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# Optical trapping assisted detection rate enhancement of single molecules on a nanopore optofluidic chip

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**We use optical trapping to deliver molecular targets to the vicinity of a nanopore for high-throughput single molecule analysis on an optofluidic chip. DNA detection rates increase over 80× to enable detection at attomolar concentrations.** © 2019 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

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Single molecule analysis using nanopores is based on detecting changes in ionic current across a membrane containing a nanoscopic hole. This simple, yet powerful principle now forms the basis of the leading next-generation DNA sequencing (NGS) technology, but is also increasingly being considered for label-free sensing of a much broader range of biological nanoparticles, including viruses, proteins and metabolites [1–3]. Thus, nanopore analysis has tremendous economic and scientific potential.

One major limitation for all nanopore application areas is the delivery of a sufficient number of analytes close enough to the pore to enable electrophoretic capture and detection. This severely limits the throughput (and extends the analysis time) and the limit of detection of the assay. Various attempts to increase the capture rate, e.g., by dielectrophoretic trapping of DNA on a nanopipette tip [4], field-enhancement at a solid-state pore [5], and electrostatic manipulation of an α-hemolysin pore [6] have seen mixed results.

Here, we demonstrate that these challenges can be overcome most elegantly by optical means. Figure 1(a) shows the new concept for trapping-assisted capture rate enhancement (TACRE) on an optofluidic chip. It relies on pre-concentrating molecular targets on microscale carrier beads (1 μm diameter), followed by delivery and optical trapping of these carrier beads in femtoliter volumes and with micrometer precision in the immediate vicinity of a nanopore. Bead capture ensures target specificity [7] and optical delivery ensures availability of analyte molecules within the micrometer-scale capture radius of the pore [4]. By implementing this concept on a waveguide-based optofluidic chip, we demonstrate DNA detection rates nearly two orders of magnitude higher than without TACRE. Targets are thermally released and electrically detected using a nanopore current modulation.

Figure 1(b) illustrates the full experimental layout on the optofluidic chip. The device has a 5 × 12 μm fluidic channel (blue) designed as an antiresonant reflecting optical waveguide (ARROW) [8] connected to solid-core (SC) ARROWs (gray). Fluidic reservoirs 1 and 3 are attached at the ends of the liquid-core (LC) channel to introduce sample solutions and to apply electrical bias. To integrate the solid-state nanopore [9], a square well was ion-milled to remove the top thick oxide of the LC channel, leaving a thin membrane [see cross-section in Fig. 1(c)]. Finally, a nanopore was ion-milled on the thin membrane [see SEM image inset of Fig. 1(c)]. An additional reservoir (#2) was attached on the nanopore and an electrical bias voltage was applied between reservoirs 1 and 2 for the electrical detection of target molecules. To implement optical trapping, fiber coupled laser light (532 nm) was introduced from the left and right SC waveguides as shown in Figs. 1(a) and 1(b) (red arrows) to form a loss-based dual-beam optical trap [10] for collection of target-carrying microbeads. It is important to note that the loss-based trap enables both particle trapping at any desired location along the LC channel [see still picture of a single trapped bead under the nanopore in Fig. 2(a)] as well as optical assembly of multiple particles in the same location [11].

To demonstrate this novel principle, 100-mer ssDNAs corresponding to a melanoma cancer gene (BRAFV600E) were selected as the target molecule [12]. Magnetic microbeads (Invitrogen) were functionalized with 14-bp pulldown oligomers to match and specifically extract the target sequence as previously reported [12]. The microbeads were brought into the central waveguide region from reservoir 3 using pressure-based flow and optically trapped under the nanopore. Once the microbeads were trapped, the chip was heated up to ∼50°C using a ceramic heater (Laird) which released the DNAs from the microbeads. A bias voltage was then applied to pull the targets through the pore at a high rate due to their proximity to the nanopore. Figure 2(b) shows the observed translocations of DNA molecules altering the current across the nanopore for different numbers of trapped beads. It is visually clear that the detection rate (event frequency) increases with the number of beads as more targets are present near the pore. We compared the measured translocation rates with the case in which the same number

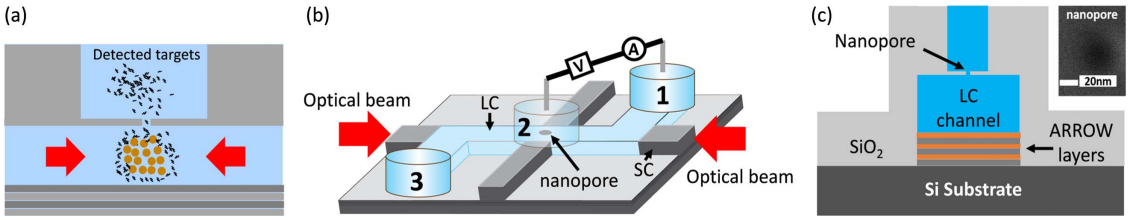


**Fig. 1.** (a) Schematic of trap assisted capture rate enhancement (TACRE) principle: Molecular targets are released for detection from microbeads (gold) held in dual beam optical trap, (b) experimental setup on a nanopore optofluidic device with intersecting liquid-core (LC, blue) and solid-core (SC, gray) waveguides, and (c) cross-section of LC channel showing nanopore integration process (Inset: SEM image of a drilled nanopore).

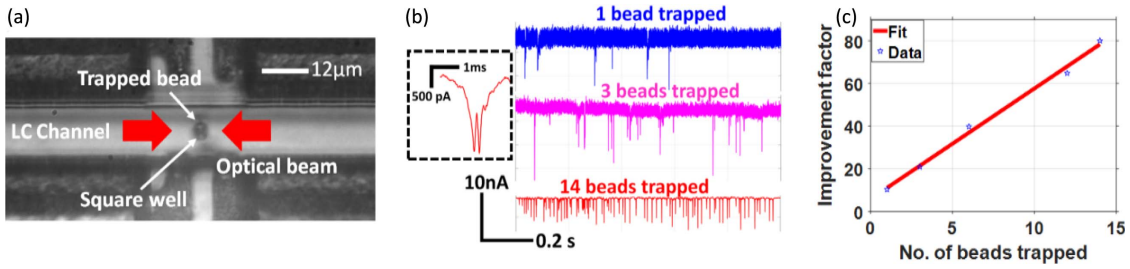


**Fig. 2.** (a) Still picture of a trapped microbead, (b) nanopore current trace after thermal release and detection of DNAs showing monotonic increase in event frequency with number of beads trapped (Inset: Example of a multi-DNA translocation), and (c) detection rate improvement with number of trapped beads relative to unconcentrated bulk solution (symbols: data; line: linear fit).

of targets as binding sites on a single bead (240,000) were uniformly distributed throughout the fluidic channel (i.e., 2 nM concentration). To count the molecules, we also had to analyze translocations with multiple sub-peaks arising from multiple DNAs moving through the pore at the same time [see inset of Fig. 2(b)]. We used a conservative threshold of 1/6th of the maximum translocation amplitude for the dip depth between sub-peaks to identify a second molecule. Figure 2(c) shows the TACRE improvement factor in the detection rate compared to the non-concentrated bulk solution as a function of the number of trapped beads. An improvement of almost two orders of magnitude (∼80×) in the detection rate is observed with a nearly linear dependence on the bead number [see linear fit in Fig. 2(c)], exceeding previous results on nanopores [5,6]. This result demonstrates the effectiveness of optically assisted target concentration for nanopore analysis and is the principal result of our Memorandum.

In the present study, the TACRE improvement factor was limited by the time delay between thermal release and applying the nanopore voltage needed to avoid electrical noise interference. During this delay, the DNA targets started to diffuse away from the pore which lowered the effective local concentration during detection. The measured improvement factor closely agrees with an estimate based on the diffusion-induced concentration reduction. The time delay can be eliminated by alternative target release methods (e.g., optical release with UV light). Then, much larger enhancements of at least 50,000× are feasible.

Nevertheless, we were already able to detect over 5,600 individual DNAs in three minutes in the present configuration (14 trapped beads). This target number corresponds to a concentration of ∼1 aM when contained in a typical blood draw of 10 mL, which corresponds to a 5,000× improvement over previous reports [4]. This corresponds to the low concentration end of infectious diseases and lies well below the current limit of detection for protein-based immunoassays.

This demonstration represents a crucial step towards fully integrated nanopore-based analysis of single molecules with broad applications. An increase of almost two orders of magnitude in molecular detection rate shows the power and ease of optical trapping to expand the applicability of nanopore analysis.

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