Sculpturing Wafer-scale Nanofluidic Devices for DNA Single Molecule Analysis

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Supplementary Information

1. Silicon Stamp Fabrication - Microchannels

The 1 cm x 1 cm microchannel layout of the fluidic devices can be seen in the sketch in Figure S 1. Two U-shaped microchannels connect four reservoirs. The microchannels are 20 µm wide. In the area where the nanochannels will be placed, we define indents, what results in a smoother flow from microstructures into the nanostructures in the devices. All these structures are defined in a computer assisted design (CAD) software and then transferred into a chromium mask using a laser writer.

Photolithography mask

For this, an opaque chromium layer is deposited on a UV transparent glass plate. A positive tone photoresist (Microposit S1813, Shiply Ltd.) is spun on the chromium at 3000 rpm for 1 minute. Then, it is baked at 90°C for 2 minutes on a hot plate. To pattern the mask, a laser writer (Heidelberg DWL 66+, Heidelberg Instruments Mikrotechnik GmbH) with a controllable laser beam (λ = 405 nm) is used. The resist is developed using MIF-319 (metal-ion-free, Shiply Ltd.) for 45 seconds and the process is immediately stopped by rinsing with deionized water (DI-water), and dried with purified nitrogen. Then, the chromium in the resist-free areas is etched in a chromium etch solution (10% Veric ammonium nitrate, 4.25% perchlorid acid, 84.95% H_2O, MicroChemicals GmbH) for 50 seconds. The etched areas are UV transparent.

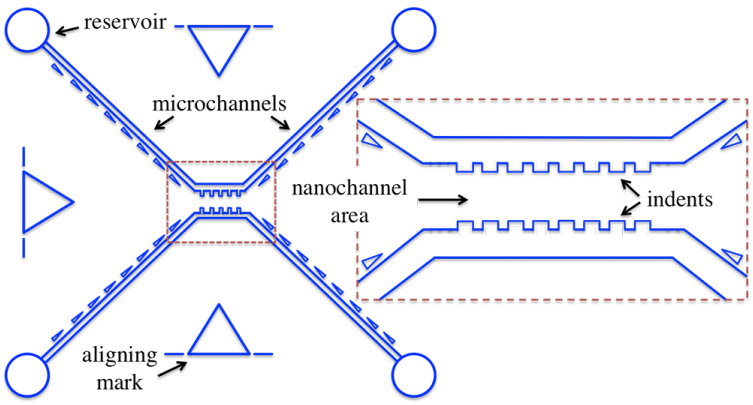

Figure S 1. Two-dimensional layout of the microchannel structures, reservoirs and aligning marks of the devices. Arrows are designed along the microchannels to facilitate the guidance under a microscope and to easily locate the nanochannels. Indents, as shown in the inset, are designed at the entrance of the nanochannels to facilitate a smoother flow from microchannels to the nanochannels. The distance between two neighboring reservoirs is 1 cm. This design is transferred onto a chromium mask for photolithography using laser writer.

Afterwards, patterns on the mask are transferred into a silicon wafer by photolithography and reactive ion etching. For this, a UV sensitive positive resist AZ4562 (MicroChemicals GmbH) is spun at 4000 rpm for 30 seconds onto a silicon substrate, followed by 2 baking steps at

60°C for 1 minute, and then at 100°C for 6 minutes. This results in a 6.5 µm thick layer. The sample is stored in air at room temperature for 10 minutes before being exposed with a mercury vapor light source with a wavelength of 365 nm and power of 13 $mWcm^{-2}$ for 15 seconds in a mask aligner MJB4 (SUSS MicroTec) with the previously patterned mask. The exposed parts of the photoresist are developed for two minutes in AZ826 MIF (metal-ion-free, MicroChemicals GmbH).

Dry etching

Then, the microchannels are etched into the silicon with an inductively coupled plasma reactive-ion etching tool (ICP-RIE SI 500 214, SENTECH Instruments GmbH). We use a gas mixture of SF6, CF4 and O2 and follow the recipe with the parameters listed in Table S 1. In these conditions, an etching time of 120 seconds leads to ~1 µm deep microchannels, as measured by profilometry. Afterwards, the resist is removed by sonication in acetone, rinsing with isopropanol and drying with purified nitrogen.

Parameter	**Value**
ICP Power	400 W
HF Power	15 W
Temperature	0 °C
Chamber Pressure	1 Pa
Gas	**Flow (sccm)**
SF_6	50
C_4F_8	70
O_2	5

Table S 1. Recipe used for etching the microchannels into the silicon master stamp by ICP-RIE

SEM images of the 20 µm wide, 1 µm deep microchannels etched down into the silicon can be seen in **Figure S 2** before (a,b), and after (c) FIB milling of the nanochannels and the 3D inlets.

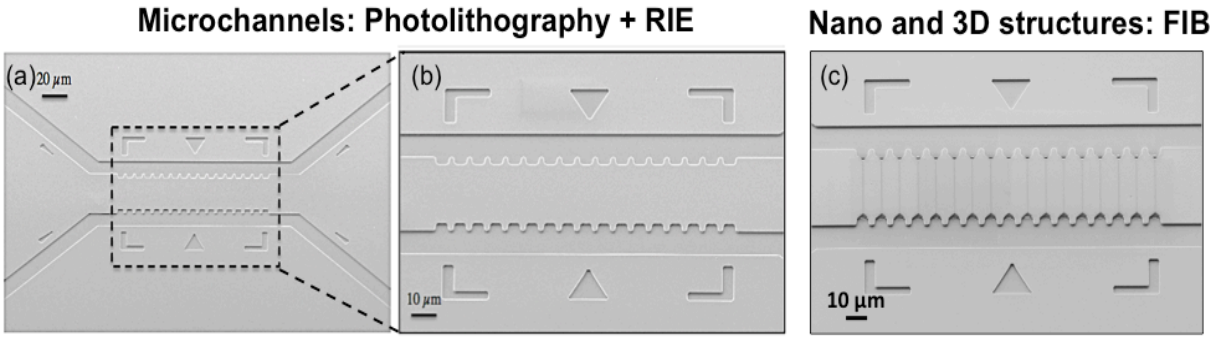

Figure S 2. SEM images of the fabrication of a silicon master stamp with linear nanochannels and 3D inlets. (a) and (b) show the microchannels, 20 µm wide, 1 µm deep, fabricated by photolithography and dry etching by ICP-RIE. (c) shows the same stamp after milling of the nanochannels and the 3D inlets by FIB.

2. Nanopatterning by FIB vs EBL

The fabrication of nanochannels with controlled shape, dimensions and position requires top-down fabrication techniques. Photolithopgraphy is a well established micro lithography method, but its resolution is limited by diffraction. Thus, for defining nanostructures, other methods like EBL or FIB are necessary. These equipments are more expensive than the tools needed for photolithography, but are now-a-days accessible to a large number of research and development labs, and even found in industry. In this work, we do not intend to replace EBL based fabrication, but we want to explore the new possibilities that FIB offers for pattering complex micro and nanostructures, which are not possible (or are very complicated) with other methods.

Total sample patterning time

FIB is often classified as a slow technique, but by using the appropriate parameters (i.e., higher ionic current) for milling structures of different sizes, the throughput can be largely improved. We show in the main text how we can pattern functional nanochannels and transient inlets within few minutes. In addition, since it is a resist free method that allows for direct milling of different sizes, depths and levels within the same step, the global time needed for the fabrication of a silicon stamp (as described in the main text) can be faster by FIB than by EBL. For EBL, spin coating, exposure, developing, etching and resist stripping are the necessary steps for defining and transfering the structures. When the structures require different depths, these steps need to be repeated each time. In addition, when more than one EBL step is necessary, aligning marks should be patterned first. And the alignment itself adds one further complication. All these steps are not necessary by FIB direct milling, what strongly simplifies the process.

		EBL	**FIB**
Process	Steps	Duration	Duration
Pattern writing	Spin coat resist	5 min	--
	Tool alignment and calibration	15 min	30 min
	Exposure/milling	20 min	30 min
	Development	5 min	--
Pattern transfer	RIE cooling down	30 min	--
	RIE etching	2 min	--
	Resist stripping	10 min	--
	TOTAL TIME	**87 min**	**60 min**
Aligning marks	Spin coat photoresist	5 min	--
	UV lithography	5 min	--
	Metal sputtering	20 min	--
	Lift off	20 min	--
	TOTAL TIME	**50 min**	**--**

Table S 2. Comparison of the total processing time for a sample patterned by EBL and FIB.

In Table S 2 we have summarized the typical time required for each processing step required for each method. As can be seen, the time needed for the fabrication of a stamp by EBL and by FIB is similar (between 1h and 1.5h). But when more than one EBL step is necessary, then this time is doubled, while patterning another depth level by FIB can be done at very little extra effort within the same step. These times have been calculated for a case where the area to be written is in the order of 100 x 100 μm^2. This is often the case in micro and nanofluidic devices, where the nanostructures are usually confined to a region that fits into the field of view of a camera. Patterning large areas becomes time consuming using FIB, and then EBL might be more convenient.

3. Flow characterization

FIB milling can sometimes lead to non-uniform and rough surfaces due to material re-deposition. In addition, milling during imaging with ions (necessary for alignment) can lead to structural and area defects. Fluid flow experiments are performed to validate the reliability of the devices, the patency of the nanochannels, and the liquid confinement. The flow measurements can also verify and examine the sealing quality, and detect possible leaks.

Figure S 3 (a) shows a fluorescent image of a device with meander nanochannels filled up with Rhodamine B diluted in isopropanol. The liquid is loaded into one of the reservoirs, and fills up the upstream microchannel immediately by capillary action. Then the liquid passes through the nanochannels to fill the opposite microchannel (downstream).

To check the liquid confinement and discard leaks, we perform photoluminescence micro-spectroscopy. A laser (532 nm wavelength, matching the absorption of Rhodamine) is focused down to a sub-micrometer spot, and the emitted photoluminescence (PL) signal is recorded with a spectrometer (from Andor). The position of the excitation spot is controlled with a piezoelectric stage. Figure S 3 (b) compares the photoluminescence signals obtained by focusing the laser spot in the microchannel (blue line), in the nanochannel (red line) and on the polymer surface in-between fluidic structures (green line). Comparing the fluorescence signals evidences the liquid confinement in the nanochannels, and discards leaks.

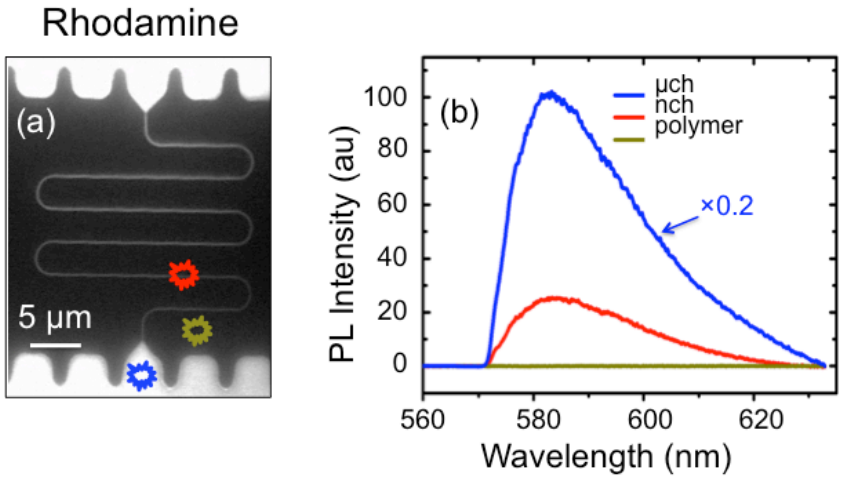

Figure S 3. (a – b) Diluted Rhodamine B in isopropanol. (a) Rhodamine B is flown in a meander nanochannel and captured with an inverted epifluorescence microscope with an exposure time of 100 ms. (b) shows the PL emission spectra of the liquid in the microchannel (µch, blue line), in a nanochannel (nch, red line) and also on the polymer as the background (green line). The spectra are taken with a focused laser, with a 532 nm excitation wavelength. The spectrum from the microchannel is divided by 5 to aid the comparison.

4. DNA in a meander nanochannel

In the main text, the flow of a DNA molecule along a meander nanochannel is shown and commented (see Figure 5). In that figure, a projection of the maximum intensity per pixel along the whole video is shown. Images of some frames of that video are shown here in Figure S 4.

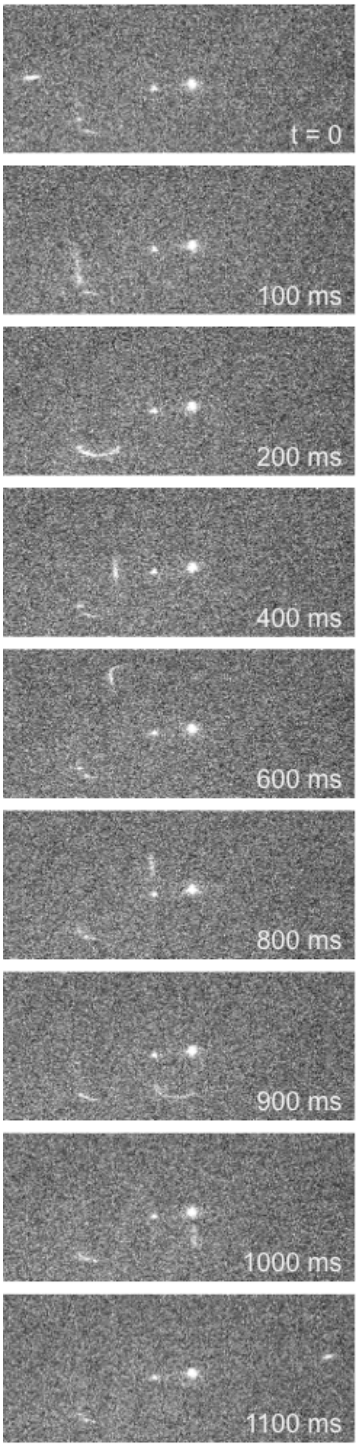

Figure S 4. Frames from a movie showing the translocation of a DNA molecule along a meander nanochannels.

5. Observation of Individual Quantum Dots in a meander nanochannel

Confined individual QDs are studied in a meander nanochannels. We used Zn-Cu-In-S/ZnS Quantum dots (PlasmaChem GmbH), 5 nm diameter, suspended in toluene with an emission maxima at λmax = ~700 ± 25 nm. These are flown into the device, and after evaporation of the solvent within few minutes, the immobilized nanocrystals can be observed individually. Figure S 5 (a) shows an epifluorescence image of a section of a meander nanochannel, where the understudied particles can clearly be seen. The image was obtained as a z-projection from a photoluminescence movie, shown in the supplementary media, by plotting the maximum intensity of each pixel, so all the quantum dots can be seen at the same time.

The photoluminescence (PL) intensity signal of one of the quantum dots along time is shown in Figure S 5 (b). The blinking behaviour is clearly observed, and the stepped signal (On and Off states) confirms that the luminescence is obtained from a single nanocrystal. This is also reflected in the histogram in (c). Other similar signals from other quantum dots can be seen in (d)-(f), corresponding to the quantum dots marked with arrows in the image in (a). All the signals are compared against the same background (black lines), obtained from a spot without nanochannel (just flat polymer).

To obtain the time scans, we recorded a time series with an EMCCD camera. Images were acquired every 100 ms. Then, using an image analysis software (Image J), the intensity profile along time for each quantum dot was plotted.

The movie where the quantum dots can be seen blinking is attached as supplementary media.

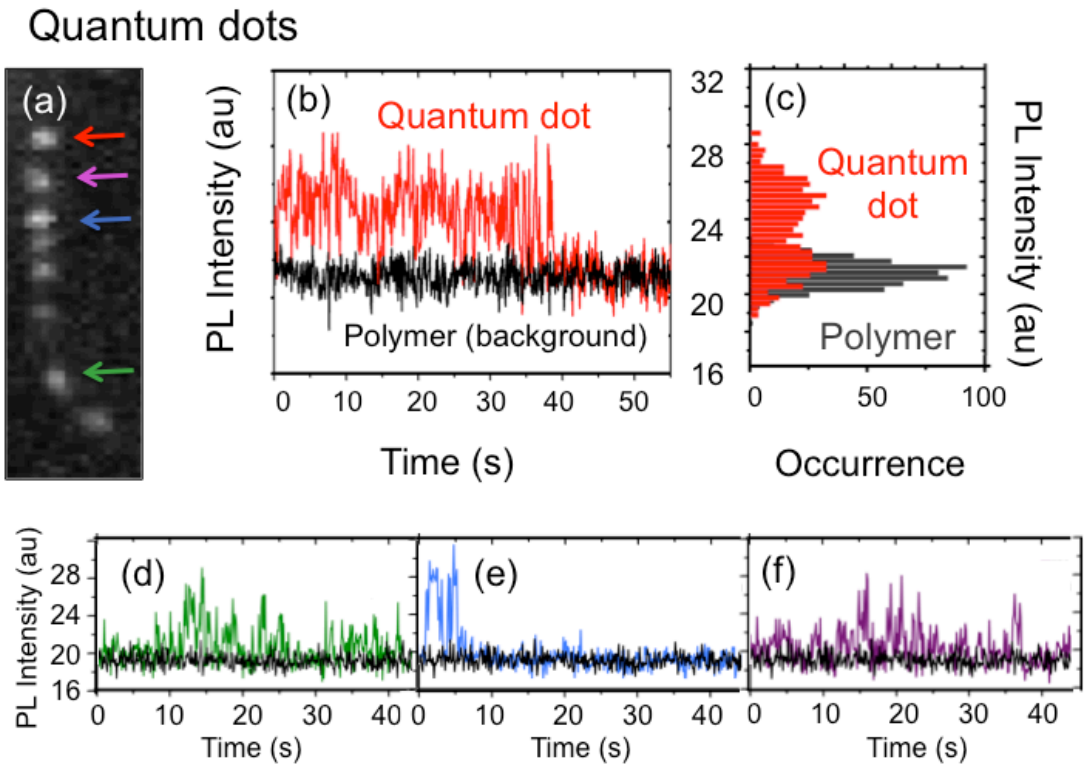

Figure S 5. Diluted quantum dots (5 nm diameter) in toluene are flown in a meander nanochannel. An epifluorescence image is shown in (a), with an exposure time of 100 ms., where they can be seen one after the other in the nanochannel. This image was obtained by plotting the maximum intensity of each pixel in a 43 s long movie, which is attached in the supplementary material. (b), (d), (e) and (f) show time traces of individual quantum dots, at positions marked in (a), where the emitted photoluminescence intensity is plotted along time, compared to the background signal obtained from a flat polymer in-between nanochannels. The histogram in (c) illustrates the on and off emission states of quantum dots, corresponding to the PL plot in (b). The signal is compared to the background (polymer).

6. Different modular inlets in the same stamp

Apart from the stamps shown and mentioned in the main text, another stamp was milled during this work, which is worth mentioning. It is shown in Figure S 6. It also has different types of inlet configurations, but, in contrast to the one presented in Figure 3 in the main text, here the nanochannels do not have the same length. The idea behind this stamp was to use different inlet elements, and combine them to see their effect in the flow. For example, we have long 3D inlets, short 3D inlets, squared transient inlets -big and small-, and abrupt deep channels. These are combined as can be seen for example in Figure S 6 (b). There, the long 3D inlet is marked as channel #1. The short 3D inlet as #2. Then, in #3, a combination of the short 3D inlet and the "big" squared transient is shown. And in #4, both, the big and small inlets are used.

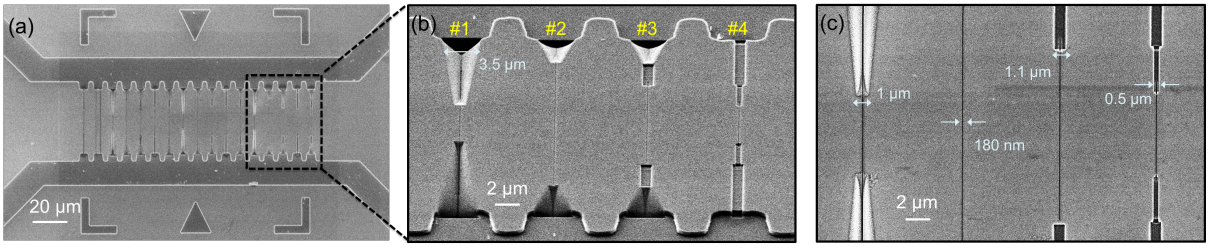

Figure S 6. A general overview of the stamp with 17 nanochannels can be seen in (a). In (b), a block of four different inlets is shown, and a detail of the nanochannels can be seen in (c). The inlets have different geometries and configurations, as can be seen from left to right in (b): long (15 µm), 3D, funnel-like triangular inlets; short (5 µm) 3D triangular inlets; a combination of a short 3D triangular inlet with a rectangular, wide (1.1 µm) and deep (440 nm) transient nanochannel; and two sets of rectangular transient nanochannels, gradually wider and deeper than the actual nanochannels (the first one is 1.1 µm wide, 440 nm deep, and the second one is 500 nm wide, 290 nm deep). In addition, the stamp has long nanochannels, which are directly connected to the microchannels. All these types of structures are milled in the same step, one after the other, which is only possible using FIB.

7. Media files

Movie 1 – Milling of a meander nanochannel
The movie shows SEM images obtained while milling a meander nanochannel in real time. The nanochannel was milled with a current of 300 pA, and a dose of 10 mC/cm^2 in one cycle. The inlets were milled with 300 pA Ga^+ ion beam current, a dose of 200 mC/cm^2 in 2 cycles with unidirectional scanning mode starting at the triangle tip. This video is 8 times faster than the real time milling.

Movie 2 – Quantum dots in a nanochannel
The movie shows quantum dots physically confined in a meander nanochannel. The liquid media is evaporated, so the quantum dots do not flow (are static) and can be observed one by one. They can be seen blinking as the photoluminescence signal goes on and off. The intensity plots along time for different dots are shown in the text and in the supplementary information.

Movie 3 - lambda DNA flowing along nanochannels with different types of inlets connected to identical nanochannels.
The movie shows lambda DNA molecules stained with YOYO1 flowing along nanochannels of an imprinted fluidic device. This device has different types of inlets, connecting the micro and nanochannels. Long, 3D inlets and squared inlets with gradually increasing width and depth work best for flowing the DNA molecules into the small nanochannels.
All the nanochannels are 260 nm wide, 220 nm deep, 20 um long. A glimpse of the topography of the sample (by bright field illumination) can be seen at the beginning of the movie for reference.

Movie 4 - lambda DNA flowing along nanochannels with different types of inlets. Modular stamp
The movie shows lambda DNA molecules stained with YOYO1 flowing along nanochannels of an imprinted fluidic device. This device has different types of modular inlets, connecting the micro and nanochannels. In min 1:10, a molecule flowing along channel 3 can be seen, and the slowing down inside the smooth 3D inlets observed.

Movie 5. DNA in meander nanochannels (background substracted)
The movie shows a lambda DNA molecule stretched and flowing along a meander nanochannels. These results are shown and discussed in the main text. To facilitate the visualization, the background has been substracted.

Movie 6. DNA in meander nanochannels (original, unmodified)
Same movie as Movie 5, but unmodified.