Electronic Supplementary Material (ESI) for Lab on a Chip.
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Supplementary Information

For

High-Throughput Roll-to-Roll Production of Polymer Biochips for Multiplexed Point-of-Care Diagnostics

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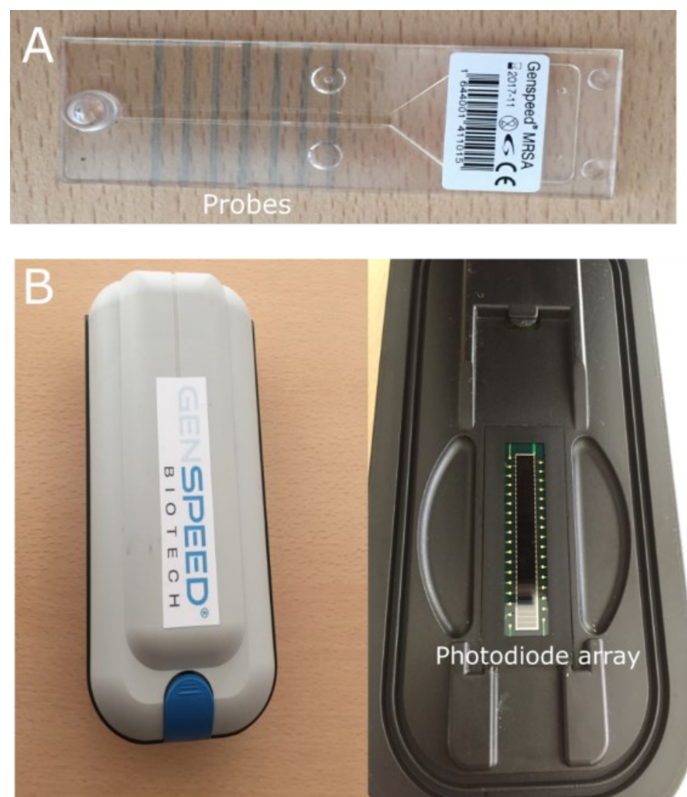
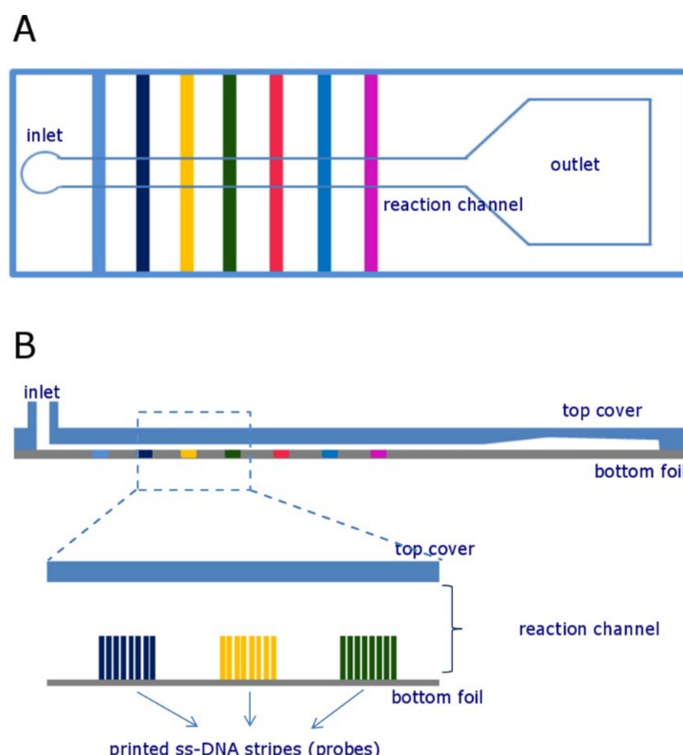
Supplementary movies available: [R2R DNA micro spotting, Capillary flow in the R2R UV-NIL produced biochips].



SFigure 1: GENSPEED® R_1 MRSA Sensing System (Genspeed-Biotech, Austria). (A) A disposable, commercial GENSPEED® chip (25 mm width and 75 mm length) with the capillary printed different probe ss-DNAs (blue stripes) at the functionalised PS surface. **(B)** The first generation, GENSPEED® R_1 (left) with a photodiode array (right) inside the tray region wherein the disposable chip is inserted. The array consists of 32 photodiodes.



SFigure 2: The disposable GENSPEED® bioanalytical chip for MRSA sensing (Genspeed-Biotech, Austria).[1] Illustrations show **(A)** top and **(B)** side views of the commercial chip. The chip consists of an upper, injection-moulded top cover and a DNA functionalised polymeric foil as the bottom surface. The top cover has an inlet, a reaction channel and an outlet. The ss-DNA stripes (i.e., probes) are capillary printed at the bottom part of the foil using a printing solution without SciPOLY3D® linker. The biochip owns seven different probe regions enabling multiplexed testing. The probes were printed at the corresponding pixel locations of 4°, 8°, 12°, 16°, 20°, 24° and 28° in the photodiode array (from left to right SE, NC, mecA, mecc, SA, PC and HC probes). A closer look of the stripes are also shown in (B). Each stripe in different colour represents a different ss-DNA probe. Also, two parts are bound together using a ultrasonic welding machine.

STable 1: The layout of probe ss-DNA printing in the disposable biochips.

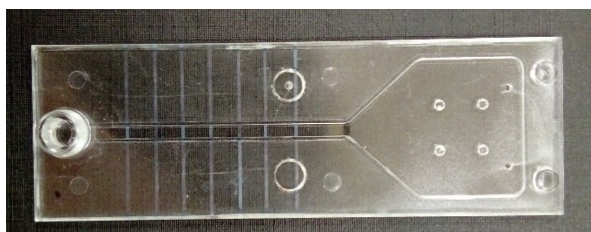
Pixel (photodiode) position	**Oligonucleotide abbreviation**	**Description**
Pixel 4	SE	S. *epidermidis* probe
Pixel 8	NC	Negative control
Pixel 12	mecA	Methicillin-resistant *mecA* probe
Pixel 16	mecC	Methicillin-resistant *mecC* probe
Pixel 20	SA	S. *aureus* probe
Pixel 24	PC	PCR control
Pixel 28	HC	Hybridisation control

STable 2: Hybridisation protocol using 1X R2R solution.

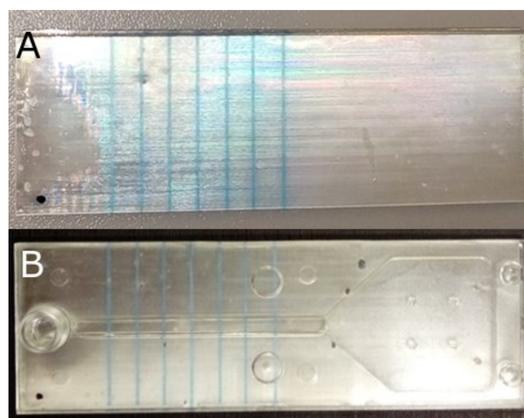
Solution	**Volume (µl)**	**Time (min:sec)**	**Duration (min:sec)**
Hybridisation (1X R2R, GENSPEED® C)	20	0:00 (start)	3:00
Enzyme (GENSPEED® D)	30	3:00	1:30
Wash (GENSPEED® E)	40, 30*	4:30	1:30
Chemiluminescent (GENSPEED® F)	30, 20*	6:00	2:30
	120, 100* (total volume)	8:30 (end)	8:30 (total duration)

**The values for the foil-based chip (i.e., $Chip_3$). The amounts of the solutions E anf F were re-adapted for $Chip_3$ since the designed total chip volume was smaller different than of the injection-moulded $Chip_1$ and $Chip_2$.*

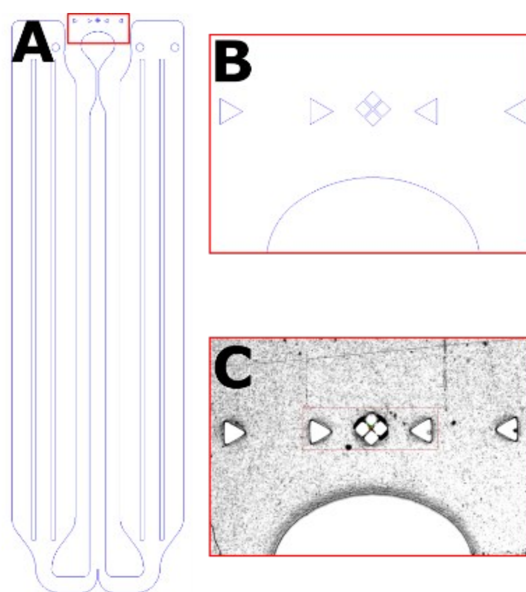
[1] *The figure was redrawn from Master thesis of Kerstin Spindlberger: Further development of a molecular diagnostic system towards integrated sample amplification and increasing multiplexing capabilities, Linz Fachhochschule, 2016 (Linz, Austria), page 16.*



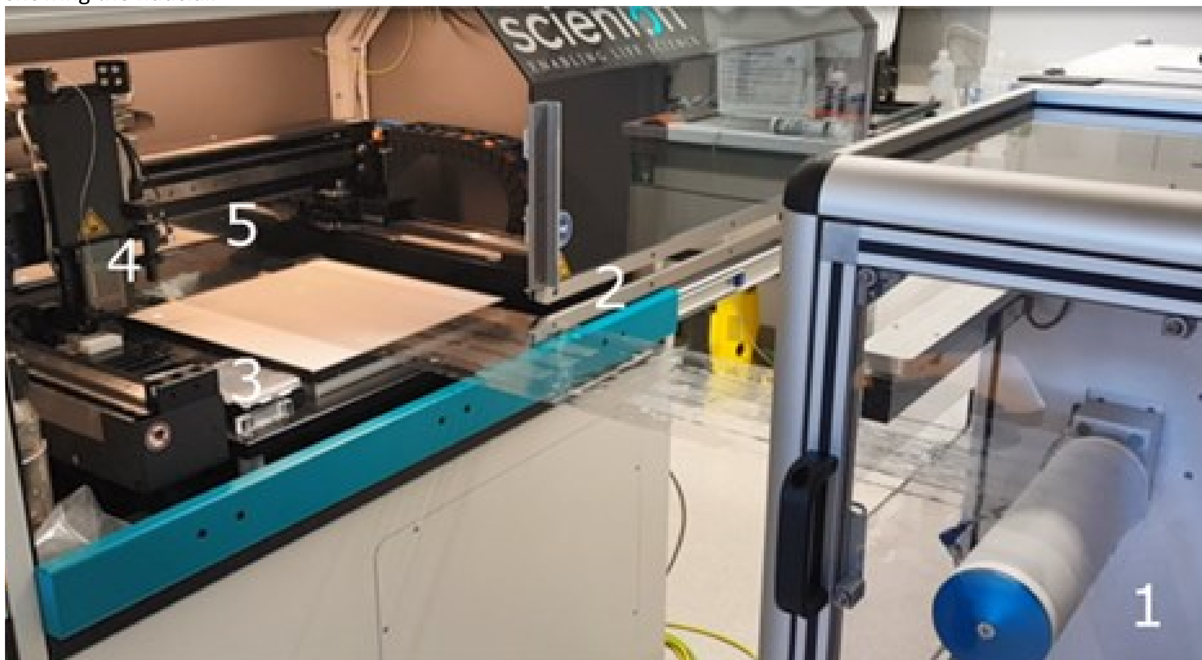
SFigure 3: The biochip with the injection-moulded top cover (i.e., Chip$_1$). The PS foil was capillary printed with 7 different ss-DNA stripes. The blue stripes from left to right are the probes of SE, NC, mecA, mecc, SA, PC and HC, respectively. **(B)** A finalised biochip, composed of the injection moulded top cover and the PS bottom part. Probe ss-DNAs (blue stripes) were capillary printed using the SciPOLY3D® DNA linker (Scienion AG, Germany). Also, the two parts were bound together inside the ultrasonic welding machine. The biochip dimensions are 75 mm (length), 25 mm (width), 1.5 mm (height).



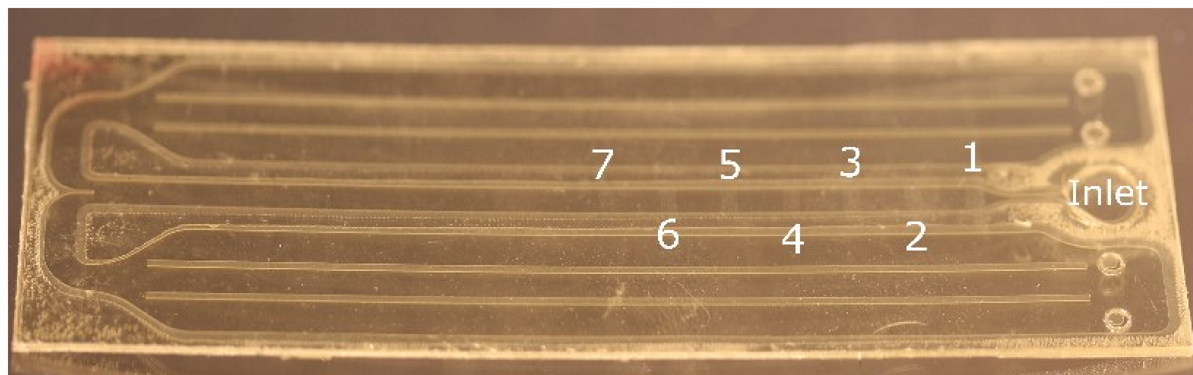
SFigure 4: The biochip with R2R UV-NIL produced optical microstructures (i.e., Chip$_2$). (A) The PS foils with imprinted optical microstructures (at the bottom side) and the capillary printed 7 different ss-DNA stripes (at the upper side. The finalised biochip composed of the injection moulded top cover and the R2R UV-NIL structured foil. Probe ss-DNAs (blue stripes) were capillary printed using SciPOLY3D® DNA linker. Also, the two parts were bound together using a ultrasonic welding machine. The biochip dimensions are 75 mm (length), 25 mm (width), 1.5 mm (height).



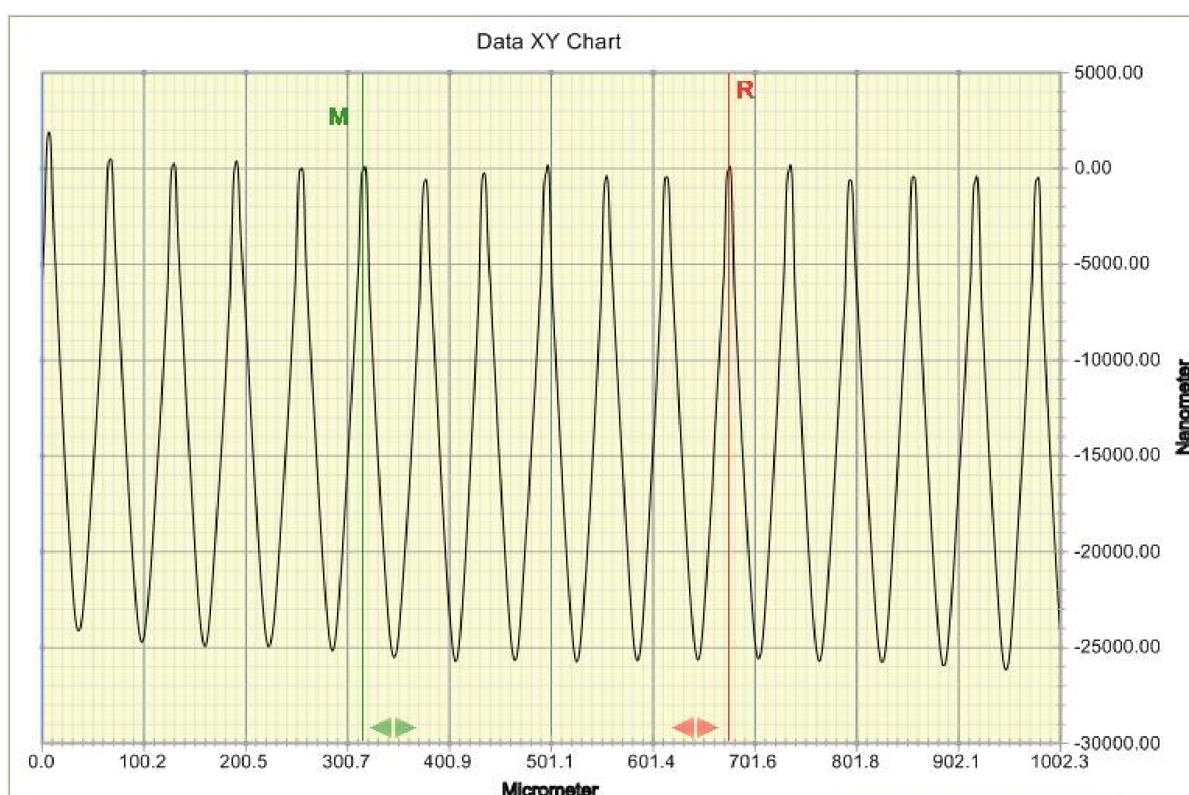
SFigure 5: A drawing showing where the fiducial was located. (A) The complete channel design. The region, in which the fiducial was located, was indicated by the red lined rectangle. **(B)** The drawing showing the fiducial inside the red rectangle. **(C)** A vertical camera image from the micro-array spotter (sciFLEXARRAYER S12™, Sscienion AG, Germany) showing the fiducial.



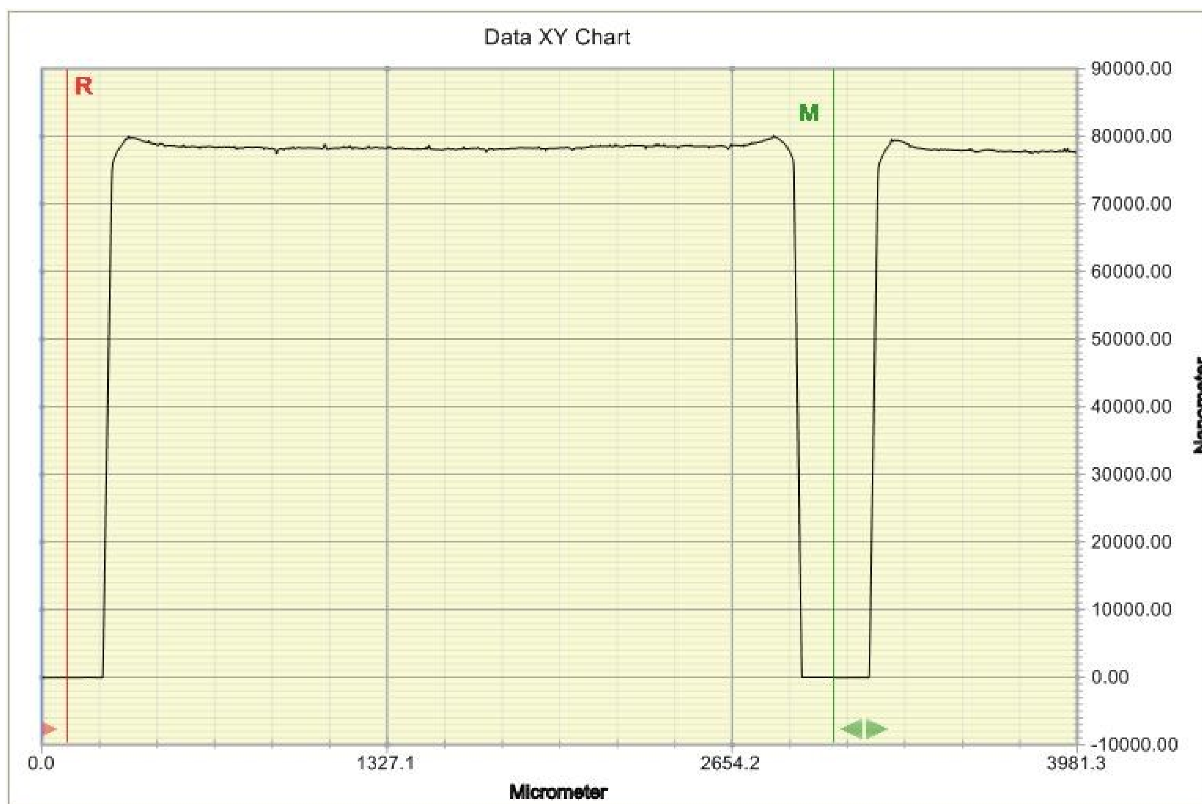
SFigure 6: The automatic R2R micro-array spotting pilot line for biofunctionalisation. (A) The R2R UV-NIL structured polymer foil is wrapped over the web inside the unwinder (1). The front side of the medium-scale micro-array spotter (i.e., sciFLEXARRAYER S12™, Scienion AG, Germany) has a slit at its sliding door, which enables the foil movement (2). The ss-DNA probes are taken from a V-bottomed 96-well source plate (3), which is controlled by a chiller to keep the temperature constant. Then, micro-spotting is done using the PDC glass nozzles installed to the nozzle head (4). During the micro-spotting, the foil area of interest is stabilized by automatically activating the vacuum table (width: 300 mm, length: 375 mm) underneath, to avoid any undesired foil movements during the process.



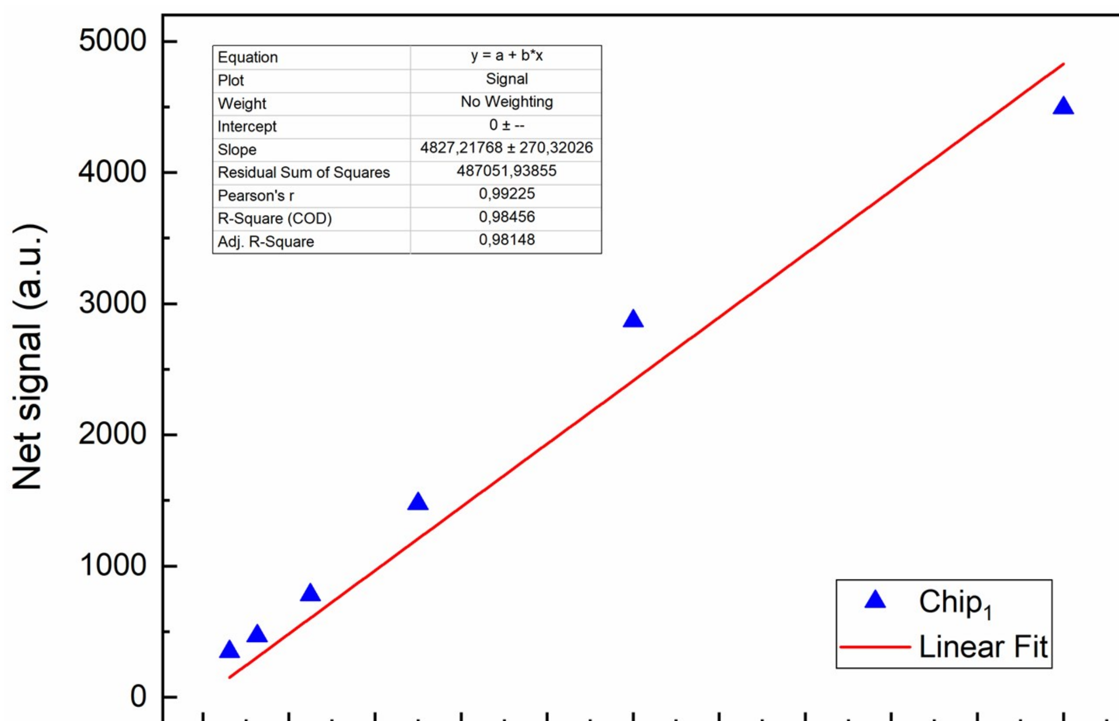
SFigure 7: The R2R UV-NIL imprinted biochip with 7 different probe ss-DNA micro-spots (i.e., Chip$_3$), allowing a multiplexed analysis. The micro-spots from 1 to 7 are the ss-DNA probes of SE, NC, mecA, mecc, SA, PC and HC, respectively. The micro-spotting was performed in the sciFLEXARRAYER S12™ (Scienion AG, Germany), at room temperature and 38 ± 2 % relative humidity. SciPOLY3D™ DNA linker (Scienion AG, Germany) was utlized during the DNA micro-spotting. The two chip parts were bound together via the glue print, in a rolling mill machine (at 50 ˚C). The biochip dimensions are 75 mm (length), 25 mm (width), 100 µm (height).

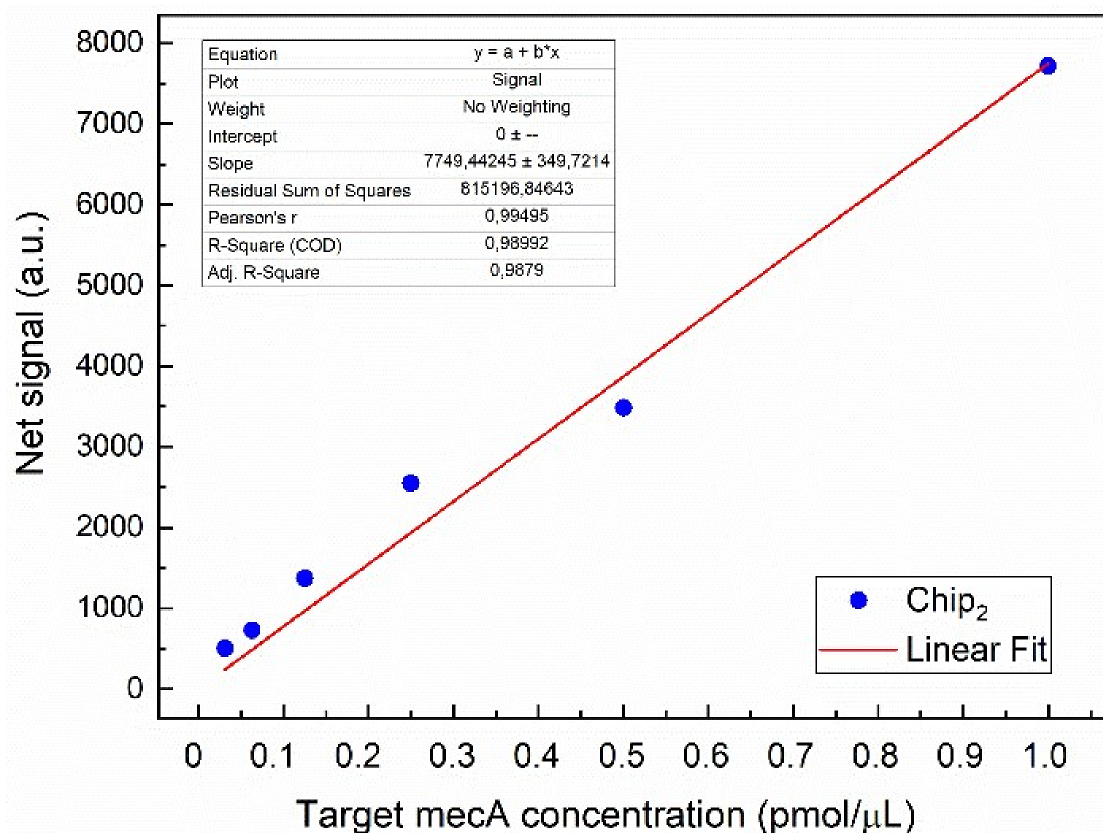


SFigure 8: The profilometry data of the R2R UV-NIL imprinted optical microstructures on PS foil. The size and depth of the imprinted structures were analysed using the profilometre (Dektak 150 Surface Profiler, Veeco, USA). The structure height and side length were measured as ~ 25 µm and ~ 50 µm, respectively.



SFigure 9: The profilometry data of the R2R UV-NIL imprinted fluidic channel on PET foil. The fluidic channel was analysed using the profilometre (Dektak 150 Surface Profiler, Veeco, USA). The channel depth and width were measured as 70 µm and ~2700 µm, respectively.





SFigure 10: The calibration curves for limit-of-detection (LOD) calculations. The target mecA concentrations of 0.03, 0.06, 0.13, 0.25, 0.50 and 1.00 pmol/µL correspond to 1:32, 1:16, 1:8, 1:4, 1:2 and 1:1 dilution ratios of 1X R2R solution, respectively. 1X R2R solution contains 1 µM of mecA target oligos. Each measurement was repeated 3 times at room temperature. Each data was provided as a mean value. The background values (a.u.) were subtracted from each data to provide a background corrected and integrated net signal (a.u.). For $Chip_1$ **(A)** and $Chip_2$ **(B)** LOD values for mecA detection, were calculated as 0.07 and 0.06 µM, respectively. *For an accurate calibration, the blank data were not included in the calibration curves for both results. Both blank intensity signals (a.u.) were below the hypothetical limit-of-blank signals (a.u.).*