Published in final edited form as:

Chem Commun (Camb). 2015 February 7; 51(11): 2084–2087. doi:10.1039/c4cc07591a.

Electro-Optical Detection of Single λ -DNA†

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Abstract

Single λ -DNA molecules are detected on a nanopore-gated optofluidic chip electrically and optically. Statistical variations in the single particle trajectories are used to predict the intensity distribution of the fluorescence signals.

A nanopore is a nanoscale opening connecting two chambers filled with conductive fluids. It forms the basis for a conceptually simple, yet powerful class of electrical single molecule sensors.¹⁻³ An ionic current through the nanopore is established when a voltage is applied across it. Meanwhile, an electric-field-induced driving force moves molecules through the nanopore, leading to characteristic current changes. Different molecules or even different nucleotides can be identified by distinguishing these current changes. Solid-state nanopores are one of two commonly used nanopore sensors. Owing to their sensitivity, robustness, and tunability, solid-state nanopores have been successfully applied to single molecule detection of proteins, viruses and nucleic acids.³ Optical detection adds analytical capabilities that cannot be provided by a nanopore, and a number of studies have shown that fluorescence microscopy can be coupled with nanopore-based electrical sensing.⁴⁻⁷ Recently, we reported the first integrated device that combines electrical and optical single particle detection on a single chip.⁸ This was enabled by integrating a solid-state nanopore with an optofluidic chip that guides both liquids and light through a microfluidic channel.⁹⁻¹² The earlier proof-of-principle demonstration was carried out using relatively large nanoparticles (100-200nm) and showed that subpopulations of H1N1 viruses can be identified from a particle mixture using the correlated electrical and optical signals. Analyzing single and double-stranded nucleic acids, which possess much smaller diameters on the order of 1-2 nm, is also of great interest as is the analysis of other small particles such as proteins or metabolites.¹³⁻¹⁵

In this work, we demonstrate dual-modality detection of single λ -DNA on our nanopore-optofluidic platform. The analysis of electrical and optical signals provides detailed information about the λ -DNA translocation dynamics and the particle velocity in the fluidic channel. Moreover, we show for the first time how statistical variations in the trajectories of

†Electronic Supplementary Information (ESI) available: Comparison between two devices. See DOI: 10.1039/c000000x/

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individual particles produce fluctuations in the detected single particle fluorescence signal. The experimental data are in excellent agreement with simulations taking into account the fluid profile in the channel and the optical mode geometry.

Shown in Fig. 1a is a layout of our optofluidic chip, which is based on anti-resonant reflecting optical waveguides (ARROW).¹² It consists of solid-core (pink) and liquid-core (blue) waveguides on the same chip. Solid-core ARROWs guide the excitation light and fluorescence signals, while a liquid-core waveguide confines light and fluid in the hollow core simultaneously. Construction details for these optofluidic chips can be found in previous reports.^{17,18} Fig. 1b illustrates a partial side view of the liquid-core waveguide. The thick top cladding layer is a natural site for nanopore integration, because of its suitable material (SiO_2)¹⁹ for solid-state nanopore fabrication, as well as its direct contact with the fluid in the optofluidic channel. To construct the nanopore, a $2 \times 2 \mu\text{m}^2$ opening is first milled into the top layer using a focused gallium ion beam (FIB), leaving a $\sim 170 \text{ nm}$ thick membrane. An 80 nm wide nanopore is then drilled through the membrane, followed by local gas-assisted SiO_2 deposition with the FIB to shrink down the nanopore size to 20 nm (Fig. 1c). Three fluid reservoirs are then glued around the liquid-core channel ends and over the nanopore for sample loading. Particle detection experiments were conducted as follows. λ -DNA was labelled with SYBR Gold (Invitrogen) intercalating dye for optical detection. The channel was filled with $1 \times \text{T50}$ buffer and then the λ -DNA solution was added into the reservoir over the nanopore. A patch clamp amplifier (Axopatch 200B) was connected to the chip via Ag/AgCl electrodes as a voltage source and an amp meter. A syringe pump maintained a continuous flow of buffer solution in the channel with a pump rate of 50 nL/min . As soon as a DNA molecule was electrically driven into the liquid-core waveguide through the nanopore, it was moved by the flow to the excitation area and optically detected. During this process, an electrical signal was recorded by the patch clamp amplifier while the λ -DNA was travelling through the nanopore, and a fluorescence signal was collected by an off-chip avalanche photo detector once the λ -DNA was optically excited.

In the experiment, voltages of 5 V , 6 V and 7 V were applied between reservoirs. Taking into account the resistance of the waveguide channel, this corresponds to voltage drops across the nanopore of 4.5 V , 5.4 V , and 6.3 V , respectively. Typical electrical signal traces at each voltage are shown in Fig. 2a. A current drop in the electrical signal is called a blockade. Fig. 2a clearly shows the electrical signal has different blockade depths under different voltages. The scatter plot of blockade depths versus durations (Fig. 2b) not only confirms that observation, but also suggests that the voltage across the pore is influencing the distribution of the blockade durations. A possible explanation is that the variation in λ -DNA shape creates a wide distribution in blockade durations when the applied voltage is lower, and there is a more uniformly stretched DNA population at higher voltages. The uniform distributions of blockade depths at different voltages indicate that only single λ -DNA molecules go through the nanopore. The average blockade depth increases linearly with incremental voltage, following the linear fitting function at a rate of 540 pA/V . The x-intercept of 2.8 V suggests there is a diffusion limited capture region above a threshold voltage of 2.8 V . Compared with lower threshold voltages found in other work,^{20,21} the higher value in our experiment is caused by the larger membrane thickness ($\sim 170 \text{ nm}$), lower buffer concentration, and lower molecular concentration. We also note a linear

dependence of the capture rate on rising voltage across the nanopore with a rate of $0.1 \text{ s}^{-1} \text{ V}^{-1}$ (Fig. 2d), indicating the capture process is governed by thermal diffusion,²² which is consistent with our finding from Fig. 2c. In a diffusion limited capture process, molecules within the capture radius migrate to the nanopore under the electrical bias. The capture radius can be calculated with,²³ where R is the capture rate, C is the concentration of λ -DNA and D is the diffusion coefficient of a particle. Here, C is 9.4×10^9 molecules/mL and D is assumed to be $6 \times 10^{-9} \text{ cm}^2/\text{s}$.²⁴ At 4.5 V, the nanopore had a capture radius of $4.9 \text{ }\mu\text{m}$. Comparable capture radii of several microns have been found in other work, albeit at different applied voltages due to the different specific experimental parameters.²⁵ Measurements on different chips yield qualitative identical results with minor variations in the extracted parameters (Supplementary Information).

The novel capability of our nanopore-optofluidic platform is the combination of electrical and optical single particle detection. This is ensured by the nanopore's gating function which limits the single particle entry into the channel. Fig. 3a shows the clearly synchronized electrical and optical signals of λ -DNA, where not only the single particle detection is confirmed, but an obvious correlation between two traces can be seen. Since electrical and optical signals have different shapes, the peak locations of all the electrical and optical signals were extracted for the construction of normalized artificial pulses. Computing the cross correlation between the artificial pulses gives us a strong cross correlation peak at 0.18 s, indicating λ -DNA travels with a rather uniform velocity of 8.3 mm/s between the nanopore and waveguides intersection.

As an independent control, fluorescence correlation spectroscopy analysis was applied to the optical signal. The autocorrelation function calculated using the experimental data is plotted in Fig. 3c. This function is also fit with a model developed for our liquid waveguides.²⁶ From the fit, we extract a flow velocity in the channel of 9 mm/s, which is a good match with the cross correlation calculation analysis, confirming the validity of both methods.

Another key novelty of the nanopore gated chip lies in exploiting the deterministic introduction of single particles into the fluidic channel. We exploit this here by taking advantage of the fact that each optical signal originates from a single molecule with similar statistical labelling efficiency. However, the location of the particle within the optical mode results in variations in the detected fluorescence signal (Fig. 3a). We can analyze these variations to learn more about the distribution of particles within the channel. Understanding this distribution is important for future improvements of optical mode placements or fluidic particle focusing for more efficient detection.

To this end, we first simulated the particle distribution in the liquid waveguide by setting up a 3D model (COMSOL) consisting of a liquid-core channel and a nanopore inlet. One hundred solid particles were introduced through the nanopore inlet and subjected to laminar Navier-Stokes flow in the channel. Particle trajectories were simulated using COMSOL's Particle Tracing Module (Fig. 4a). The positions of the particles at the excitation area were obtained from the simulations and imported in Matlab. This distribution across the $5 \times 12 \text{ }\mu\text{m}$ liquid core is shown in Fig. 4b (top) and shows a clear concentration of molecules near the top of the channel due to the entrance pathway via the nanopore. The mode location of

the excitation light was acquired by taking a mode image at the end of output solid-core waveguide (Fig. 4b (bottom)); however, where the optical mode locates inside the liquid-core channel cannot be acquired directly without destroying the chip. Fig. 4c shows the intensity distribution of optical signals obtained from the experiment. The brightness histogram is well matched by a fit to a Poissonian distribution. By combining the predicted particle locations from the flow-based simulations with the optical excitation and collection mode profiles we can create predicted brightness histograms for various vertical locations of the optical mode in the channel. The best fit with the experimental statistics of Fig. 4c was obtained when the center of the optical mode was positioned 3.6 μm above the bottom of the liquid channel, in excellent agreement with the mode location observed at the solid-core output. This shows that single particle analysis is able to provide meaningful statistical analysis tools beyond bulk averages to analyze the individual electrical and optical signals, or, alternatively, the optical (mode position) or fluidic (flow profile) device properties.

In conclusion, we have demonstrated opto-electrical single λ -DNA detection on a nanopore-optofluidic chip. Analysis of the electrical signal generated by λ -DNA translocating through the nanopore indicates that the capture process is governed by thermal diffusion. Correlated electrical and optical signals verify single molecule sensitivity and furthermore reveal their flow velocity through a fluid channel. Moreover, we were able to explain the measured fluorescence intensity distribution based on the statistical variations of the particle position across the channel. This analysis also confirmed the optical excitation mode location in the liquid-core channel without a physical measurement. Multiple nanopores can also be defined along one or more waveguide channels. The demonstration of this multi-modal single molecule detection platform lays the foundation for exciting applications of specific detection of molecules, and provides a candidate solution for solid-state nanopore sequencing assisted by optical methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge support by the W.M. Keck Center for Nanoscale Optofluidics at University of California, Santa Cruz, the NSF under grants CBET-1402848 and CBET-1402880, and the NIH under grants R01EB006097 and R21EB008802. J.W.P. acknowledges support by the National Science Foundation Graduate Fellowship Research Program under grant DGE 0809125.

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Figure 1.

(a) Layout of the experimental setup. An Argon ion laser (wavelength: 488 nm) is used as the light source for excitation (blue arrow). A syringe pump is connected with the optofluidic chip using a PDMS adapter.¹⁶ (b) Side view diagram of liquid-core waveguide and the detection process. (c) Top-view scanning electron microscope image on a nanopore. The nanopore's diameter is shrunk from 80 nm (left) to 20 nm (right). (scale bar: 50 nm)

Figure 2.

(a) Typical electrical signal traces at voltages across the nanopore of 4.5V, 5.4V, and 6.3V. (b) Scatter plot of blockade depth versus duration. (c) Blockade depth versus voltage. (line: linear fit) (d) Capture rate versus voltage. (line: linear fit) Voltage values used in c and d are the calculated voltages dropped across a 20 nm wide and 170 nm deep cylindrical nanopore.

Figure 3.

(a) Typical electrical blockades (black) and optical signals (red). Inset: a zoomed-in view of an optical signal. (b) The cross correlation function between electrical and optical signals. (c) The autocorrelation function of the optical signal. (line: fit curve).

Figure 4.

(a) Blue lines present the calculated trajectories of 100 particles in the liquid-core channel. The dark red cylinder is a diagram of the excitation laser beam. (b) Top: A cross section view of the particle distribution. Bottom: The optical mode in the solid-core waveguide. The dotted lines show the location of the center of the optical mode. (scale bar: 2 μm) (c) Experimental intensity distribution. (line: Poisson fit) (d) Simulated intensity distribution when center of the optical mode is located 3.6 μm above the bottom of the channel. (line: Poisson fit)