Tandem array of nanoelectronic readers embedded coplanar to a fluidic nanochannel for correlated single biopolymer analysis

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We have developed a two-step electron-beam lithography process to fabricate a tandem array of three pairs of tip-like gold nanoelectronic detectors with electrode gap size as small as 9 nm, embedded in a coplanar fashion to 60 nm deep, 100 nm wide, and up to 150 μm long nanochannels coupled to a world-micro-nanofluidic interface for easy sample introduction. Experimental tests with a sealed device using DNA-protein complexes demonstrate the coplanarity of the nanoelectrodes to the nanochannel surface. Further, this device could improve transverse current detection by correlated time-of-flight measurements of translocating samples, and serve as an autocalibrated velocimeter and nanoscale tandem Coulter counters for single molecule analysis of heterogeneous samples. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4861435>]

INTRODUCTION

Electronic detection has been a preferred platform for nanoscale analytical tool developments due to its portability, sensitivity, temporal response, and the ease of multiplexity. Applications of electronic analysis are pervasive ranging from the characterization of solid-state nanomaterials,¹ quantum devices,² to the study of single molecules.³ Particularly, biological and solid-state nanopores have been reported to provide unique ionic blockade current signals characteristic of nucleic acids that translocate through such structures.^{4,5} To increase the spatial resolution, nanometer-scale electrodes embedded on the side walls of a nanopore were proposed and shown to be an excellent way to measure lateral (transverse) ionic or tunneling currents when DNA molecules are driven through,^{6,7} where analytical information about the backbone of DNA and its complex forms may be revealed.^{8–12} However, conventional nanopore detection schemes for biopolymer analysis present some challenges, such as the random coil configuration in free solution prior to their entry through the pore, high translocation speed due to enhanced local electric field, and the inability of distinguishing false-positive signals.¹³ These challenges could be overcome by implementing physical confinement provided by nanofluidic channel and, within which, a tandem array of embedded electrode nanogap detectors. This scenario offers the possibility to measure the characteristic electrical conduction perpendicular to the DNA backbone in a correlated manner as it moves through the tandem gaps, while taking

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advantage of the confinement-induced DNA stretching in nanochannel when driven in by external forces, as the bending of DNA in nanoconfined geometry is not energetically favorable.^{14–17}

Various techniques like electro-migration,¹⁸ chemical etching, E-beam lithography (EBL),¹⁹ mechanically controlled break junctions (MCBJ),²⁰ shadow mask evaporation,²¹ etc., have been reported to fabricate nanoelectrode gaps. Electro-migration could be used to achieve sub-10 nm nanogaps, but electrode structures formed by this technique have irregular features and lack of process control, though feedback controlled electro-migration could overcome some of the issues.²² While MCBJ is a promising technique to achieve nanogaps smaller than 1 nm,²³ it lacks control over the exact shape of the cross sectional area of the electrodes and is poorly suited for building extended arrays of molecular circuits. Features obtained with shadow mask evaporation technique depend on the evaporation angle and may result in inconsistent electrode gaps due to granularity of the metal deposits.²⁴ Instead, EBL is the most common way adopted for the fabrication of electrode nanogaps.^{25,26} It also provides a reliable and reproducible way to fabricate sub-10 nm nanogap, but the complexity increases many folds when such nanogaps are to be embedded inside a sub-100 nm nanofluidic channel, a size comparable to the bending stiffness of a double-stranded DNA, in a co-planar fashion for leak-free bonding.^{10,27,28}

For example, an approach using a combined EBL, nanoimprint, and tilted shadow evaporation has been used to integrate sub-10 nm nanoelectrode gap within a nanochannel,¹⁰ but electron microscopy images show that the electrode sidewalls are isotropic, which could be problematic to single molecule detection, as such variations may generate amplitude differences of the electrical signal while molecules passing by. There are also demonstrations of fabricating electrode nanogaps, suspended above or on the substrate, embedded inside a microchannel using MCBJ technique²⁸ or nanochannel using a PMMA sacrificial layer.²⁹ As these structures cannot be sealed coplanarly, the molecules may diffuse either above or below the nanogap without being detected. Recent report of 50 nm gap nanoelectrodes bridged (not coplanar) across 400 nm wide, 60 nm deep nanochannels,³⁰ with blunt end electrodes (200 nm long), could not take full advantage of the spatial resolution provided by the sharp tip electrodes and the confinement-induced DNA stretching (Kuhn length ~ 100 nm).

In this work, we realized a tandem array of co-planar electrodes to overcome some of the challenges mentioned above which may serve as a tool for correlated time-of-flight studies of translocating samples. We adopt a two-step EBL-assisted fabrication approach for embedding a tandem array of electrode nanogaps inside a nanofluidic channel that relies in its first step on the precise exposure of three e-beam-resist nanostripes that are later used as masks to transfer three pairs of electrodes coplanar to the surface with their corresponding nanogaps. This process is followed by a second EBL step using precise nanoscale alignment to superimpose the nanofluidic channel (100 nm wide, 60 nm deep, and 150 μ m long), encompassing the gap tips of the previously defined electrodes. The precise alignment and encapsulation of the nanofluidic channel also provides the necessary integration of the detection nanostructures with a larger microfluidic interface that allows the loading of biomolecules onto the chip. Test measurements with DNA-protein complexes were performed to show a proof-of-principle of the tandem time-of-flight detection scheme demonstrated with our device.

MATERIALS AND METHODS

Device fabrication

Microfluidic channels with an H-shaped geometry^{15,17,31} were defined on a fused silica wafer by means of photolithography using a mask aligner (EVG-620, Austria) with S1813 photoresist (Shipley) spin-coated at 4000 rpm for 25 s. Obtained features were etched using an inductively coupled plasma etcher (ICP Samco RIE-10iP, Japan) to 1 μ m depth (Fig. 1(a)). Then, contact pads and microwires to connect the tandem nanoelectrode detectors (Fig. 1(b)) were mask-aligned and defined also by means of S1813 positive-tone photolithography. These patterns were then etched down 60 nm using reactive ion etching (RIE, Plasmalab 80, Oxford Instruments). Then, a Ti/Au bilayer of similar thickness (10 nm Ti, 50 nm Au) was e-beam evaporated on the trench with a Peva-600E High Precision Electron Beam Evaporator (AST

FIG. 1. Schematics of device fabrication process: (a) First exposure and double alignment of a contact shadow mask to define an H-shape microfluidic channel with a step-like micro-nano interface. (b) Microlithography for the definition of micro-contact wires. (c) First EBL, pattern transfer, metal evaporation and lift-off to define nanoelectrodes and alignment nanokeys. (d) Second EBL after double alignment with nanokeys and RIE pattern transfer. (e) Encapsulation at room temperature with a gasket PSQ polymer layer coated and cured on a glass coverslip. (f) Bonding of the loading reservoirs on the back of the chip.

Instruments, Taiwan) and later lifted off with an acetone soak, assisted by low-power ultrasonication. This defined a metal structure coplanar with the surface (to obtain better device encapsulation) as well as metal alignment keys needed for the alignment of the first EBL process.

For the first EBL step, we coated a layer of ZEP520A e-beam resist (Zeon Chemicals, Japan), and a top layer of Espacer300z charge dissipating material (Showa Denko K.K., Japan) to achieve the e-beam patterning on fused silica substrates with an Elionix ELS-7000 Ultra High Precision Electron Beam Lithography System (at 20 pA emission current) (see also a recent review on the fabrication of nanofluidic devices by Duan *et al.*³²). A simple fabrication scheme using electron beam overlapping was adopted to create a small strip of e-beam resist between the exposed electrode tip regions.³³ Nano-registration keys (12 μm long, 500 nm wide and sharp tips of 20 nm in radius) were also written during this step, to allow the alignment of the nanochannel structure to the nanoelectrodes during the second e-beam step. The obtained features were then subjected to a two-step RIE etching process to transfer the e-beam defined pattern into the substrate.

Following RIE, an e-gun metal evaporation step was performed to fill up the pre-etched trenches with the nanoelectrode shapes using a home-assembled UHV 6 kW e-gun evaporator. E-gun metal thin-film deposition was performed under high-vacuum conditions (1.1×10^{-8} Torr) and with a slow deposition rate to allow the filling of the metal film in the pre-etched portions on the silica substrate. A 10 nm thin film of Ti was deposited as an adhesion layer followed by a 50 nm thick Au layer and a final 8 nm Ti layer which was used to convert the metal sandwich into a metal mask able to withstand the high power etching process that followed nanochannel e-beam writing, as gold is prone to sputtering during RIE processes and its re-deposition results in severe grass formation.³⁴ The residual metal and mask were

lifted off leaving just the buried electrode metal structures with nanogap between tips and alignment nanokeys (Fig. 1(c)). The section of the geometry, defined by a loading nanoslit with a funnel shape, provides an overlap with the micro-to-nanofluidic interface, and an array of 500 nm diameter posts present in the funnel aids prestretching and uncoiling of the DNA molecules (Fig. 1(d) and Fig. S1 in the supplementary material³⁵). After development, nanochannel features were etched down to 60 nm using the same etching recipe and substrate coupling technique as it was used for the transfer of the electrode tips' shape to the surface (Fig. 1(d)). Though focused ion beams (FIB) milling technique may be used to define channel structures,³⁶ here our microwires and resulting embedded nanoelectrode pairs were connected using an ion beam assisted deposition (IBID) process using a dual-beam FIB (DBFIB, FEI, NOVA-600) to render Pt patches coplanar to the surface and overlapping both ends of the metal structures. The device was then cleaned and encapsulated (Fig. 1(e)) with a thin $18 \times 18 \text{ mm}^2$ glass coverslip, spin-coated with polysilsesquioxane (PSQ, Gelest, Morrisville, PA), following an oxygen plasma treatment step.^{37,38} Finally, the loading reservoirs were bonded onto the backside of the chip (Fig. 1(f)) with UV curing adhesive (Norland Optical Adhesive No.108, Cranbury, NJ). See the supplementary material for more device fabrication details.³⁵

Electronic and optical measurements

After sealing, the chip package was wedge-bonded to a chip carrier with home-built on-board electronic system for signal amplification, filtration, and data acquisition, in order to record net pA/nA current changes across the nanogaps (see Fig. S2 in the supplementary material for the electronic board³⁵). The board was mechanically mounted onto a specimen holder of an inverted Leica fluorescence microscope (Leica DMI 4000B), with mercury lamp light source and a K3 filter cube (Ex/em BP 470-490 nm/LP 515, Leica). A $100\times$ oil-immersion objective (N.A. 1.4) and an electron-multiplying charge-coupled device (EMCCD) camera (iXon888, Andor Technologies) with an exposure time of 0.1 s/frame were used to make simultaneous fluorescence microscopy.

Preparation of DNA-protein complexes

Test experiments were conducted with genomic T4 (166 kbp, Wako, Japan) and λ -phage (48.5 kbp, Sigma) DNA molecules labeled with YOYO-1 dye (1:5 dye/base-pair ratio) suspended in observation buffer ($0.5\times$ Tris/Borate/EDTA (TBE), 10% (w/v) glucose, 2.5% (w/v) polyvinylpyrrolidone (PVP), and 0.1% (v/v) Tween 20) containing an oxygen scavenger system (50 $\mu\text{g/ml}$ glucose oxidase, 10 $\mu\text{g/ml}$ catalase and 0.5% (v/v) β -mercaptoethanol) to minimize photobleaching.^{17,39} DNA-protein complex was prepared by formaldehyde crosslinking mechanism⁴⁰ using genomic λ -DNA conjugated with *E. coli* RNA polymerase holoenzyme (RNAP, Epicentre Biotechnologies) (detailed bioconjugation protocol will be published elsewhere).

RESULTS AND DISCUSSION

To find out the smallest electrode gaps that could be created, an approach using the Gaussian overlapping of the electron beam exposure³³ and special CHF_3 -based etching techniques were used (see the supplementary material for more device fabrication details).³⁵ Figure 2 shows the results, with nanoelectrodes coplanar to the surface with effective separations down to 3–4 nm that could be accomplished using this method. However, the yield varies for different size of nanogaps, which is $\sim 40\%$ for 3–4 nm gaps (Fig. 2(a)), $\sim 95\%$ for 8–9 nm gaps (Fig. 2(b)), and $\sim 100\%$ for gaps larger than 10 nm.

E. coli RNA polymerase holoenzyme (RNAP) used in our experiments were reported to be around 15 nm (Ref. 41) and DNA-RNAP complex can be around 20 nm. Although the creation of sub-10 nm gaps is hereby demonstrated, bigger gaps in the order of 20 to 30 nm across tips were expected to provide the required geometry to allow the passage and sensing of these complexes. The resistance of our electrode gaps was tested in vacuum using an *in-situ* probe system that locates a pair of probe needles with the aid of a nanomanipulator (Zyvex S200-4) inside an SEM (FEI-Inspect) coupled to a semiconductor characterization system (Keithley 4200).

FIG. 2. (a) and (b) SEM images of electrode nanogap structures with gap sizes as small as 3–4 nm. (c) Optical image of a nanochannel overlapping electrode nanogaps after resist exposure and development. Nanokeys (see inset for details) are used to register the nanochannel structure to the nanoelectrodes during the second EBL step.

Leakage currents can be measured with sub-fA resolution and typical resistance to be in the order of 10^{11} – 10^{12} Ω for the 20–30 nm gaps, indicating excellent insulation properties.

Conventional EBL process use laser interference calibration to reduce overlay mismatch, but is limited to tens of nanometer error range. Recent works using EBL to demonstrate very high overlay accuracy relies mainly on secondary electron detector signals using complicated mathematical algorithms and expensive software to measure the overlay error based on micrometer sized alignment keys and provides feedback to correct such errors.^{42,43} These methods lack accuracy when considerable changes occur in the alignment keys, like rotation, scaling or degradation of alignment keys during the multi-step fabrication process.⁴⁴

In our system, a 0.31 nm beam positioning resolution is realized in the ELS-7000 e-beam writer by utilizing an 18-bit DAC. Also, by employing a laser interferometer with surface reading resolution of 0.6 nm, both the stitching accuracy and overlay accuracy are attained within 30–40 nm. With the aid of nano-alignment keys, featured by their sharp tips (Fig. 2(c)), we narrow down the dimensions of the central coordinates near the tips to just few tens of nanometers, while their effective tip radius (20 nm) is comparatively smaller (~ 2 – 3 times) than the nanochannel. A pair of such nanoalignment keys functions as registration marks, used by the EBL alignment software to effectively calculate the rotational and positional displacements of the work piece. The accurate location of the center of the nanokey defines the degree of alignment between the nanochannel structure and the nanogaps. Consequently, the nanoalignment key system presented in this work constitutes a reliable method to perform multistep EBL processes with a high degree of alignment precision. Shrinking the size of alignment keys from conventionally used micrometer sizes to nanometer size enhances the alignment capability and reduces unpredictable overlay mismatch, rendering precisely aligned nanogap array across a nanochannel (Fig. 3(a)). Here, we would like to stress the important function our nanokeys serve to ensure the coplanarity of the nanochannel and the electrode nanogaps. Conventional alignment key designs are micron-sized and deposited (or etched) with certain thickness above/below the surface, in order to provide sufficient contrast for the (edge or shape location) recognition algorithms.^{42,43,45,46} Lack of surface co-planarity could induce large strains in the polymer to surface bonding front, leading to poor bonding and/or detachment of that region and later to leakage, as observed during our earlier experiments involving non co-planar alignment keys. In addition to surface co-planarity, large metal surfaces seem to be detrimental for leak-free sealing, as PSQ polymer used in our experiments showed poor bonding for such large

FIG. 3. (a) SEM tilted angle images of three tandem nanoelectrode gaps integrated into a single 100 nm wide nanochannel from (b), with the gap distance ~ 30 nm in all cases. AFM characterization confirms the planarity of electrodes with the channel surface to be within 10 nm (see Fig. S3 in the supplementary material for AFM characterization³⁵). (b) Optical micrograph of embedded structures and connecting wires integrated by IBID. (c) Optical micrograph of the resulting structures after PSQ bonding where dark regions correspond to the bonded surface.

metal region, due to its much higher rigidity than polydimethylsiloxane (PDMS).³⁷ Using coplanar nanoalignment keys eliminates these problems. Disregarding their small size, the nanokeys still provide a good contrast for the secondary electron detector, utilized by the commercial EBL tool during the mark registration process. This is proven with the high quality alignment of three nanogaps inside a nanofluidic channel, as shown in Fig. 3(a). We have designed the nanoalignment key position in a way that it is in the same electron-beam writing field as the nanogaps. This is important, as the proximity of one of the nanoalignment marks to the intended position of the later nanochannel overlay affects the rotational accuracy of the overall alignment (see below). In addition to this, the alignment strategy does not require any post-processing algorithms, besides the one that calculates the rotational and translational matrixes for the manual registration strategy of the EBL manufacturer (Elionix Corporation, Inc.).

Optical bright-field imaging was used as the preliminary assessment tool to determine the effectiveness of the channel's overlapping (Fig. 2(c)). Results showed that effective overlapping between the first and the second e-beam steps allows a sub-100 nm alignment precision, and that variations in the key tip geometry and size will lead to mismatches. Nanochannel width dimensions ranging from 90 to 200 nm were demonstrated by this technique maintaining the same overlap near the center between electrodes, but side shifts from the center are noticeable when the dimensions of the nanochannel are further narrowed down.

Following the alignment, exposure and development of the nanochannel structure, an RIE process permits effective overlapping between the tips of the electrode structures and the nanochannel thereby creates a free passage in the nanogap between tips as the insulating material is etched away, leaving an electrode wall with a high degree of anisotropy. Topographical images by Atomic Force Microscopy (AFM, Innova, Veeco, US) confirmed the existence of the overlapped structures with the claimed width and depth dimensions (see Fig. S3 in the supplementary material for AFM characterization³⁵). The degree of coplanarity ranges between 5 and 10 nm, but metal blur structure formation as reported⁴⁷ is reduced by the allowing use of

ZEP520A as e-beam resist which has slightly negatively tapered profile that aids the lift-off process in the coplanar electrode structure.

Our technique offers the possibility of writing tandem gaps simultaneously, the existence of multiple electrode pairs along one nanochannel. Tilted angle SEM images (45°) of a channel 100 nm wide and 60 nm deep, with 3 embedded tandem electrode nanogaps, with gap size of 30 nm and inter-electrode pair spacing of $5\ \mu\text{m}$, are exemplified in Fig. 3(a) with the corresponding layout in Fig. 3(b). The planarity of the nanoelectrodes and the channel surface has been further verified by a leak-free PSQ bonding (Fig. 3(c)). Electrode nanogaps in the same channel have similar widths with size variation below 5% according to the information extracted from the electron micrographs. The existence of different detector pairs of similar size and shape along the nanochannel opens up the possibility of studying cross-correlation in ionic current blockade events, as different electrode pairs that sample simultaneously while the same DNA molecule is passing by in the same channel could provide time-correlated information about the sample.³⁹

Preliminary results obtained from translocation of genomic DNA molecules and DNA-RNAP complexes across the nanogaps show the possibility of such measurements. Initial fluorescence microscopy investigations showed that T4 DNA could effectively make its way into the nanochannel after applying an initial pulse of 1–2 V to overcome the entropic barrier at the micro-to-nano step interface, after which an array of nanoposts present in the funnel-shaped region (Fig. 4(a)) provided the final uncoiling before DNA entered the nanochannel. DNA then acquired a linear conformation while effectively passing the nanogap regions (Fig. 4(b)) without leaving behind ionic current blockade signatures distinctive from the background noise, because of its small diameter ($\sim 2\ \text{nm}$) compared to the electrode gap size of 30 nm (Fig. 4(c) top).

However, when λ DNA-RNAP complexes are driven through the structures, with a driving potential of $\sim 1\ \text{V}$ applied across the nanochannel from the reservoirs (V_{drive}) and 10 mV transversal bias potential (V_b) across the nanoelectrode gaps, they leave characteristic signatures of a current drop from the background of $\sim 150\ \text{pA}$ ($< 30\ \text{pA}$ r.m.s.) to less than 40 pA (Fig. 4(c) bottom). A close-up look of such signatures (10-point moving averaged) indicates the distinctive translocation event for a DNA-RNAP complex recorded simultaneously in three tandem gaps (Fig. 4(d)). As the molecule is passing rapidly through the detector and due to the limitation in the temporal resolution of the present electronic setup (1 ms), detailed information of the protein complex cannot be inferred. To be noted, though the measurements are only a single bit lower than the baseline (Fig. 4(c) bottom), they are not single pixel time events, but consist of at least 9 to 10 points acquired at a resolution of 1 data-point/ms. Moreover, these events are away from the mean values by more than 3σ after 10-point moving average process, statistically significant to confirm them as real events (the mean values from the 3 tandem electrode array are 140, 166, and 154 pA and the corresponding 3σ values are 41, 48, and 61 pA, while the respective translocation peaks read 55, 82, and 91 pA). For all peak values fall within the 3σ of the baseline means are disregarded as non-translocation events. Moreover, the fact that the same event is observed with a small time delay in all three electrode nanogaps (Fig. 4(d)), confirms that these events arise from DNA-protein complex translocation and not random noise. This further supports that a tandem array setup is advantageous in distinguishing real events from false positives than single pair of nanoelectrodes.

Additionally, from our data, time-of-flight information could be extracted using the shift in the curves. The full width at half maximum of the current blockade obtained in three tandem nanogap detectors rounds 9–10 ms and the time difference between detectors is 1 ms. Simple estimation of the speed of molecules is $\sim 4\text{--}5 \times 10^3\ \mu\text{m/s}$ as the distance between different pairs is $5\ \mu\text{m}$. Differences in the length of the molecule arising from the calculations ($45\text{--}50\ \mu\text{m}$) to the expected fully stretched contour length of YOYO-1 labeled λ -DNA in a similar nanochannel setup ($22\ \mu\text{m}$)^{48,49} could account for the limited time resolution (1 ms), conformational rearrangements upon the entrance of head of the molecule complex to the nanogaps,^{30,39,50} as well as friction in the nanogap surface due to the applied detecting bias.³⁰

To increase the signal-to-noise ratio of translocation events, we tried to slow down the translocation speed by reducing V_{drive} . In such cases, we observed current blockade signatures

FIG. 4. (a) Assorted fluorescence images of the stretching of YOYO-1 labeled T4 DNA molecule in a nanochannel. The posts in the nanoslit region and the funnel-shaped region help pre-stretching DNA molecules before entering the nanochannel. (b) Fluorescence image of a YOYO-1 labeled λ DNA molecule in the nanochannel with embedded nanoelectrodes. (c) Electronic readouts from the tandem detectors show ionic current blockades when λ DNA only (top) and λ DNA-RNAP complexes (bottom) translocate across the nanogaps, with only the latter leaving evident signature distinguishable from the background under 1 V of driving potential (V_{drive}) and 10 mV bias (V_b). (d) A close-up look of DNA-RNAP signatures recorded simultaneously in three tandem gaps, shown with 10-point moving average of the raw data. (e) Much slower DNA-RNAP translocation events observed with $V_{\text{drive}} = 50$ mV and $V_b = 10$ mV (black: raw data; red: 10-point moving averaged data).

3–4 times different than the baseline with $V_{\text{drive}} = 50$ mV (Fig. 4(e)). The duration of such events could be 20–30 times longer than that with faster translocation (Figs. 4(c) and 4(d)). However, at very small V_{drive} , V_b becomes a retarding potential, which could deter the molecule from passing through the gaps.³⁰ Hence, for practical applications the ratio of V_b/V_{drive} needs to be optimized.

Finally, we will discuss the resolution improvement strategy for our electronics. The reason of the relatively poor resolution (1 ms, 100 pA) in our current setup, when comparing to existing commercial patch-clamp amplifiers, such as Axopatch 200B Amplifier (Molecular Devices) which has a time resolution of 10 μ s and an open-circuit noise level (root-mean-square value) of ~ 1 pA at 100 kHz bandwidth, is due to the fact that our original electronic design was meant to be potentially incorporating AC dielectrophoresis (DEP) driven by high-frequency AC signal (up to MHz) for molecular manipulation^{51,52} (though not implemented and demonstrated in this device) that limits the bandwidth and sensitivity related to the filtering of the AC trapping field

for future experiments. However, we considered that this was good enough to find the parameters needed for the construction of an optimized later version of the detection electronics. The improvement to a new instrument version is currently undergoing, which is targeting performance of 100s of kHz sampling rate and few pA sensitivity by removing the AC DEP-enabling functionality to optimize the speed and length of biopolymer translocation study. Despite the current limit in detection capability, our instrument nevertheless constitutes an improvement of the state-of-the-art in this field, provided a chip redesign and the aforementioned nanoalignment key assisted method disclosed in this article are implemented. For example, commonly used Axopatch 200B Amplifiers, with a time resolution of $10\ \mu\text{s}$ and an open-circuit noise level (root-mean-square value) of $\sim 1\ \text{pA}$ at 100 kHz bandwidth, are made of bulky electronic boxes that have up to 2 parallel readers (mostly comes with single channel) capability. While being expensive, it also requires the use of a separate head plus very special shielding techniques and interconnects to the chip to avoid Faradaic currents on the circuitry. On the other hand, our design is a compact modular instrument that can fit into most microscope stages without the need of complex shielding nor fixture systems, and has already been designed and constructed to host up to 15 individual detecting channels which could also render an expanded sequential tandem array of nanogaps embedded coplanarly in the same nanochannel for later leak-free sealing. We believe our device design presents itself a novelty in the reduced electronic detection costs, increased portability and modularity of nanoelectronic sensors for complex analysis of translocating samples. Particularly our approach can apply equally to the study of other DNA/protein mapping applications, such as DNA methylation profiling¹⁶ and chromatin modification mapping.⁵³

CONCLUSIONS

To summarize, a two-step EBL process that uses nanoscale alignment keys to effectively overlap two different geometries from different materials on the nanoscale has been presented. This strategy has been demonstrated for the first time by fabricating embedded nanoelectronic detector gaps coplanar, within 10 nm, to the nanochannel surface. Further, three sets of tandem electrode pairs have been fabricated inside a sub-100 nm nanochannel with a spacing of $5\ \mu\text{m}$ and size variations less than 5% between consecutive electrode pairs. The device was successfully bonded through a thin polymer gasket layer and demonstrated to detect DNA-RNAP complex with reproducible tandem blockage current signals. This opens up the possibility of simultaneous time-of-flight data acquisition for correlated study of biopolymers that could reduce false positive signals and shed light on the conformation, length, mobility, and other dynamic information of the sample molecules. With on-going improvement of time resolution of the reader to microseconds, our coplanar tandem detectors could be used to study, in general, high-resolution protein binding events or epigenetic modifications along a DNA backbone and serve as a nanoscale correlated Coulter counter for single molecule analysis of heterogeneous samples in general.

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