Special Issue on Advances in Life Science Systems and Applications: Guest Editorial

Life sciences encompass aspects of living organisms, their organization and behavior, as well as their relationship to each other and the environment. Historically, biology has played a central role in the life sciences [1], but other fields such as medicine, ecology, and ethology have also played important roles. Technological advances in the life sciences have inevitably followed advances in physics, chemistry, and engineering.

Since the invention of the integrated system in the 1950’s, the microelectronics industry has seen a remarkable evolution—from the centimeter-scale devices created by Kilby [2]—to millimeter-scale integrated circuits fabricated by Noyce—to today’s 8-nm feature size MOS transistors [3]. During this time, not only have exponential improvements been made in the size of the devices [4], but the computer-aided design and workstation technologies have advanced at a similar pace, enabling the design of complete truly complex systems on a chip. Such advances in the microelectronics industry have also enabled the proliferation of computational fields for bio-informatics, systems biology [5], imaging, and multiscale multidomain modeling [6].

The stunning convergence of information technology and life-sciences research is transforming the landscape of the pharmaceutical, biotechnology, and healthcare industries, signaling the arrival of personalized and molecular imaging diagnosis and treatment, speeding up the pace of scientific discovery, and changing the practice and delivery of patient care.

Many of the biggest engineering and technological challenges in advancing the state-of-the-art are how to collect, process, compress, integrate, archive, model, analyze, interpret, and mine the massive quantities of heterogeneous biological, chemical, imaging, clinical, and cognitive data available as a result of the rapid advances made in biotechnology, imaging, and informatics during the past few decades. Never before has there been a merger between engineering and biomedicine been so strong, and the IEEE Circuits and Systems Society (CAS-S) believes it can make a major contribution to the interdisciplinary glue bonding the two disciplines.

The paper contributions in this Special Issue of the IEEE Transactions on Circuits and Systems—I: Regular Papers provide a snapshot of advances and cutting-edge research results from a community of scientists and engineers in the Circuits and Systems community that has ventured outside their traditional research areas and into the life sciences arena. Out of 43 original contributions, a rigorous review process yielded 16 papers, an eclectic mix ranging from integrated circuits for life science and applications to bioinformatics—to signal and imaging processing and their applications in the life sciences—to dynamical systems models of biological structures at different scales, from molecules to behavior. All papers had at least three reviews, and, in many instances, four and five substantial reviews. Also notable is the observation that even within a paper, the authors come from diverse backgrounds, ranging from applied mathematics and computer science—to radiology and electrical engineering—to bioengineering and applied physics.

In a group of papers that have a hardware-oriented focus, we see some truly novel approaches to signal conditioning and quantization, central functional blocks in modern integrated circuits enabling the conversion of real-world signal into digital information. For example, a state-of-the-art ultra-low-power analog-to-digital converter architecture for biointerfaces is presented in the paper by Yang and Sarpeshkar. The authors report data-conversion efficiency from fabricated chips of 0.12 pJ per quantization level, which is the best efficiency reported in the literature to date. In the paper by Gore et al., the authors present a multichannel architecture for a potentiostat array, aimed at endowing biosensors with digital interfaces. The system is employed to measure conductance variations in antigen–antibody binding.

Often, advances in technology give the opportunity to re-invent a classical experimental technique; in this instance, the patch-clamp method that allows the study of ion transport in the channels of cell membranes. Twenty five years ago, Sigworth, working in the laboratory of Nobel Laureates Neher and Sakmann, made key contributions to the basic patch-clamp technique. Today he is a co-author of a paper with Laiwalla et al., reporting on an integrated electronic interface for a high-throughput planar patch-clamp system. The chip is fabricated in silicon-on-insulator CMOS and because of the insulating substrate, the authors report background noise level lower than a similar system designed and fabricated in bulk CMOS technology.

For applications requiring tissue-level sensing, Genov et al. report on an interface integrated circuit for sensing and quantizing chemical signals. Working at the molecular level, Bhattcharaya et al. present a microsystem architecture for biological analysis and novel diagnosis methodology using DNA sensing. By exploiting the properties of fabricated silicon nanopores, with a sophisticated, adaptive ac measuring technique, the authors demonstrate convincingly that they can analyze the size distribution of molecules that modulate the conductance of the nanopore. Finally, in the hardware category, we have a lab-on-chip architecture for the handling, immobilization, and probing of bio-molecules such as DNA and antibodies. In the next paper, Piedade et al. also provide a lumped parameter circuit model for the various components on the chip. It is worthwhile noting that in all but the last paper in the hardware area, the devices were tested with real biological systems.
signals. Moving from the molecular and cellular to the human scale, El-Sharkawy et al. report on a system architecture and algorithms for adaptive calibration of a noninvasive temperature sensing device, an RF radiometer suitable for use in an MRI scanner.

In the algorithms and applications, three contributions link advanced mathematics and computational techniques. In the paper by Yang et al., the authors describe an algorithm to automate the image processing at the individual cell level in time-lapse fluorescence microscopy. Xiong et al. present algorithms based on deformable models for image analysis of data derived from high-throughput RNA interference experiments. Pham et al. report on algorithmic developments using two spectral pattern methods for gene expression data for cancer classification.

A set of four papers report on algorithmic and basic mathematical contributions that relate to gene networks. Xiao and Daugherty tackle data inconsistencies through probabilistic Boolean networks. In their paper, they report on optimization methods for gene regulatory network structure design. Alterovitz et al. employed topological analysis to detect and characterize gene lethality. The paper by Chen and Wang is a synthetic biology paper aimed at designing gene regulatory networks that have specific functions. Their formulation employs fundamental notions of control systems and feedback both continuous and discrete time. Of more theoretical nature is the paper by Li et al., where the authors analyze the stability of genetic networks with sum regulatory logic.

Parlikar et al. report on an equivalent circuit model that is aimed at capturing cardiovascular dynamics. Their paper demonstrates nicely how one can gain insights on problems in the life sciences through linear and nonlinear lumped parameter circuit models. Finally, at the behavior level, we have an intriguing contribution by Lin et al. In their paper, the authors report on an adaptive EEG-based alertness estimation system to monitor the human cognitive state with applications in car driver safety.

In selecting the papers for this Special Issue, the Editorial Board and reviewers had to make difficult paper-acceptance decisions that go beyond the regular editorial and reviewer duties of members in our community. Guided by the advice of the Editor-in-Chief, Dr. Sankar Basu, many good papers did not make it into the Special Issue as the Editorial Board strived to give some focus to an otherwise very diverse submission pool of papers. It is our hope that this Special Issue will generate further interest in life sciences applications for circuits and systems either to this journal or to the newly established CAS journal, IEEE Transactions on Biomedical Circuits and Systems. We encourage our community to work and make advances in a field that is so central to our quality of life.

As we begin to make small strides in the 21st century, we are witnessing the birth of Life e-Science, an integrative discipline that goes beyond the individual domains and is aimed at the automation of the processes and systems that will enable sophisticated real-time prediction as well as closed-loop diagnosis and disease treatment.

With an ever more accelerating technological and scientific progress in the life sciences, in 50 years from now, we predict that many of the world’s premier medicine and health care research institutions will be turned into historical museums. Visitors will be able to get a glimpse of how top-tier medical care and research was carried out in the 20th century. What used to be laboratory space and patient rooms, will host high-performance redundant data storage servers and supercomputers. Health care will be delivered at home using patient monitor services and chip-level implantable instrumentation. Wireless, electronically programmed chips will control delivery of drugs in a timely and precise fashion based on algorithms that monitor the individual daily function and behavior. Molecular imaging technologies will allow cellular and tissue level real-time monitoring of electrical, chemical and other physiological signals, enabling closed-loop drug therapies. The pharmaceutical industry would have survived the downhill spiral in the early part of the century as the model of drug testing on rats and mice was abandoned. A new paradigm for research will evolve around engineered organs and whole animal models from stem cells.

In 50 years from now, a century would have passed from the invention of the first microchip and Moore’s Law will be no more. However, we will be living in an era where the chip, short for the microchip, such as the one depicted in Fig. 1 will provide the underpinnings and the foundation for affordable state-of-the-art global medicine and health care.

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He currently serves on the National Institutes of Health scientific review panels, editorial boards of several international journals, and program committees of several conferences and workshops. He is the past Chair of the Life Science Systems and Applications (LSSA) Technical Committee of IEEE Circuits and Systems Society. He has been a Licensed Professional Engineer of Electrical Engineering since 1991.
Abstract—Nanopore-based biomolecular sensing is an emerging nanotechnology which relies on the ability to measure changes in ionic conductance of single nanoscale pores as biomolecular analytes are driven through them, one at a time, by an applied electric field. Nanopores constructed from self-assembled proteins as well as using silicon-based fabrication techniques have been demonstrated to allow sizing and identification of DNA, RNA, proteins, and other biomolecules many times faster than with current technology. Despite the potential of nanopore sensing to produce “next generation” biomolecule analysis devices, its current demonstrations are based on the use of a simple dc stimulus across the nanopore. As a result, the resolution obtained is insufficient for many practical applications. In this paper, we report a novel optimization, translocation.

I. INTRODUCTION

In biotechnology and medicine, sequencing and separating chain-like biomolecules (e.g., single-stranded DNA, RNA, and proteins) is a process of vital importance [1], [2]. For DNA sequencing using current methods, an “amplified” sample of DNA strands of varying sizes is first generated by the well-established polymerase chain reaction (PCR) [3]. The PCR process generates DNA strands of varying lengths from the original sample, such that the length of a generated strand reflects the identity (A, C, G, or T) of the base at the fluorescently labeled termination position. The sequencing problem is thus reduced to size separating (or sizing) DNA strands. This step is carried out via electrophoresis in a gel or a capillary bundle, wherein the molecules are separated into bands, by virtue of differences in their transport rates through the medium as a function of their size and charge under an applied electric field. However, efforts to substantially raise the throughput rates of these devices are impeded by the “short read length” problem, i.e., inefficient operation at long sequence lengths due to very slow transport rates of long strands through the medium [1]–[4]. Furthermore, the sophisticated equipment and laboratory environment required does not permit “on-the-fly” field applications. In this light, overcoming the limitations of current DNA sequencing methods will result in a large technological impact, in terms of new applications such as “personalized medicine” (routine, patient-specific genome sequencing to diagnose genetic health risks), and fast genotyping of new pathogens or biological warfare agents to allow a rapid therapeutic response [5].

In recent years, research at the biology–nanotechnology interface has shown potential for creating revolutionary advances in speed, efficiency, reliability and portability of biomolecule sensors. An underlying advantage of a truly nanoscale biomolecule sensing technique is the ability to detect single molecules at a nanometer length scale and at very short time scales, using only small amounts of sample. Operation at such short-length scales would remove the transport limitations associated with electrophoresis technology. Of several proposed strategies for sizing DNA, the use of nanoscale ion channels is particularly attractive [6]–[10]. The sensing element is a nanometer-scale pore (∼2–5 nm in diameter and a few nanometers long) embedded in a substrate. Sensing occurs by measuring the partial blockage of ionic current through a single nanopore as individual analyte molecules are driven through the pore by an applied electric field (Fig. 1). Such measurement techniques were originally developed to study ion channels in living cell membranes [11]. For chain-like molecules such as DNA, RNA, and proteins, the duration of current blockage strongly correlates with their chain length. The advantages offered by this technique include very high detection sensitivity (single molecule levels), extremely rapid and reversible response due to the short detection length and small time scales (c.a. 1 nm and ∼1 ms, respectively), good signal-to-noise ratio even at low analyte concentrations (since single molecules are detected irrespective of their concentration), and concurrent multiple-analyte sensing using arrays of nanopores. It is estimated that these devices can increase sizing speeds from 10¹¹–10¹² bases per day on a single electrophoresis...
instrument, to levels of $10^7$–$10^8$ bases per day; i.e., 3–4 orders of magnitude higher [12].

Different types of nanopores [6]–[10], [13], [14] have been proposed or demonstrated for use in the above devices, and are currently under further development in several research groups. Concurrently, there is an important requirement for developing efficient diagnostic methodologies and algorithms for operating the nanopore sensor and analyzing its output. Preliminary demonstrations of nanopore devices have invariably used a dc voltage [6]–[10] to translocate the biomolecules through the pore. As a result, the performance of the sensor is limited by the physics of transport of molecules under a dc voltage, whereas the use of more generalized as well as properly optimized stimuli can, in principle, allow much better performance. Hence, more sophisticated operating protocols must be developed to enable the processing of real samples in an efficient and reliable manner, and to optimize the characteristics of the sensor, such as sensitivity and signal-to-noise ratio. These diagnostic tools should be based on the essential physics of translocation of chain-like biomolecules through a nanoscale channel driven by an applied voltage stimulus, and must take into account the stochastic nature of this single-molecule process.

II. OVERVIEW OF DNA DIAGNOSIS SYSTEM

The physical basis of the present sensing technology is the measurement and interpretation of ionic current (picoamperes to nanoamperes levels) through individually addressable nanoporous ion channels (of 2–5-nm diameter) in a substrate (e.g., silicon nitride membrane of $\sim$5–20-nm thickness). An aqueous medium with a dissolved electrolyte such as KCl enables the flow of ions between the electrodes. Fig. 1 illustrates the proposed setup of a nanopore-based biomolecule analysis system. When a biomolecule is driven through the nanopore, the ionic current flowing through the pore is partially blocked due to the portion of the molecule within the pore [6]. The duration of the current blockage correlates with the translocation time for the molecule through the pore, which is in turn directly correlated to its length [6]–[10].

The electrodes that are used to supply the driving stimulus may be positioned close to the nanopore to detect small fluctuations of currents during translocation. In previous experiments, the nanopores and the electrodes were submerged in a polymeric (e.g., teflon) sample cell of fluid volume approximately 0.1–1 mL [10]. Based on current research efforts, it is anticipated that this apparatus will be replaced in the near future with a nano/microfabricated system on a silicon chip, containing an array of individually addressable nanopores enclosed in fabricated micro-chambers with microelectrodes at appropriate locations. This system would be combined with a circuit chip that analyzes the signals from the nanopore array, as well as a micro-fluidic system for handling input and output of analyte samples, as shown in Fig. 1.

![Fig. 1. (a) Internal diagram. (b) Operating principle of the sensor: in “open” mode, the pore permits a high current. During translocation, the current is reduced to a low level due to partial pore blockage by the molecule. The blockage duration ($t_B$) correlates to the molecule length.](image)

III. OBJECTIVES

DNA is a highly charged biopolymer, and can carry a maximum charge of approximately $-4e$ per monomer, where $e$ is the charge quantum ($1.67 \times 10^{-19}$ C). The motion of the DNA monomers under the influence of an applied electrical potential is governed by three main forces: electrical, viscous drag and a rapidly fluctuating random thermal force. The random force and the drag force are related to each other, since the molecular origin of both forces is the same, viz., bombardment of the biomolecule by solvent molecules. The random force follows a normal distribution with zero mean and standard deviation proportional to the drag force, temperature and the mass of the molecule. The various forces acting on the DNA monomers are explained later in this section.

The change in the channel resistance due to the passage of a DNA molecule through the nanopore causes a change (ca. 50–100 pA) in the ionic current. The DNA strand length can
be calculated based on the duration for which the channel is blocked, known as translocation time \( t_B \). The translocation time for DNA polymers of the same length (under an applied dc potential) shows a stochastic variation due to the random force effects operating during each translocation event. This reduces the resolution of the sensor for distinguishing between two different chain lengths that are close in value. Even for two chains that can be clearly distinguished, the process may become time consuming and inefficient due to the measurement time required for improving the statistical quality of the data. To increase the resolution, we propose to use an optimized ac voltage stimulus (called ac stimulus from now on) that is “tuned” to the time scale of DNA transport through the nanopore, which is in turn a strong function of its length. By doing so, the difference between the translocation times of DNA strands of comparable lengths can be increased significantly compared to using a dc voltage stimulus (called dc stimulus from now on) alone. As presented in the paper, this results in an efficient method for sizing an unknown mixture of DNA molecules.

IV. PHYSICS OF DNA TRANSPORT IN A NANOPORE

In this paper, we are concerned with using a simple model that captures the essential physics of DNA translocation in order to demonstrate the proposed approach. The DNA molecule is modeled as a rigid cylindrical rod [18]. However, more complicated simulation engines for DNA translocation can also be coupled to our proposed optimization procedure. For simplicity, it is also assumed that the motions along \( x \) and \( y \) directions are restricted and hence the only degree of freedom of movement for the molecule is along \( z \)-axis, i.e., along the nanopore axis. The DNA strand has \( N \) monomers (“bases”), each of length \( l \sim 0.4 \text{ nm} \) [18], giving a total length of \( L = N l \). The DNA strand has a diameter \( d \sim 1.5 \text{ nm} \). The lengths (>50 nm) of DNA strands of interest are much greater than the pore length (\( \sim 5 \text{ nm} \)), so that the interactions of the molecule with the pore walls do not affect the translocation process.

A. Forces Acting on the Molecule

Electric Force \( (F_E) \): The total charge on the DNA strand is \( Q = -4qN \), where \( q = 1.6 \times 10^{-19} \text{ C} \). We assume a potential difference of \( V(\omega, t) \) applied to the electrodes resulting in an electrical force given by

\[
F_E = \frac{QV(\omega, t)}{s}
\]

where \( s \) is the separation between the electrodes.

Viscous Drag Force \( (F_D) \): The magnitude of drag force acting on the DNA molecule is given by [19]

\[
F_D = -M\gamma \frac{d\vec{v}}{dt}
\]

where \( M \) is the mass of the DNA strand \( (M = Nm, \text{ with } m \text{ being the mass of a single monomer of DNA, } ca. 307 \text{ a.m.u.}) \), \( \vec{v} \) is the velocity of the DNA strand, and \( \gamma \) is the drag coefficient of the solution given by [19], [20]

\[
\gamma \equiv \frac{3\pi\eta L}{M\ln \left( \frac{d}{4} \right) + 1}
\]

where \( \eta \) is the viscosity of water. The drag force is proportional to \( N/\ln(N) \), and increases with increasing chain length. The drag force is operational on the part of the molecule outside the pore.

Random Thermal Force \( (F_R) \): The fluctuating random force \( F_R \) [20] acting on a molecule follows a gaussian distribution with zero mean and a variance given by \( 2 M kT / \Delta t \). Here, \( k = 1.38 \times 10^{-23} \text{ J/K} \) is the Boltzmann constant and \( \Delta t \) is the time step used for numerical integration (typically \( \sim 1 \mu s \)). As mentioned earlier, the random force is strongly related to the viscous force, as the drag coefficient \( \gamma \) appears in both force definitions. Its origin, like that of the drag force, lies in the random collisions of the solvent molecules with the molecule. The viscous drag captures the time-averaged macroscopic force on the DNA strand due to collisions of solvent molecules, whereas the random thermal force captures the instantaneous fluctuations. The larger the integration time step, the smaller the fluctuations in random force, essentially “smoothened out” by time averaging.

B. Equation of Motion

The translocation of the DNA molecule through the nanopore in the presence of an electric field can be modeled using the equation of motion

\[
M \frac{d^2\vec{v}}{dt^2} = F_E + F_D + F_R.
\]

The initial velocity for the molecule can be obtained by equating its kinetic energy along the \( z \)-direction to the one-dimensional thermal energy \( 1/2kT \)

\[
\frac{1}{2} M \nu_0^2 \rightarrow v_0 = \sqrt{\frac{kT}{M}}.
\]

Using (1)–(3) and substituting in (4), we get a differential equation in \( v(t) \), with the initial condition given by (5). Algebraic manipulation of (4) gives us the translocation time. Due to the random forces, the translocation time for the same molecule will be different for repeated simulation instances.

C. Correlation to Experimental Data

As reported in [21], the measurements of translocation time using a \( \alpha \)-HL protein nanopore were performed at 120 mV, 25°C. As reported earlier in [22], preliminary simulations were performed without correlation between the experiments and the simulation data. It is highly desirable that the simulated translocation time should be matched with existing experimental data. In [21], the translocation time was computed from the measured current blockage duration. Using the translocation time and the length of molecules in the calibrated solution (a solution with a known mixture of molecules), the terminal velocity was estimated. The terminal (steady-state) velocities of the molecules remain almost constant (0.15 \( \text{ Å} / \mu \text{s} \)) for molecules with more than 15 bases. Fig. 2 shows the terminal velocities of DNA molecules for base lengths 4–100.

In this paper, our primary goal is to demonstrate our approach using a simple but realistic model, which is first matched to experimental data. We use the viscosity of the solution as a parameter that is adjusted such that the simulated translocation time
D. Effect of dc Stimulus on Detection Sensitivity

Translocation was first simulated with a 120 mV dc driving voltage. The differential equation (4) was solved to obtain the translocation time for molecules with different values of $N$. The sensitivity of the translocation time to the length of the strand is given by

$$\frac{\Delta t_B}{\Delta N} = \frac{d}{dN} \frac{N}{t_B} = \frac{d}{dN} \frac{d}{d(N)} (\ln t_B).$$

The dc sensitivity curve is shown in Fig. 4. As evident from the figure, the sensitivity of the device is practically constant for almost the entire range of molecule sizes investigated, especially for long molecules, which are the primary target of the ultrarapid sizing technique.

E. Effect of ac Stimulus on Translocation

If a sinusoidal voltage waveform with carefully adjusted frequency, amplitude, and phase is added to the dc signal, the sensitivity for certain regions of strand lengths can be enhanced significantly. This is evident from Fig. 5, where the translocation time versus base length is plotted for an applied ac+dc stimulus, along with that for a pure dc stimulus. For some regions of $N$, the slope of the translocation time curve (and hence the sensitivity) increases sharply when an ac stimulus is applied. However, for other regions, sensitivity is lower than dc when an ac stimulus is applied.

We thus found that the sensitivity obtained varies with the frequency, amplitude, phase and the dc offset of the input ac and dc stimuli. A particular ac stimulus, i.e., a combination of frequency, phase, amplitude, and dc offset values provides better performance than dc for a certain pair of base lengths, and at the same time is worse than dc for another pair of base lengths.

The distributions of translocation times for two different values of $N$ (2000 and 2100) are shown in Fig. 6. The distributions are obtained by running repeated translocation simulations including the thermal force in the equation of motion. The introduction of an ac stimulus separates the two histograms further compared to a pure dc stimulus, providing better resolution and diagnosis. The ac stimulus used in this case has amplitude of 100 mV @ 30.7 Hz. For the pair of molecules shown in Fig. 6, the detection resolution (efficiency) is more than 3.5 compared to the dc stimulus. As described in
the next section, the amplitude, frequency, and phase of the signal can be optimized together to enhance the accuracy and increase the detection resolution of bases with similar lengths.

V. Optimal Diagnosis Algorithm

The diagnosis algorithm optimizes the frequency, amplitude, and phase of the ac stimulus to increase the sensitivity for a pair of molecules compared to dc. The algorithm consists of two main parts. The first part uses a dc stimulus to identify different base length regions that may be present in a given sample. In the next part, the ac stimulus is optimized for these different regions. Each region is investigated by optimizing the values of input parameters, i.e., frequency, amplitude, and phase of the input stimulus, to increase the detection sensitivity (i.e., the distance between the mean translocation times of the different DNA strands of comparable lengths). Fig. 7 shows the pseudocode for the proposed algorithm. The function “decision” uses a gradient search algorithm to determine the optimal frequency, amplitude, and phase for the ac stimulus to increase the sensitivity of detection. It also determines whether to increase or decrease the optimization parameters and the step size for the same. In addition, the algorithm maintains a history of the optimization steps. If the optimization parameters move away from a target value, it reverts to the previous point where best sensitivity was obtained, and changes the search direction.

The algorithm targets the attainment of sensitivity (using ac stimulus) equal to a user-specified performance enhancement factor ($K$) times the dc sensitivity. The algorithm stops when a combination of the optimization parameters achieves this condition, which gives the required resolution in the samples studied here. In our study, we used $K = 3$. However, we emphasize that considerably higher values of $K$ can be obtained if desired, by allowing the optimization to proceed further. The time required to run such an optimization is in the order of tens of minutes, usually less than an hour.

VI. Results and Discussion

A. Case Study I

The optimization algorithm described in Section V was used to simulate and diagnose a solution of arbitrary base lengths. We first consider a solution with the following composition, shown in Table I.

![Fig. 6. Distribution of translocation times for repeated translocation events of DNA molecules of two different base lengths (2000 and 2100) under dc and dc+ac stimulus.](image)

![Fig. 7. Pseudocode for the proposed algorithm.](image)

<table>
<thead>
<tr>
<th>Base length</th>
<th>Number of molecules</th>
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<tbody>
<tr>
<td>2000</td>
<td>939</td>
</tr>
<tr>
<td>2010</td>
<td>957</td>
</tr>
<tr>
<td>2020</td>
<td>299</td>
</tr>
<tr>
<td>2030</td>
<td>985</td>
</tr>
<tr>
<td>2040</td>
<td>595</td>
</tr>
<tr>
<td>2050</td>
<td>284</td>
</tr>
<tr>
<td>2060</td>
<td>305</td>
</tr>
<tr>
<td>2070</td>
<td>734</td>
</tr>
<tr>
<td>2080</td>
<td>237</td>
</tr>
<tr>
<td>2090</td>
<td>349</td>
</tr>
<tr>
<td>2100</td>
<td>382</td>
</tr>
</tbody>
</table>

The sensor output from this solution was simulated with a dc stimulus of 120 mV and the result is shown in Fig. 8. All the eleven base lengths are not distinguishable from the histogram. Four regions could be identified for separate analysis to find the optimal ac stimulus for each region. In addition, the mean of each region and the corresponding base lengths were determined.

Next, each region was simulated with an ac stimulus added to the initial dc stimulus. The initial frequency for the ac stimulus was calculated from the inverse of the mean translocation
Fig. 8. Histogram of translocation times for initial analysis with dc stimulus. Eleven base lengths of Table I (shown by vertical lines) are present, but several are not distinguishable.

Fig. 9. Analysis of region I with an optimized ac stimulus, showing increased resolution for base lengths 2000 and 2010. Dotted lines are a guide to the eye.

time of the region. The initial amplitude of the ac stimulus was chosen to be 50 mV with a frequency of 25 Hz for the optimization procedure conducted for each of the regions. For region I, the optimal stimulus was a sinusoidal waveform with peak-to-peak amplitude of 110 mV and frequency of 31.45 Hz. This stimulus split the region I considerably to reveal the different base lengths that it consists of, as shown in Fig. 9.

Although the optimization increased the resolution for DNA molecules with 2000 and 2010 bases, the optimized stimulus performed worse than pure dc on regions III and IV. This is acceptable, as the purpose of using the ac stimulus is to investigate each region separately. Using an optimal ac stimulus with the dc essentially redistributes the translocation times nonlinearly, while increasing the resolution in the region of interest. Therefore, a loss in resolution in the other regions does not impair the measurement process.

The optimal stimulus to clearly detect molecules of base length 2020 was 95 mV, 31.35 Hz. These molecules are present in low concentration (Table I) and cannot be detected by pure dc because of overlap with other base lengths. For region II, the optimal stimulus was 120 mV, 31 Hz. This stimulus was able to clearly separate the base lengths 2030 and 2040. dc analysis of region III revealed the presence of a molecule with large concentration. Two separate optimizations showed two additional molecules in the region. The optimized stimuli were 110 mV, 30.65 Hz and 105 mV, 30.45 Hz, respectively. For region IV, the optimal stimulus was a sinusoid with amplitude of 100 mV, 30.25 Hz. Thus, all the base lengths constituting the solution were diagnosed with a high degree of accuracy.

<table>
<thead>
<tr>
<th>Base length</th>
<th>Number of molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>316</td>
</tr>
<tr>
<td>51</td>
<td>328</td>
</tr>
<tr>
<td>52</td>
<td>920</td>
</tr>
<tr>
<td>53</td>
<td>505</td>
</tr>
<tr>
<td>54</td>
<td>860</td>
</tr>
<tr>
<td>55</td>
<td>779</td>
</tr>
</tbody>
</table>

Fig. 10. Optimized analysis of sample in Table II. All molecules were distinguishable with a single optimized stimulus.

B. Case Study II

The ultimate objective of DNA sequencing with nanopores is to obtain “single base resolution,” i.e., to distinguish DNA molecules that differ from each other by a single monomer (i.e., unit base length). To test the applicability of the proposed technique to this objective, another set of simulations were performed for DNA molecules with base lengths differing by only a monomer length (Table II). The solution had six different types of molecules with a total of 3708 molecules. From the sensitivity analysis performed earlier, it is evident that lowering the dc stimulus increases the sensitivity. As the molecules had base lengths closer to each other compared to the previous study, a lower dc value was used as the starting point for the optimization. But it was found that a higher dc voltage, along with a higher ac voltage was necessary to optimally detect the molecules. So, the optimization used a dc offset of 200 mV.

As expected, an initial dc analysis showed a coarse size distribution in which the six molecules were not clearly resolved. In this case, the spread of base lengths in the entire sample is 5, as opposed to 100 in the previous case study. Hence, the power of the technique could be further demonstrated by optimizing the entire sample at once. The optimized stimulus obtained was 165 mV, 1180.5 Hz. As the translocation time for the molecules in this case was much smaller, the optimal ac frequency also increased significantly. Fig. 10 shows the results of the optimized stimulus.

VII. CONCLUSION

A diagnostic methodology for high-speed sizing of mixtures of chain biomolecules like DNA by fabricated nanopores has been developed based on optimization of the externally applied electrical driving potential. An initial dc driving force produces a coarse size distribution assay of the sample, which can then be refined by optimized ac stimuli specific to each region of the size distribution. The electrical and molecular (viscous and thermal) forces acting on the molecule, and hence the characteristic time
scale of transport through the nanopore, are strong functions of its length. As a result, an ac stimulus that is “tuned” to this intrinsic time scale of the transport process considerably increases the performance.

We note that for real-time applications, the sensitivity function can be computed and stored in a database, thereby making the time cost negligible. In addition, the fast time scale (\(\sim 1\) ms) of translocation and the fabrication of parallelized nanopore arrays will allow sample analysis at much faster rates than currently possible, even if optimized analysis is done for various regions of molecule lengths present in the sample. Although a simplified transport model is used here, the approach is general and can be applied to atomistically detailed descriptions of nanopore transport emerging in current literature [16]. The new diagnostic methodology, together with ongoing device fabrication research, strengthens the potential of nanopore sensing to provide next-generation biomolecule sensing analysis methods.

REFERENCES


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