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

Electronic Supplementary Informations

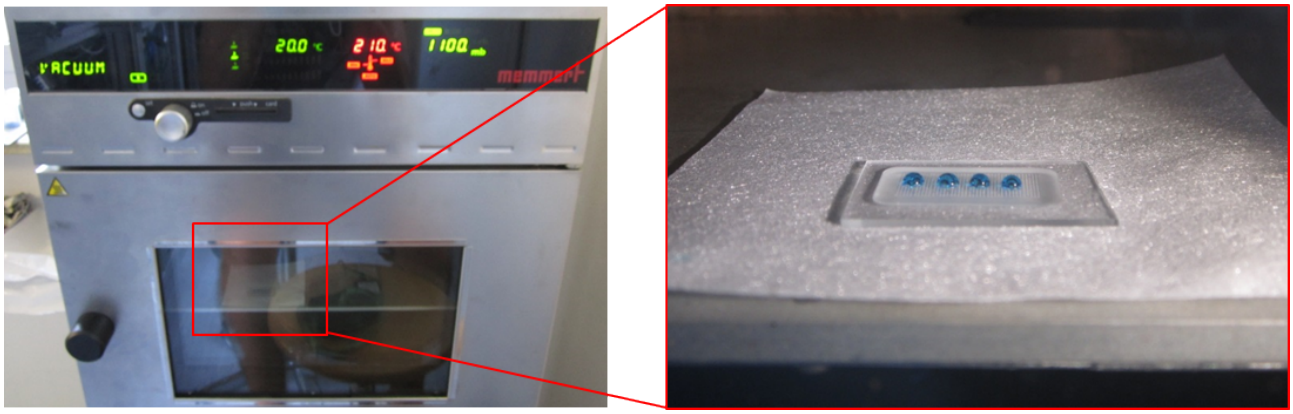

Figure S1. Digital photographs of the experimental set up for trapped air evacuation from the PDMS membrane microchannels.

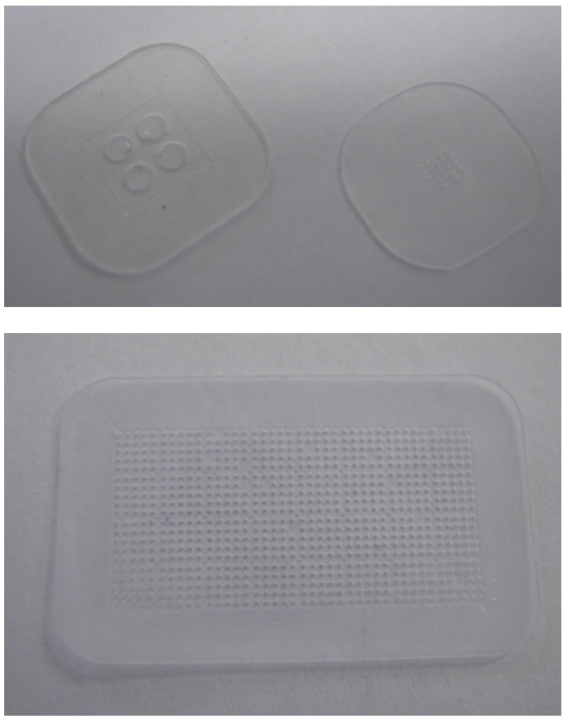

Figure S2. Digital photographs of PDMS membranes with different layouts, areas and hole diameters.

PDMS membrane fabrication

Benchman VMC4000 Numerical Control milling machine was used to fabricate the PMMA masters for the casting of PDMS membranes. The as-fabricated PMMA mould was cleaned several times in ethanol and dried under nitrogen stream to remove the residual deriving from the mechanical micromachining. PDMS pre-polymer and curing agent (Sylgard 184, Dow Corning) were manually

mixed for 5 minutes and degassed at room temperature for 1 h. The mixture was then poured into the mould and cured in a convection oven for 1 h at 70 °C. Subsequently, the 200 μm thick membrane was peeled off from the mould, clean in ethanol, drien under N_2 flow and dehydrated on hot plate for 5 minutes at 80 °C.

Surface functionalization and activation protocol

Glass slices were used as test substrates. Before exposure to organosilanes, the substrates were cleaned into piranha solution (70% H_2SO_4 : 30% H_2O_2) for 10 minutes to remove organic contaminants, then rinsed with deionised water and dried in a stream of nitrogen.

Freshly prepared samples were incubated in 3-aminopropyltriehoxysilane (APTES) 1% v/v solution in toluene reflux at 70 °C for 10 min, following an anhydrous protocol (reaction conducted under argon flux using glassware dried in oven at 140 °C overnight), in order to reduce the presence of water that, acting as a catalyst, causes APTES hydrolysis in ethanol and trisilanols. Silane-coated substrates were rinsed deeply with toluene and well dried under nitrogen flux. Just after the reaction, they were incubated in a 0.5 % (v/v) glutaraldehyde (GA) solution in borate buffer 0.1M (pH 8.5) for 1 h, using an orbital shaker at 40 rpm. Borate buffer pH value ensures that amino groups exposed at surface are not protonated ($–NH_2$), so available to react with GA aldehyde groups (–CHO). 300 μL of sodiumcyanoborohydride solution (5M) in sodium hydroxyde were added after 15 minutes, in order to create a more stable bond, reducing the imine formed by the reaction between $–NH_2$ and –CHO groups. After incubation, substrates were rinsed several times with deionized water, dried under nitrogen stream and stored into a dessicator.

Cleaning procedure for membrane reusability

The sample were cleaned by ultrasonicating the sample in Acetone, Ethanol and deionized water 10 min each.

Fourier transformed infrared spectroscopy

Fourier transformed infrared (FTIR) spectra were collected using a Nicolet 5700 FTIR Spectrometer used in attenuated total reflectance (ATR) mode with 4 cm^{-1} resolution and an average of 64 scans. FTIR was performed on PDMS membrane to check the effectiveness of the cleaning procedure after spotting.

Optical measurements

A ZEISS optical microscope (Scope A1-Axio) was used to collect images of the spotted surfaces in bright field mode.

Fluorescence microscopy measurements

The samples were characterized by fluorescence microscope using the customized setup schematically depict in Figure S2. Fluorescence is excited by means of a collimated Nd:YAG laser beam (532 nm) impinging on an area larger than the spot size. Fluorescence is then collected through a lens with Numerical Aperture 0.07 and imaged, via a tube lens, on a CMOS RGB camera (Thorlabs DCC1645C). Along the collection arm, a spectral filter (RazorEdge LongPass 532) is used to filter out the laser radiation.

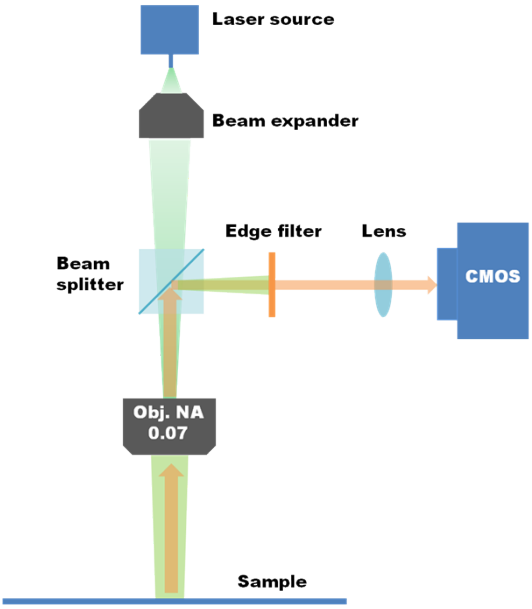

Figure S3. Scheme of the fluorescence microscopy customized setup used to characterize the spotted samples.

Multiple solution spotting procedure

Figure S4 shows how to create an array of spots containing multiple proteins by filling each column of wells with corresponding target samples. In this way different configurations become feasible. Optical microscope image showing the alignment of a PDMS pad having microchannels on its bottom side onto the microarray PDMS spotter (inset Figure S4). This arrangement demonstrates the feasibility of this approach for multiple solution spotting.

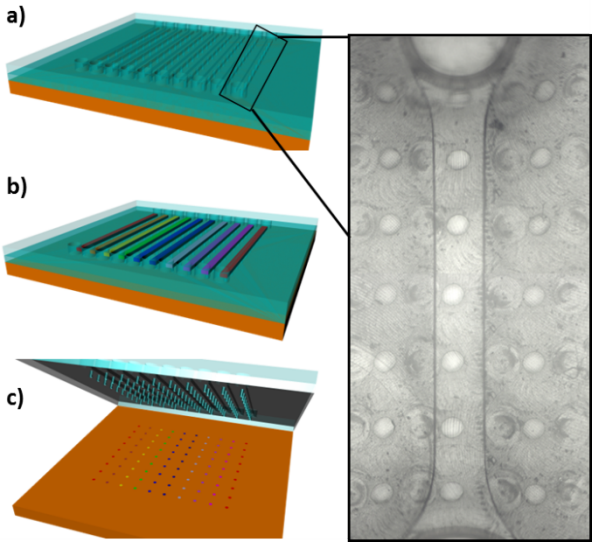

Figure S4. 3D scheme showing the alignment of an ad-hoc designed microchannel array PDMS pad onto the holed PDMS membrane (a). By simply filling each channel with different solutions and applying the vacuum (b) it is possible to print multiple analyte spot array.

Cascade reaction procedure

The proposed approach is based on the simple realignment of a micromachined PDMS chip containing straight channels on top of a pre-spotted surface. Different solutions can be convoyed towards the various columns and/or rows of the microarray, therefore allowing cascade reaction handling (Figure S5). An example of alignment of a PDMS pad having microchannels machined on its bottom side onto pre-spotted surface is shown in the inset of Figure S5a.

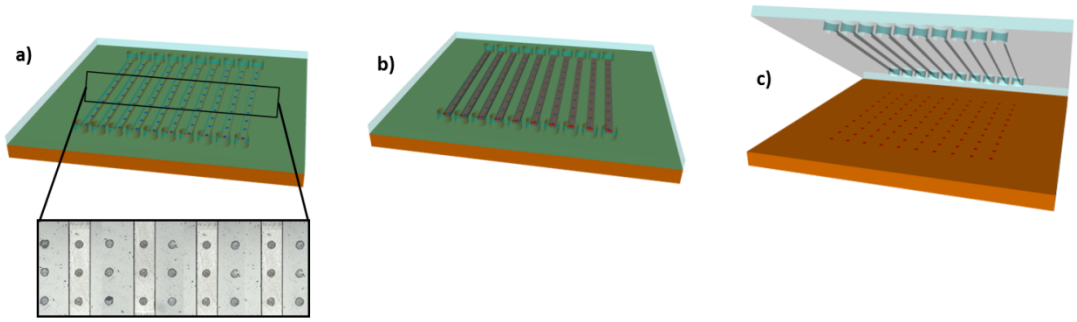

Figure S5. 3D scheme showing the alignment of an ad-hoc designed microchannel array PDMS pad onto a pre-patterned surface (a). By simply filling each channel with different solution (b) it is possible to perform cascade reactions.