

## SUPPORTING INFORMATION

### Orthogonal *tip-to-tip* nanocapillary alignment allows for easy detection of fluorescent emitters in femtomolar concentrations

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## Experimental details

**Chemicals and Materials.** Tris(hydroxymethyl)aminomethane, borate, polyvinylpyrrolidone (PVP, average  $M_w \sim 1,300,000$ ), capillary column butt connector, double-tapered ferrule (0.4 mm I.D.) were purchase from Sigma-Aldrich (St. Louis, USA). The polydimethylsiloxane (PDMS) and curing agent (DOW CORNING Sylgard 184) were obtained from Sil-More Industrial Ltd. (Taipei, Taiwan). Black chloroprene rubber was purchased from KS BOND (GS30, Taipei, Taiwan). HPLC purified, Alexa Fluor 647-labeled oligonucleotide (20 nt) was ordered from Integrated DNA Technologies (Coralville, USA). The synthesized DNA were dissolved in double deionized water to bring a final concentration in 0.1 mM. The DNA sample used for translocation were prepared at 1.0 nM to 1.0 fM concentration range by serial dilution of 100 or 400 mM Tris-borate buffer (TB buffer, pH 8.0) or double deionized water (Millipore, USA) just before direct sample filling (Figure 3a-d) or electrokinetic injection (Figure 3e-g). The polyimide coated, fused silica capillaries (365  $\mu\text{m}$  O.D., 50  $\mu\text{m}$  I.D.) were obtained from Polymicro Technologies (Phoenix, USA). The coverslips (No. 1.5) used for building up the base of the nanocapillary holder were purchased from Marienfeld (Lauda-Königshofen, Germany). TFE Teflon tubing (0.3 mm) that was used for connecting the fused silica capillary to a No. 23 needle was obtained from Supelco (Bellefonte, USA). The buffer used in this work is filtered by sterilized 0.2  $\mu\text{m}$

surfactant-free cellulose acetate Minisart NML syringe filter (Sartorius, Goettingen, Germany) and 20 nm Whatman Anotop syringe filter (GE Healthcare Life Sciences, Freiburg, Germany) before filling into the buffer containers or nanocapillaries.

**Capillary pulling and shrinking.** A CO<sub>2</sub> laser-based micropipette puller (Sutter Instrument, Novato, USA) was utilized for the capillary pulling according to previously study.<sup>25</sup> Briefly, the fused silica capillary was cut into ~12 cm length by a ceramic tile and the polyimide was removed by burning the center of capillary followed by methanol cleaning. The capillary was kept on the puller bars and the cleaned part of the capillary located on the laser-focused position. The program parameters for pulling 50-100 nm capillary were as follows: heat: 540, filament: 4, velocity: 10, delay: 145, pull: 0 followed by heat: 540, filament: 4, velocity: 10, delay: 145, pull: 160. For the pulling of capillaries that have pore size ranges from 150-200 nm, the program parameter were: heat: 600, filament: 4, velocity: 10, delay: 145, pull: 0 followed by heat: 600, filament: 4, velocity: 10, delay: 145, pull: 140. The resulting capillaries were then transferred on a metallic holder for capillary shrinking by SEM. The capillary shrinking and imaging was performed by Merlin SEM (Zeiss, Germany) with on-axis, in-lens detection. The working distance of SEM is about 7 mm with 500 pA probe current and 3.0 kV acceleration voltage.

**Instrumentation of dual-nanocapillary device.** All the fluorescence imaging was accomplished on an IX73 microscopy (Olympus, Tokyo, Japan) (Figure S4). The focusing nanocapillaries were fabricated by depositing platinum by atomic layer deposition (ALD, Beneq TFS200, Beneq, Espoo, Finland) on the pulled nanocapillaries (150-200 nm). The precursors used for ALD of Pt were trimethyl(methylcyclopentadienyl) platinum (IV) and O<sub>2</sub>. The ALD was performed at 280 °C in a deposition rate of 0.5 Å per cycle. The established thickness of Pt-ALD should be 50 nm, resulting the 50-100 nm pore size of Pt-coated nanocapillary. A hollow square aluminum-alloy was sealed on the coverslip to form a capillary holder (also as buffer receiver) that can also be used for the buffer container for electrophoresis (Figure 1-3). The aluminum-alloy holder contains a 0.5 mm hole that allow to insert the Pt-coated nanocapillary. The tip of the metal-nanocapillary was carefully faced to the coverslip as close as possible in order to keep the tip within the working distance of a UPlanFLN 40x, 0.75 NA objective. A 647 nm, 5 mW laser beam was focused into the Pt-nanocapillary and a bright light spot (Figure S5a) can be observed from the tip of focusing-nanocapillary. Briefly, a laser was installed on a xz translational stage and kept a few centimeters above the microscope stage. The laser beam was guided to a broadband mirror that was fixed on a 3 adjuster kinematic mirror mount (Figure S4a). The reflected beam was then focused into the opening of a Pt-nanocapillary (50 μm)

via a 15 cm plano-convex lens. Once the scattering observed on the tip of the nanocapillary, the dye-labeled DNA (1.0 nM) was delivered into the capillary holder to visualize the beam profile. Inappropriate alignment can lead to a disordered fluorescence profile as shown in Figure S5b. By carefully aligning the laser position and mirror angles, the laser beam can be coaxially focused on the tip of the nanocapillary and present a sharp dispersive profile (Figure S5c).

On the other hand, the shrunken nanocapillary (20-30 nm pore size, length about 1.5 cm) was filled with DNA (for Figure 3a-d) that contained in 100 mM TB buffer (pH 8.0) or only buffer for electrophoresis (Figure 3e-g) in a desiccator using a vacuum pump. The nanocapillary was then inserted to a double-tapered ferrule and connected to a polyimide-coated capillary on the other end of ferrule followed by screwing the butt connector tightly. The remaining bubble at the tip of the nanocapillary can then be completely removed by a NE-1010 syringe pump (New Era Pump Systems Inc., Farmingdale, USA) after two hours pumping. The electrophoretic nanocapillary (~1.5 cm) was connected to a buffer filled microcapillary (40 cm, I.D. 50  $\mu\text{m}$ ) and aligned to the tip of the focusing-nanocapillary by a xyz translation stage with differential adjusters (Thorlabs, USA). For the alignment of the nanocapillaries, the tip of Pt-nanocapillary was placed on the image center of an EMCCD (low noise mode) with bright field illumination. The electrophoretic nanocapillary was then moved into the

holder and the tip was left just above the tip of Pt-nanocapillary in ~5 mm distance by manual alignment of the right-angle post clamp. Now, the electrophoretic nanocapillary can be approach to the focusing nanocapillary by lowering the z-axis of the translational stage. During this alignment the beam of a flashlight was used to visualize the electrophoretic nanocapillary. Once the shadow of the nanocapillary appears on the image of the eyepiece, tip-to-tip arrangement can be easily achieved by adjusting the differential adjusters on the stage. After alignment, the outlet holder was filled with 100 mM TB (pH 8.0) immediately in order to avoid bubble formation in the tip of the nanocapillary. At this point the image of the tip might out of focus after the buffer is added. Additional alignment of the objective is needed. First the Pt-nanocapillary is aligned, followed by a z-axis movement of the translational stage for the electrophoretic nanocapillary so that both tips of the nanocapillaries will be in-focus once more. The two nanocapillary are aligned *tip-to-tip* as close as possible but leave a few micrometer free space in order to avoid capillary breaking during handling. The electrophoretic nanocapillary was then aligned below the focus plane of the objective by going down the z-axis of the translational stage. A scattering from the wall of the nanocapillary can be observed by the EMCCD right now as EM gain was switched on. We then raise the z-stage until the scattering disappears from the image. Last of all, we lock the three differential adjusters by fastening their screws in order to avoid any drifting during the

experiment. The microcapillary was then inserted into the sample for electrokinetic injection (10 kV) or just buffer solution for electromigration of prefilled DNA molecule. Nanocapillary electrophoresis was performed by about 10 kV driving voltage (Gamma High Voltage Research, Ormond Beach, USA), resulting in an electric field about 240 V/cm. Imaging was performed by a back-illuminated EMCCD (Princeton Instruments, Trenton, USA) with a EM gain of 100 and 30 millisecond exposure time. The images and movies were collected by either the WinView32 or  $\mu$ Manager software.

**Nanocapillary chamber for imaging of DNA migration.** For observation of DNA behavior during nanocapillary electrophoresis (Figure 4 and 5) the filled nanocapillary (about 2 cm) was transferred to a coverslip that was sealed with a hollow square acrylic to form the insulate buffer chamber. A little bit of PDMS was added on the middle of nanocapillary and two drops of buffer were added to cover the two end of asymmetric nanocapillary. The nanocapillary can then be fixed on a coverslip and forms a two chamber (*cis/trans*) buffer container by heating the PDMS at 65 °C for 15 minutes within an oven. Another coverslip was covered on the capillary chamber to avoid the evaporation of buffer solution during the PDMS heating process. When the nanocapillary is fixed in the capillary chamber, chloroprene rubber can be added on the hardened PDMS middle of nanocapillary so as to increase the buffer volume of *cis* and *trans* end. The electro-migration of DNA was accomplished by applying 500 V to the

*cis* end of the nanocapillary. A 647 nm OBIS LX laser (Coherent Inc., Taipei, Taiwan) with 5-mW radiant power was utilized for the excitation of Alexa Fluor 647 by epifluorescence configuration. The resulting photons were collected by an UPlanSApo 10x, 0.4 NA objective (Olympus, Tokyo, Japan).



## Legends for Supplementary Movie

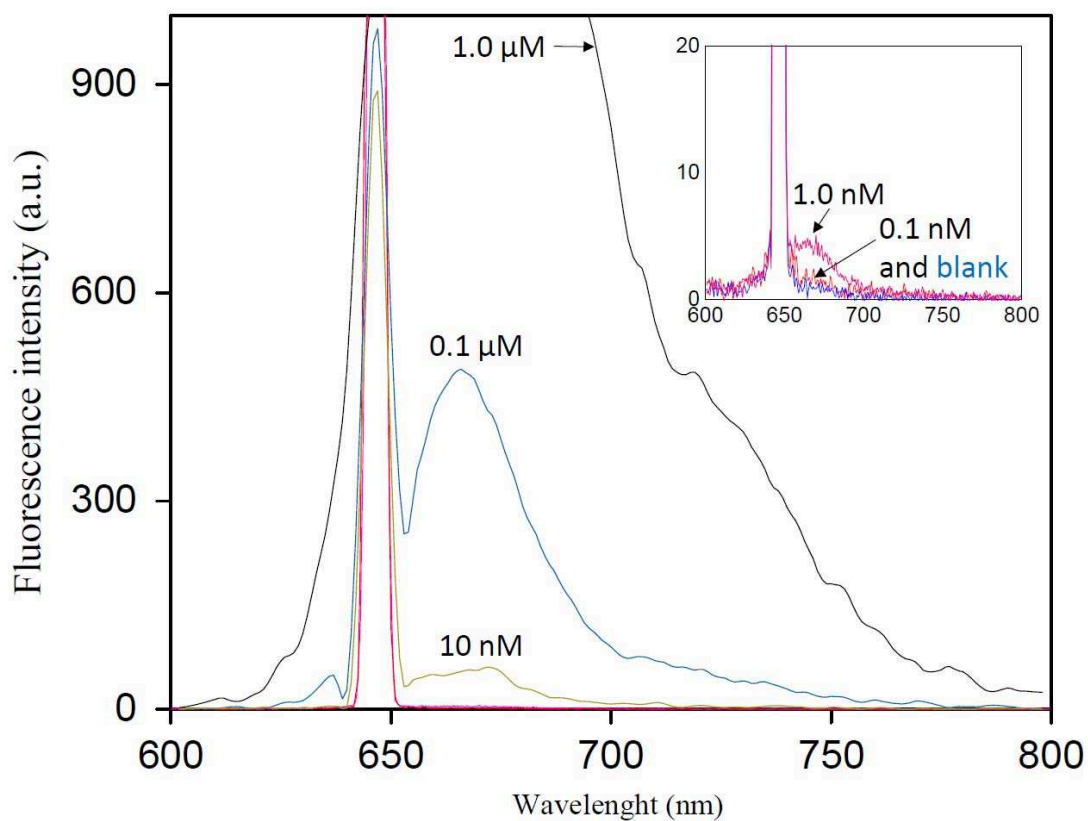
**Movie S1** Observation of fluorescence burst from the orifice of a nanocapillary. The DNA concentration is 10 fM and the driving voltage is 10 kV, resulting an electric field about 240V/cm.

**Movie S2** Dynamics of the fluorescence intensity under constant DNA electrophoresis conditions. At the start of the experiment, DNA molecules accumulate at the tip, near the tip of the nanocapillary or at both zones. As most of the molecules are ejected both zones display decrease in the fluorescence but forming a bright fluorescence burst near the orifice of nanocapillary.

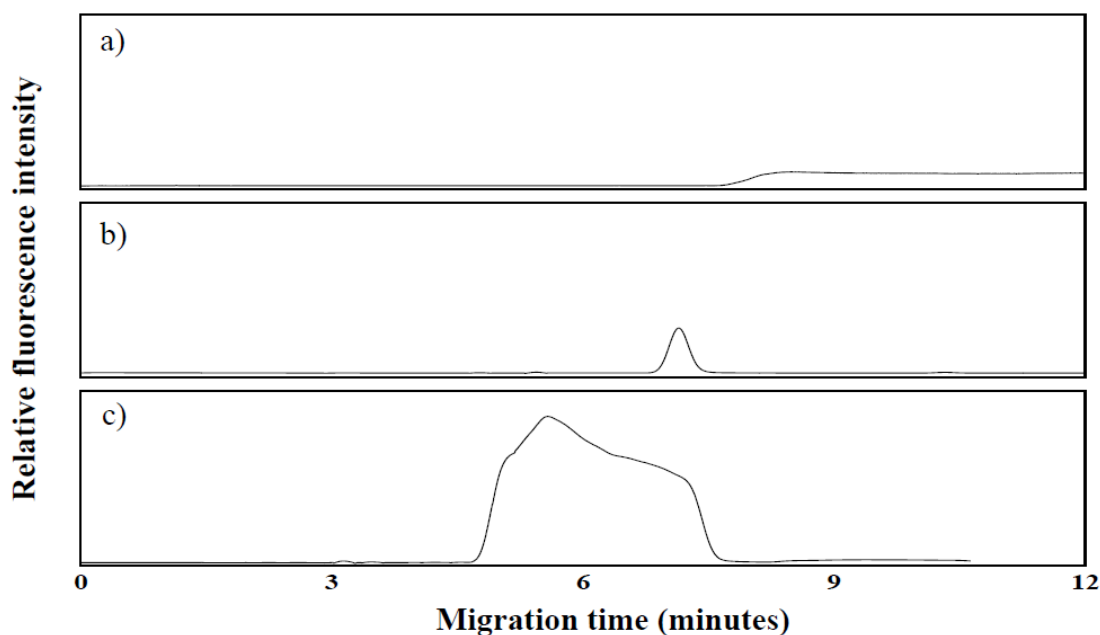
**Movie S3** The electroosmotic flow affects DNA migration and the fluorescence signal during nanocapillary electrophoresis. Under constant electrophoresis condition buffer gets depleted and the *trans* side becomes more basic increasing the EOF mobility. Consequently, the DNA molecules are pushed back to the *cis* side and can form a highly concentrated DNA zone within the nanocapillary. The concentrated DNA zone moves toward the *cis* side by EOF and returns to the *trans* side once we add ethanol to the buffer to destroy the electric double layer on the capillary wall.

**Movie S4** Free migration of fluorescent beads in a condition where the EOF was suppressed. The fast movement of the beads indicates that no obvious change of velocity occurs near the outlet of capillary.

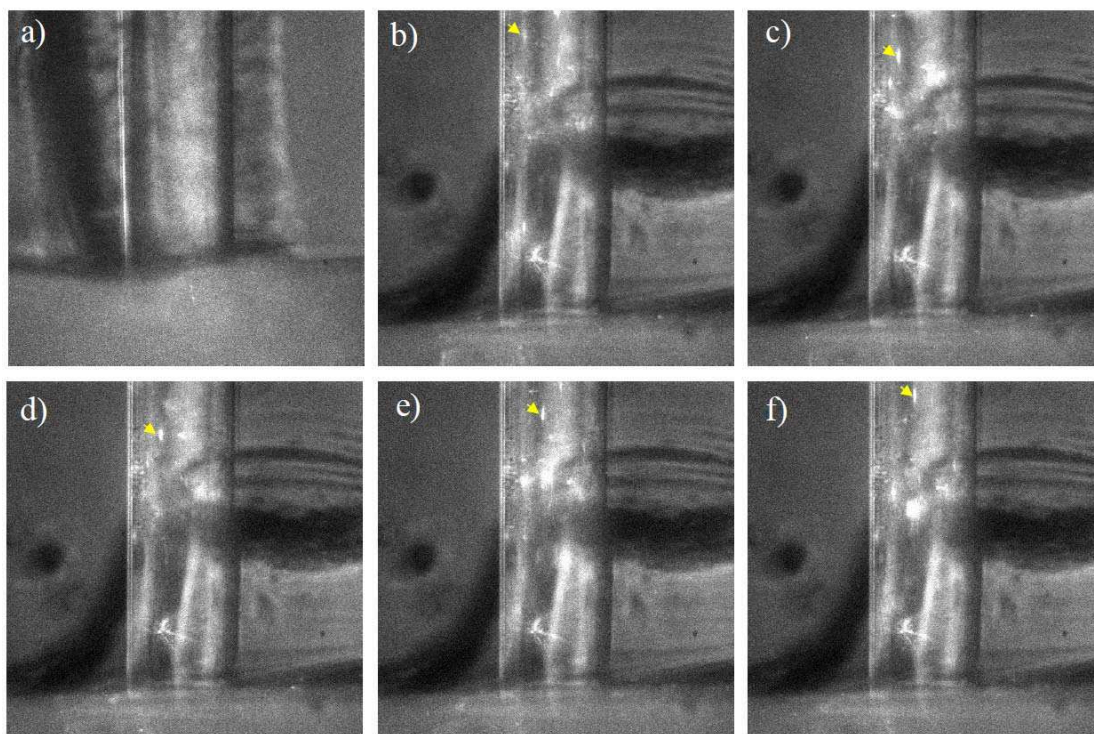
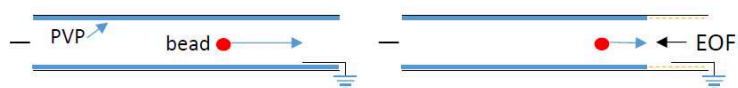
**Movie S5** The beads may slowdown in the *trans* side if EOF is partially generated by removing polyvinylpyrrolidone from the inner wall of the capillary close to outlet. Some of the beads may have a U-turn behavior as they get closer to the outlet of capillary. Brighter locations also indicate the aggregation of beads occurring if the EOF moves against the bead migration.



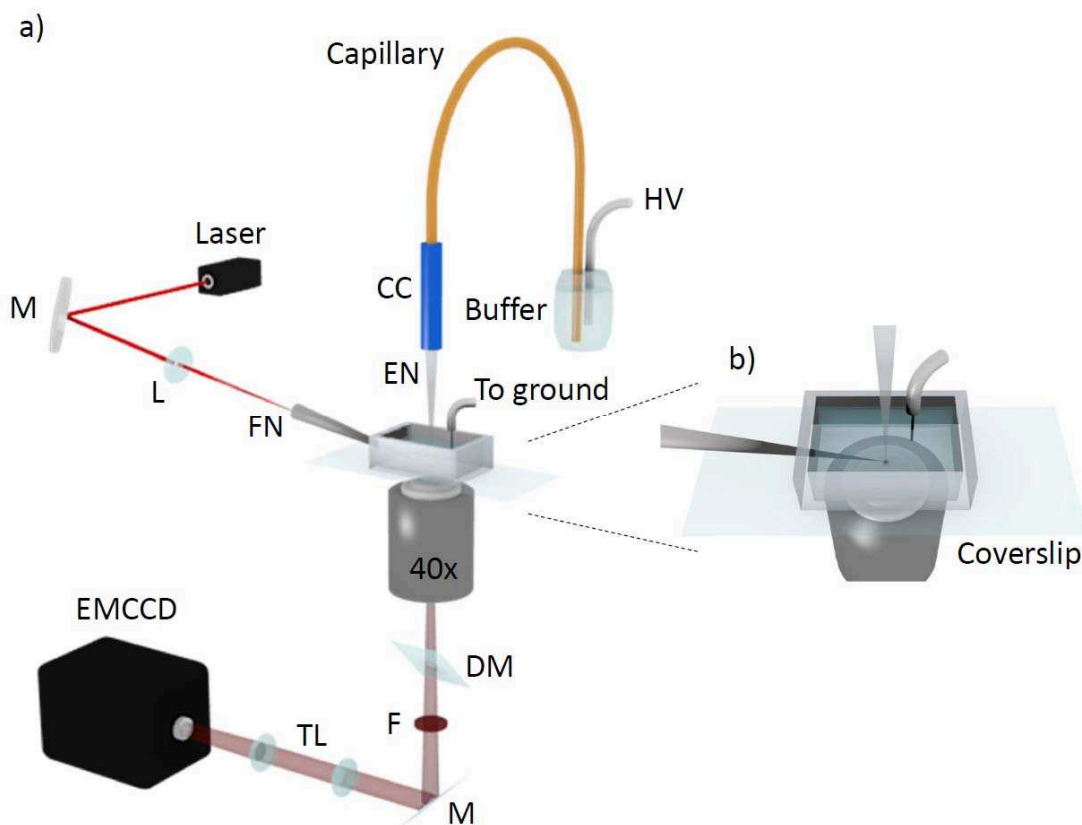
**Figure S1.** Fluorescence spectra of AlexaFluor 647 labeled DNA in concentrations ranging from 1.0  $\mu\text{M}$  to 0.1 nM. The highest PMT voltage setting (800 Volt) was used to collect the fluorescence signals. Both emission and excitation slit widths were 2.5 nm. The DNA samples were dissolved in a blank, 400 mM Tris-borate buffer solution (pH 8.0).



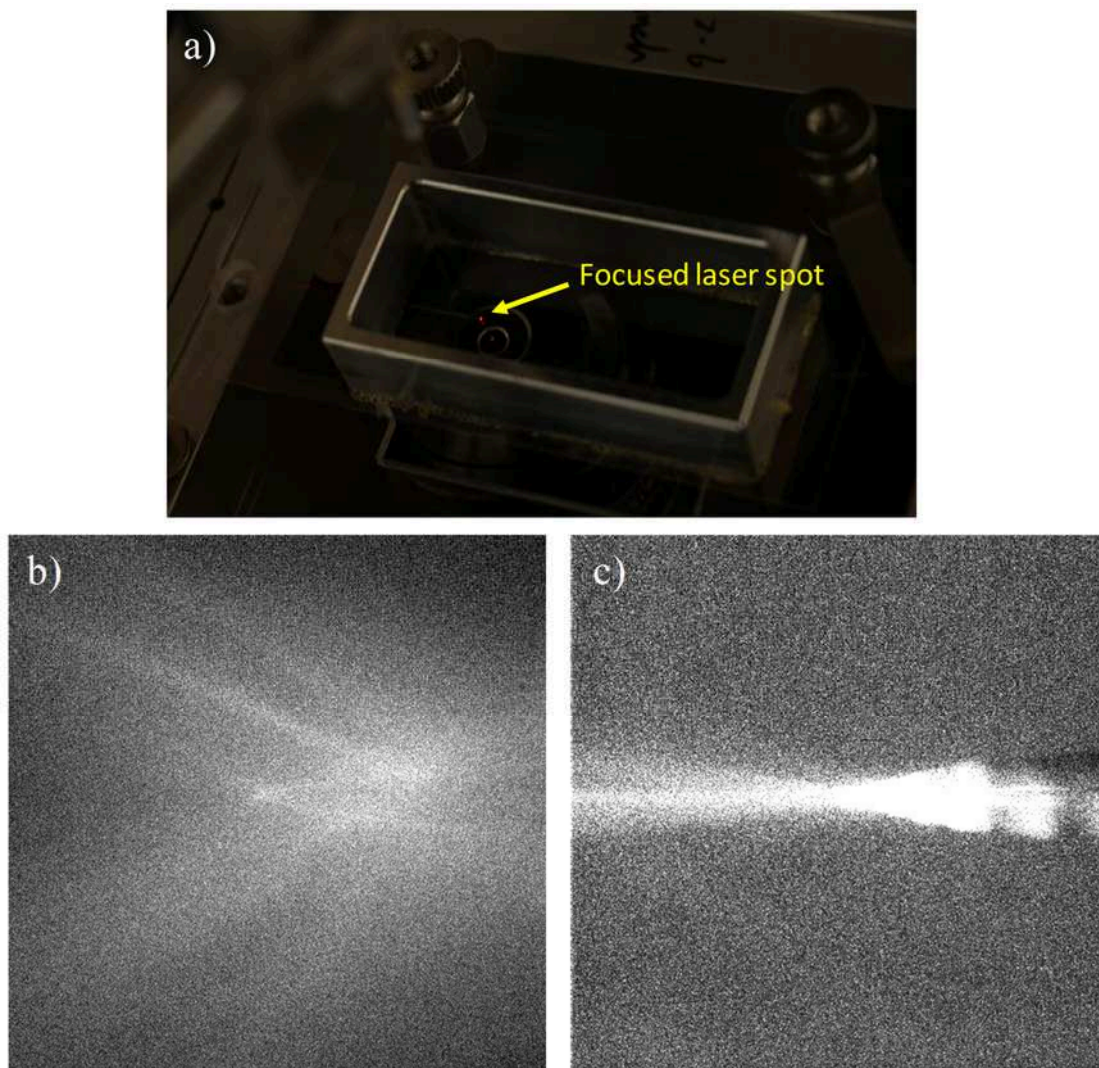
**Figure S2.** Electropherograms of emitter-labeled DNA in free solution. a) Continuous hydrodynamic injection of DNA sample without applied voltage. Electrokinetic injection at an electric field of 240 V/cm for b) 10 seconds and c) 3 minutes. The electrophoresis was performed with an electric field of 240 V/cm, identical to the conditions of the nanocapillary electrophoresis experiments presented in Fig. 3. The total capillary length is 35 cm (effective length 28 cm) and filled with 400 mM Tris-borate buffer (pH 8.0). The ratio of fluorescence intensity for a) static excitation, b) 10 second injection and c) 3 minutes injection are 1 : 833 : 15493. The size of the focused laser beam is located near the capillary diameter of 75  $\mu\text{m}$ .



**Figure S3.** Polyvinylpyrrolidone (PVP) effects on the electrophoretic migration of fluorescent beads in a) a completely PVP coated microcapillary (Supplementary Movie 4) and b)-f) a microcapillary with partially removed PVP. PVP was removed by burning the capillary about 3 mm from the *trans* side (Supplementary Movie 5). The arrow indicates a U-turn behavior of the beads as they get close to the outlet of capillary.



**Figure S4.** Instrumentation of the nanocapillary electrophoresis setup with Pt-deposited nanocapillary assisted laser-induced fluorescence. a) The electrophoretic nanocapillary is connected with another PVP coated fused silica capillary (i.d. 50  $\mu\text{m}$ ) by a capillary column butt connector with a double-tapered ferrule for the electrokinetic sample injection and electrophoresis. A 647-nm laser beam (5 mW) was focused into a Pt coated (50 nm) focusing nanocapillary, which resulted in a measured radiant power of about 50  $\mu\text{W}$  for the excitation of dye-labeled DNA molecules. b) The nanopore of the electrophoretic nanocapillary is aligned to the focusing nanocapillary tip-to-tip in order to perform laser-induced fluorescence. EMCCD: electron multiplying charge coupled device; HV: high voltage; M: mirror; L: lens; DM: dichroic mirror; F: emission filter; CC: capillary connector; TL: tube lens.



**Figure S5.** Images of the laser spot after passing through the Pt-nanocapillary. a) A bright red spot can be observed from the tip of the nanocapillary if the laser beam has been focused through the Pt-nanocapillary. By immersing the nanocapillary in 1.0 nM dye-labeled DNA the fluorescence profile can indicate whether the laser beam is b) misalignment or c) well aligned to the orifice of the Pt-nanocapillary.