Supplementary material (ESI) for Lab on a Chip

Supplementary information

High-Sensitivity Miniaturized Immunoassays for Tumor Necrosis Factor α using Microfluidic Systems

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Surface treatment of the chips.

A hydrophobic self-assembled monolayer was deposited on the upper surface of the Au-coated chip. This was done by inking a slab of PDMS with a 0.5-mM solution of eicosanethiol in ethanol for 1–2 min, drying the stamp with compressed air, and microcontact printing the chip for 1 min. The etched structures (filling ports, microchannels and capillary pumps) were subsequently coated by immersing the entire chip in a 10-mM solution of HS-PEG in ethanol for 20 s, and the chip was rinsed for 10 s with a stream of ethanol, and dried. This compound made the recessed structures wettable (advancing contact angle with water of ~35º) and protein-repellent.

Background fluorescence of PDMS substrates

The background fluorescence of the PDMS substrates shown here was measured for the excitation wavelengths of the fluorescent labels most commonly used in biological assays. Measurements were taken using a 1-mm-thick PDMS. The substrate was scanned in a fluorescence spectrophotometer (F-4500, Hitachi) with an excitation wavelength of 450 to 650 nm; the fluorescence emission between 500 and 700 nm was recorded. The

sampling interval was 2.0 nm and the scanning rate 30,000 nm min^{-1}. The excitation and emission signals both had a width of 10 nm. A glass microscope slide (49 × 49 × 1 mm^3, Menzel, Braunschweig, Germany) was used as a carrier for the PDMS. This glass did not exhibit auto-fluorescence within this wavelength range (data not shown). PDMS is largely transparent to visible light (see J. C. McDonald and G. M. Whitesides, *Acc. Chem. Res.*, 2002, **35**, 491–499) and can even be employed as a light-coupling mask for photolithography or to form optical waveguide structures. Figure S1 reveals, however, that PDMS fluoresces to some extent when excited at ~570 nm and shorter wavelengths. The Pt complexes that appear as traces in Sylgard 184 and are used to catalyze the polymerization reaction might account for this fluorescence. The fluorescence of TRITC, FITC, and Cy3 overlaps with the autofluorescence of PDMS; it is therefore preferable to select longer-wavelength fluorophores such as Cy5 or Alexa 647 to perform high-sensitivity assays.

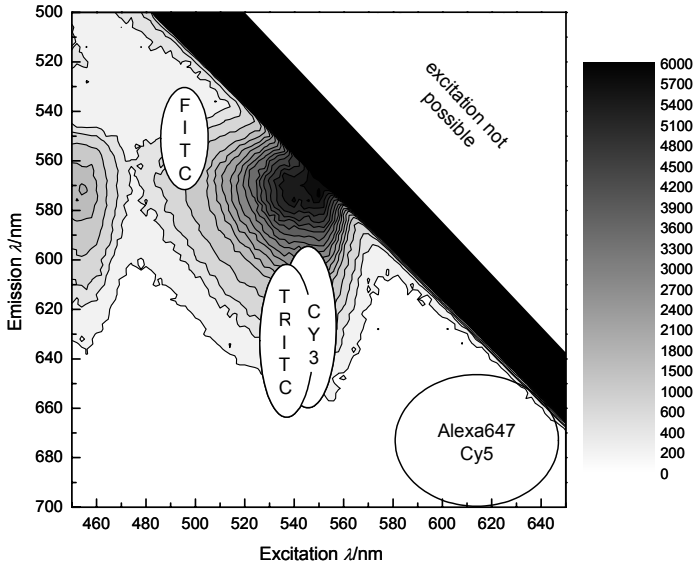

Figure S1. Fluorescence spectrum of PDMS Sylgard 184. The fluorescence emitted by PDMS as a function of the excitation wavelength reveals that PDMS has a small but significant fluorescence for the excitation wavelengths of TRITC and FITC fluorophores. Alexa 647 and Cy5 can be used as signal generating dyes in the assays while keeping

background fluorescence from the PDMS substrate minimal. The diagonal black bar corresponds to saturation of the detector (> 65,000 counts) by the excitation signal.