Supporting Information

Straightforward micro-patterning of oligonucleotides in microfluidic by novel spin-on ZrO2 surfaces

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Spin curves of ZrO_2 based system

HOI films have been deposited by spin coating technique on silicon wafer (100) at different spinning rates (1200, 2000, 3000, 4000, 5000 rpm) for 30 sec. Thickness of the samples has been measured by means of spectroscopic ellipsometry (V-Vase J.A.Woollam Ellipsometer).

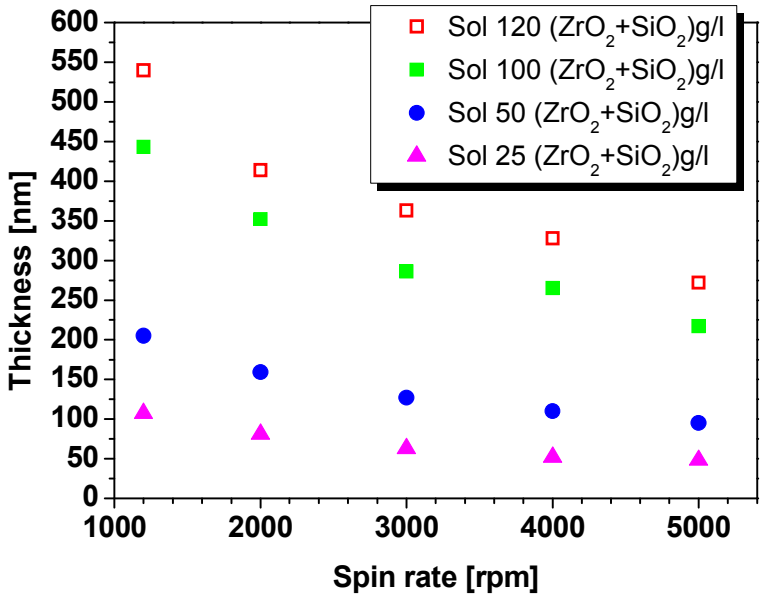

Figure S1. Spin curve of ZrO_2 based system with sol concentration of 120, 100, 50, 25 (SiO_2+ZrO_2) g/l.

Bonding test

The PDMS microfluidic channels have been bonded via UV-ozone treatment to specific substrates. Figure S2 shows the resulting breakage pressure on different samples. We first tested the UV-ozone treatment effect on quartz and we observed that the treatment on the substrates is fundamental for the achievement of strong bonding with PDMS. Secondly, we tested the bounding protocol to others substrates: ZrO_2 flat and patterned ZrO_2 and we obtained analogous results of pressure resistance.

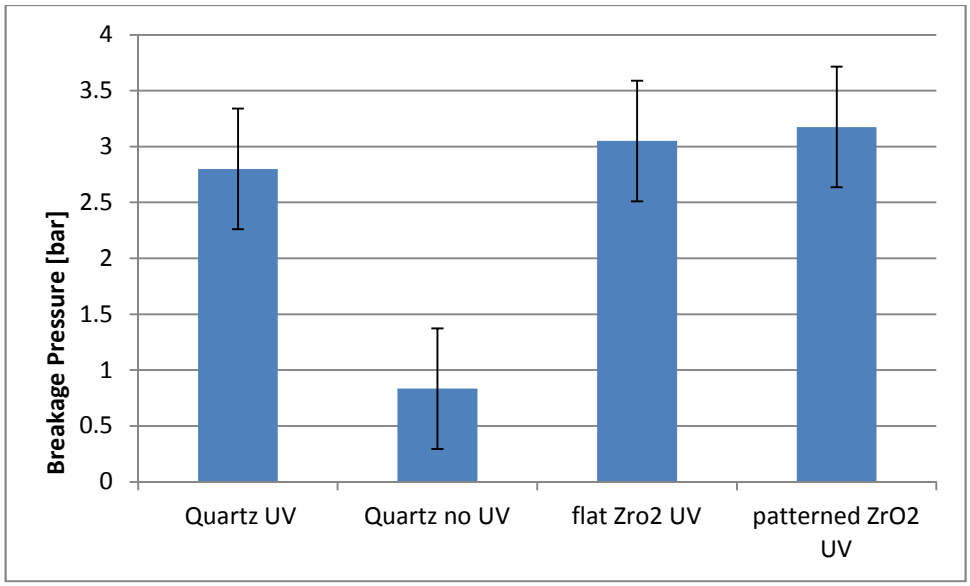

Figure S2. The histogram shows the effect of UV-ozone pre-treatment on PDMS bonding.

Both quartz and zirconia substrates have been tested showing high resistance to breakage.

Surface activation

The UV-ozone treatment has an effect on surface activation.[1] We observed that UV ozone treatment of glass slides creates a non-specific bonding of target molecules on the surface. In order to avoid non-specific absorption, the channels have been rinsed with acetone for a few minutes.

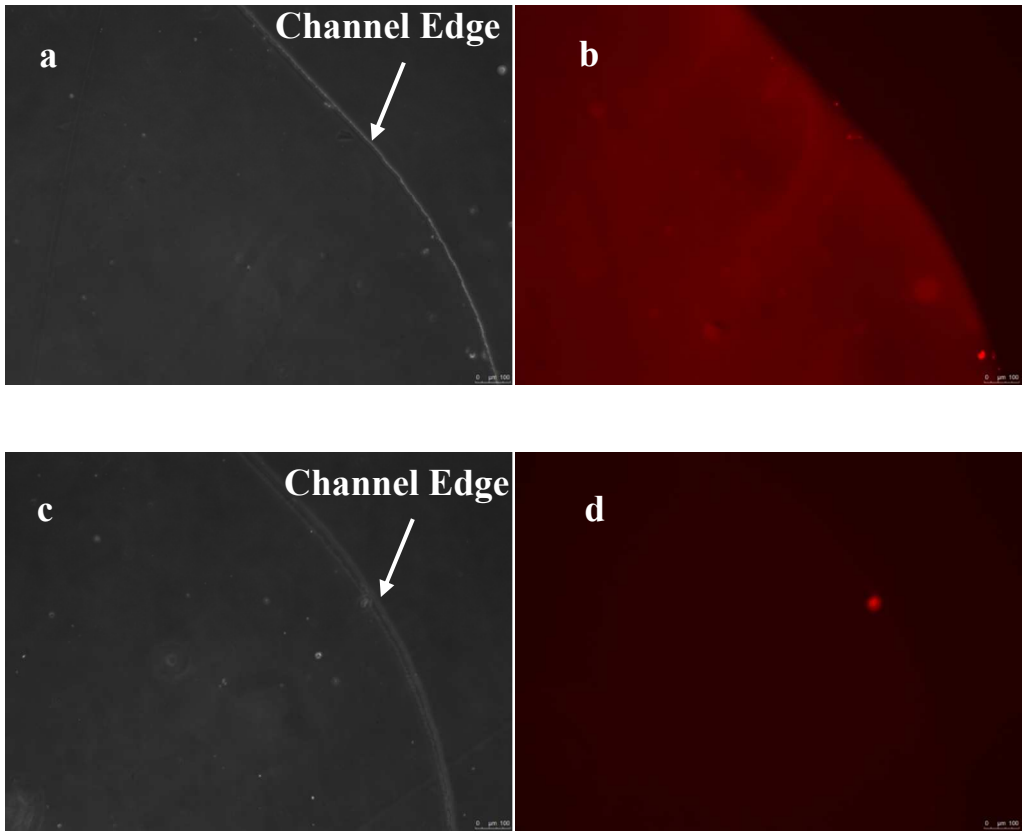

Figure S3. Optical microscopy and fluorescence image of UV-ozone-activated quartz samples for PDMS bonding. The slide a) and b) has been functionalized with NCT strand labeled in 5' with Alexa for 30min without any rinsing after the UV-ozone treatment. Samples c) and d) have been previously rinsed with acetone.

Melting point of ssDNA strands

Melting temperatures of the ssDNA oligos used in the immobilization experiments, non-specific absorption tests and hybridization/denaturation cycle were reported in table S1. The melting temperatures were calculated for a concentration of 1μM, by using an online software (https://eu.idtdna.com/calc/analyzer).

Table S1. Melting temperatures of ssDNA sequences used in immobilization experiments, non-specific absorption tests and during the DNA-DNA hybridization tests.

DNA Denomination	**Base sequence (5'-3')**	**Melting point T_M (°C)**
Probe 1	GGGCAGGCCATTCCTCCTTCA	**64**
FCT (Full Complementary Target)	TGAAGGAGGAATGGCCTGCCC	**64**
NCT (Non Complementary Target)	ACTTCCTCCTTACCGGACGGG	**62.7**
Probe 2	CCCGTCCGGTAAGGAGGAAGT	**62.7**

Non-specific absorption tests

Fluorescently labeled ssDNA strands (FCT and NCT) without the terminal phosphate group have been used to investigate the non-specific attachment on the ZrO_2 surface. The non-phosphorylated oligonucleotide solution (1 uM) has been deposited onto the patterned features, treated at 800°C and left to interact for 30 min at RT. The experimental evidence, reported in Figure S4, is that no fluorescence signal can be detected. This result confirms the non absorption

of ssDNA through phosphate ester backbone and supports the specificity of terminal phosphate group to the zirconium oxide surface.

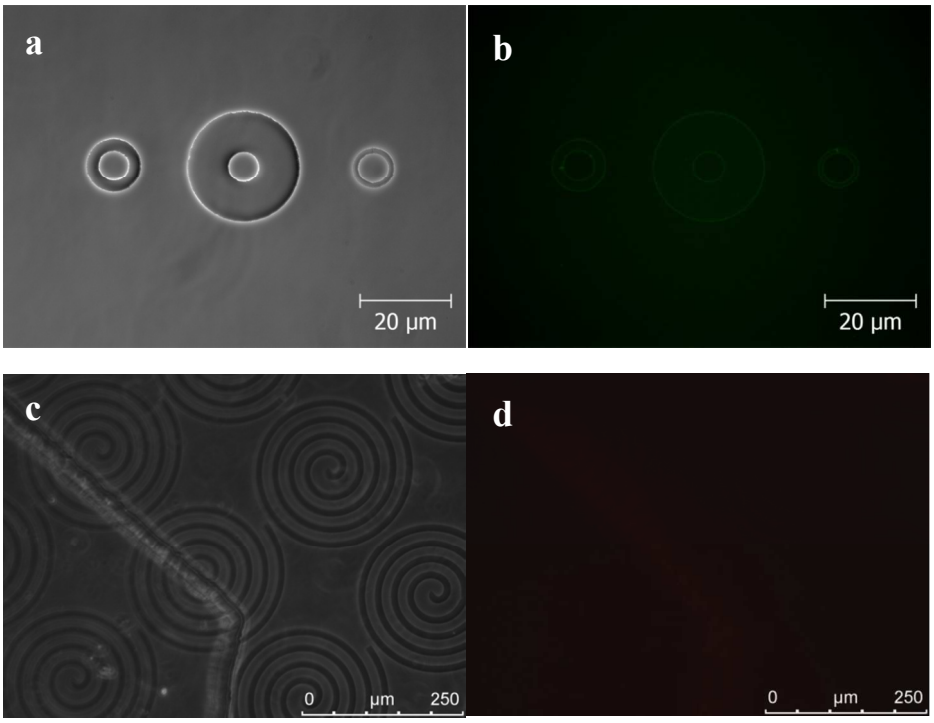

Figure S4. Optical microscopy and fluorescence image of thermal treated ZrO_2 features at 800°C. The samples a) and b) have been functionalized with single strand DNA molecule labeled in 5' with fluorescein (FCT) for 30 min. The sample c) and d) have been functionalized for 30 min with the NCT strand labeled in 5' with Alexa 594.

References

(1) Vig, J.R. UV\Ozone Cleaning of Surfaces. *J. Vac. Sci. Technol. A* **1983**, 3, 1027-1034.