *Z*-coordinate by measuring the input voltage of the high-voltage amplifier, switch SW2 is closed, the integrator output signal is reset to zero, and the piezoscanner returns to its initial „zero“ position (zero at the high-voltage amplifier (HVA) input). The NP then retracts to the maximum possible distance from the surface. Subsequently, the NP moves to a new point with coordinates X and Y, and the process repeats (Fig. 1, *b*). The SCM image of the sample surface represents the dependence $Z(X, Y)$.

In the conventional algorithm, the scanner returns to its initial „zero“ position, and the NP retracts to the maximum possible distance from the surface, thereby maximizing the approach/retract cycle time. To reduce this cycle time, an adder is introduced in the FB loop downstream of the integrator and amplifier. This adder adds the voltage generated by a digital-to-analog converter (DAC) to the amplifier output signal. Consequently, even when switch SW2 is closed and the integrator's output voltage is zero, the NP retracts from the sample surface to a reduced height determined by the DAC output voltage. This adjustment reduces the scanning time significantly (Fig. 1, *c*).

The control circuit shown in Fig. 1, *a* allows for measuring the so-called approach curve, i.e., the ion current dependence on the NP-to-surface distance. For this purpose, the FB loop is opened using switch SW1, and scanner voltage is stored (via a sampling-storage scheme that fixes the integrator.s output signal in time). The DAC signal added to the initial signal at the HVA input enables piezoscanner

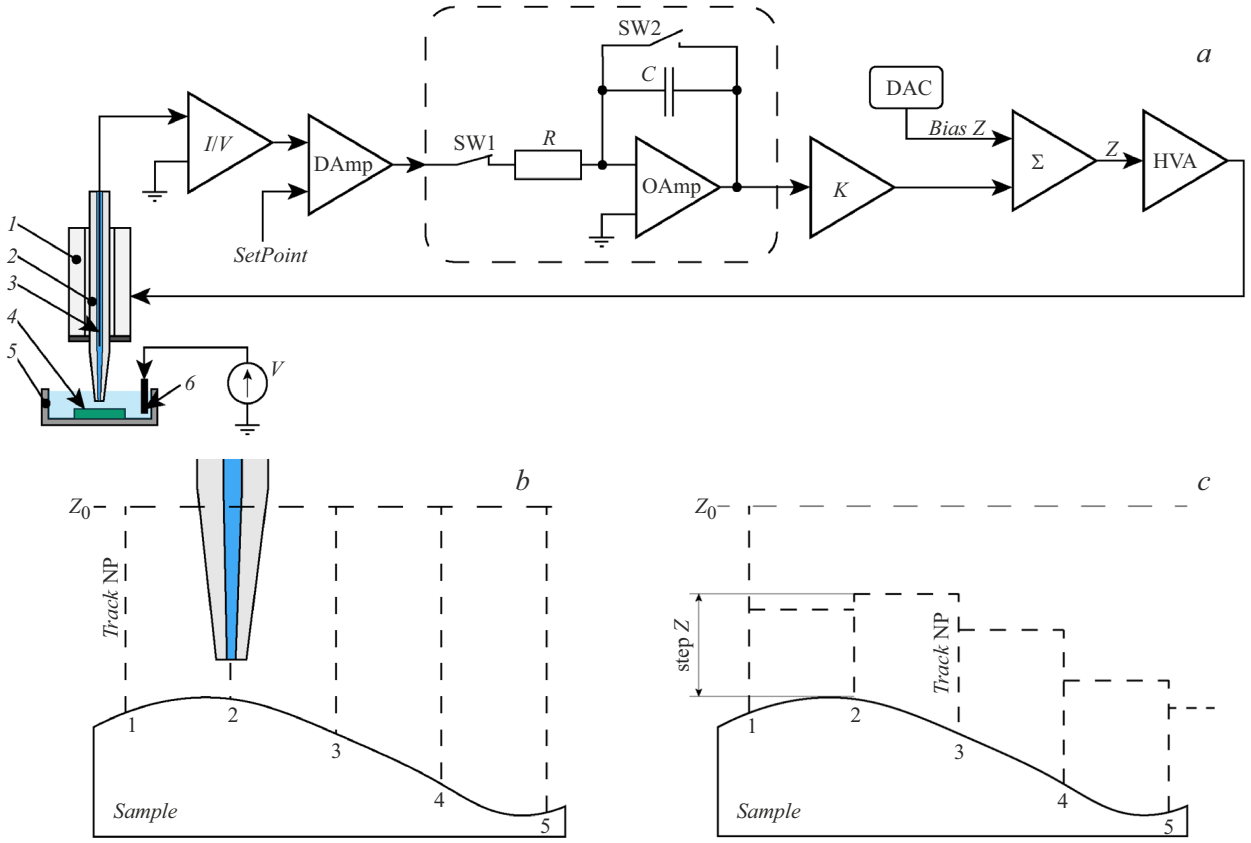


Figure 1. *a* — TS circuit with an analog FB loop used in SCM in the „hopping“mode. *1* — piezo scanner; *2* — NP; *3*, *6* — AgCl electrodes; *4* — sample; *5* — Petri dish with 5% NaCl solution. *V* — voltage source on the ring electrode in the Petri dish, *I*/*V* — „current– voltage“converter, DAmp — differential amplifier, OAmp — operational amplifier, *K* — FB loop amplifier, DAC — digitaltoanalog converter, Σ — adder, HVA — high voltage amplifier. Adder Σ is used in implementing the algorithm that reduces the measurement time. *b* — NP trajectory in scanning the sample without using DAC. *c* — NP trajectory in scanning the sample using DAC.

extension or shortening, thereby displacing the NP relative to its initial position. This proposed control circuit facilitates approach curve measurements at each scan point, aiding the study of charge distribution on the sample surface.

Fig. 2 illustrates the algorithm for single-line scanning in the „hopping“ mode, which incorporates an adaptive reduction of the nanopipette (NP) retraction height from the sample surface at each scan line point. At the start of the first scan line, when the probe reaches the initial point (Line start point), the voltage offset is set to zero, and the scanner fully retracts, maximizing the distance of the NP from the surface. Subsequently, through integration of the input signal within the closed feedback (FB) loop, the NP is moved from the „zero“ position to the sample surface (Capture current drop), traversing the maximum distance before stabilizing the operating current.

At the first point, the integration time of the input signal (scanner retraction time) is the longest compared to subsequent scan line points. This results in a longer delay (t_1) between the initiation of input signal integration (by opening the SW2 switch) and the measurement of the Z coordinate. After recording the Z coordinate at the first point, the scanner retracts completely from the sample (by closing switch SW2), and the fixed NP retraction height (step Z) is subtracted from Z. The resulting difference is shaped using the DAC and gradually applied to the adder (Scanner retract and set bias Z).

For subsequent scan line points (Line next point), the NP traverses a shorter distance during the approach/retract cycle, as determined by the measured Z coordinate and the scanner's initial „zero“ position. This shorter distance reduces the time required for the cycle. The delay time (t_2) for all subsequent points is set to a value smaller than t1, which corresponds to the point of maximum scanner elevation.

The step Z value can be determined either by measuring the surface relief of the test sample using the conventional single-line algorithm or empirically from prior data about the sample. Typically, it is set to a value 30–40% greater than the height of the pre-measured or estimated relief.

Conflict of interests

The authors declare that they have no conflict of interests.

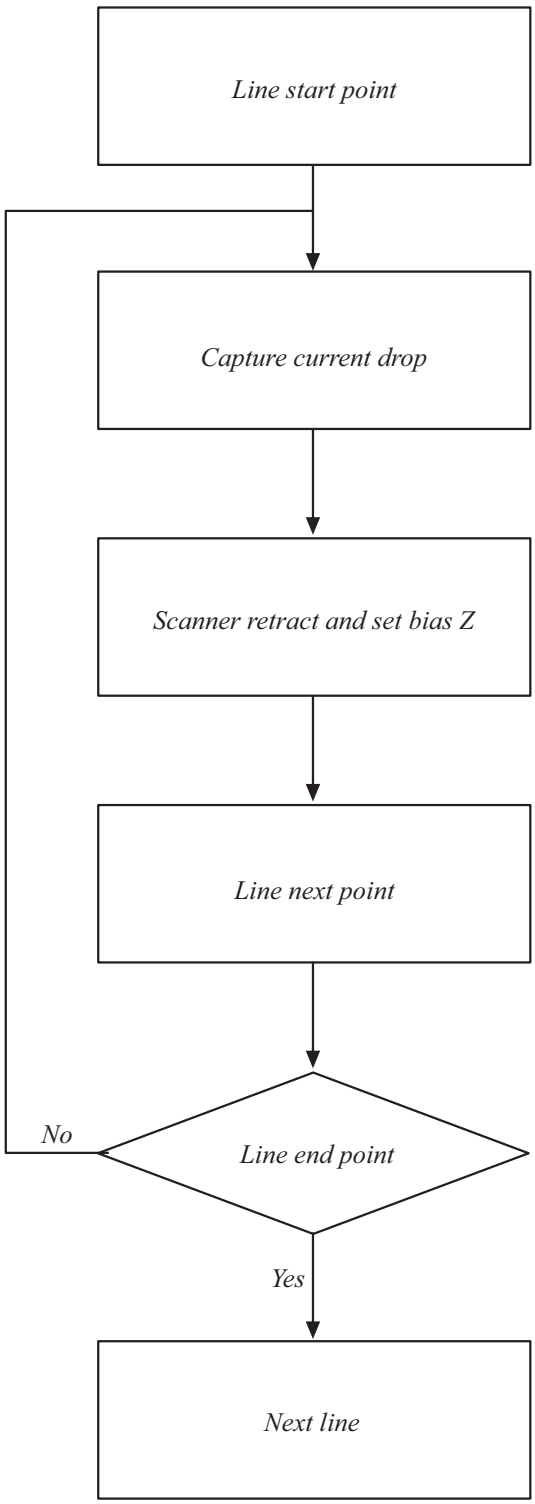


Figure 2. Schematic representation of the adaptive algorithm for single-line scanning in the SCM „hopping“ mode, illustrating the reduction in nanopipette (NP) retraction height from the sample surface.

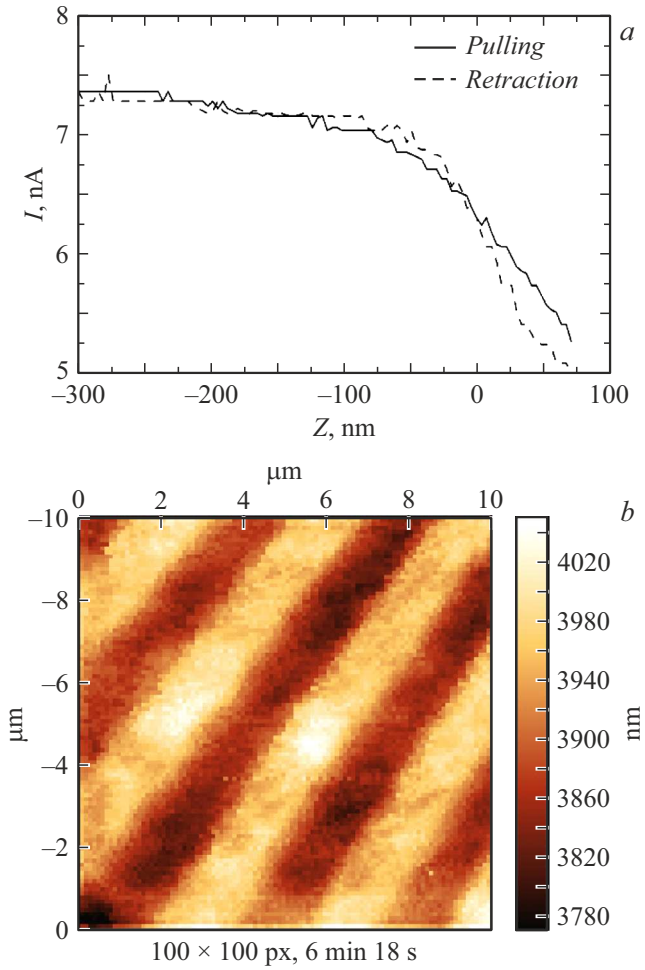


Figure 3. Results of measuring the approach curve (*a*) and scanning the test sample in the „hopping“ mode using the proposed adaptive algorithm (*b*). The frame consists of 100×100 points, the nanopipette (NP) aperture diameter is approximately 100 nm, the offset voltage at the AgCl electrodes is 0.3 V, the ion current is 1.2 nA, and the NP retract height in the adaptive algorithm (step *Z*) is 700 nm.

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