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

Supporting Information

Multiplexed microfluidic fluorescence immunoassay with photodiode array signal acquisition for sub-minute and point-of-need detection of mycotoxins

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Section S1. Functionalization and packing of beads in microchannels

To functionalize the agarose beads with antibodies against each of the target mycotoxins, commercial protein G conjugated beads were first incubated in anti-mycotoxin IgG solutions for 15 min in PBS buffer using a rotator mixer (20 revolutions per min) to avoid settling. For the incubation, 3 µL of a bead solution in PBS (≈ 50% (v/v) bead resin) were transferred to 12 µL anti-AFB1, 12 µL anti-DON or 0.5 µL anti-OTA IgG solutions, prepared also in PBS to a final IgG concentration of 50 µg/mL, except for the anti-OTA IgG where the final concentration was 41.7 µg/mL. To pack the beads in the single microchannels, 110 µL of a solution of 1 mg/mL BSA in PBS was added to each set of beads. Then, after suspending the beads in solution, 40 µL of each bead solution were pipetted and the pipette tip subsequently released after being fitted to the inlet hole of the microchannels. Afterwards, a syringe pump (NE-1200 from New Era Pump Systems, Farmingdale, NY, USA) was immediately used to exert a negative pressure at the outlet of the microchannel at a flow-rate of 15 µL/min. The microchannels with the packed beads were stored at 4 °C until further use.

Section S2. Labelling of mycotoxin-BSA conjugates with Alexa 430

The mycotoxin-bovine serum albumin (BSA)-Alexa 430 conjugates were prepared by labelling BSA-mycotoxin conjugates with an amine reactive Alexa 430-NHS ester dye (Thermo Fisher Scientific). Prior to the labelling procedure, 500 µL of BSA-mycotoxin conjugate in phosphate-buffered saline (PBS) at an initial concentration of 1 mg/mL were buffer exchanged and concentrated to a final volume of ≈25 µL using Amicon Ultra-0.5 centrifugal filter units (MWCO of 10 kDa), purchased from Merck, at 14,000 g for 10 min, resulting in a final concentration of ≈20 mg/mL in 100 mM bicarbonate buffer (pH = 9.2). Then, 24 µL of BSA-mycotoxin conjugate solution were mixed with 6 µL of amine reactive dye solution (dissolved in DMSO at 10 mg/mL) and continuously agitated in the dark for 60 min on a benchtop orbital shaker at 600 rpm. The free reactive dye was subsequently removed by a series of 7 diafiltration steps using the same Amicon filter units and protocol described above, until the filtrate (initially containing the free dye) was visibly clear. Finally, the purified mycotoxin-BSA-Alexa 430 conjugates were diluted to 1 mg/mL and stored at -20 °C. BSA, PBS tabs, sodium bicarbonate, anhydrous dimethyl sulfoxide (DMSO), carbonyldiimidazole (CDI), ochratoxin A (OTA), deoxynivalenol (DON) and aflatoxin B1 (AFB1), OTA-BSA

conjugates and AFB1-BSA conjugates were purchased from Sigma-Aldrich. OTA, DON and AFB1 solutions were prepared in methanol (33% methanol (v/v) at 100 µg/mL), anhydrous methanol (1 mg/mL) and anhydrous ethanol (200 µg/mL), respectively and stored at 4 °C. The DON-BSA conjugates were prepared using CDI chemistry as described in detail elsewhere [1].

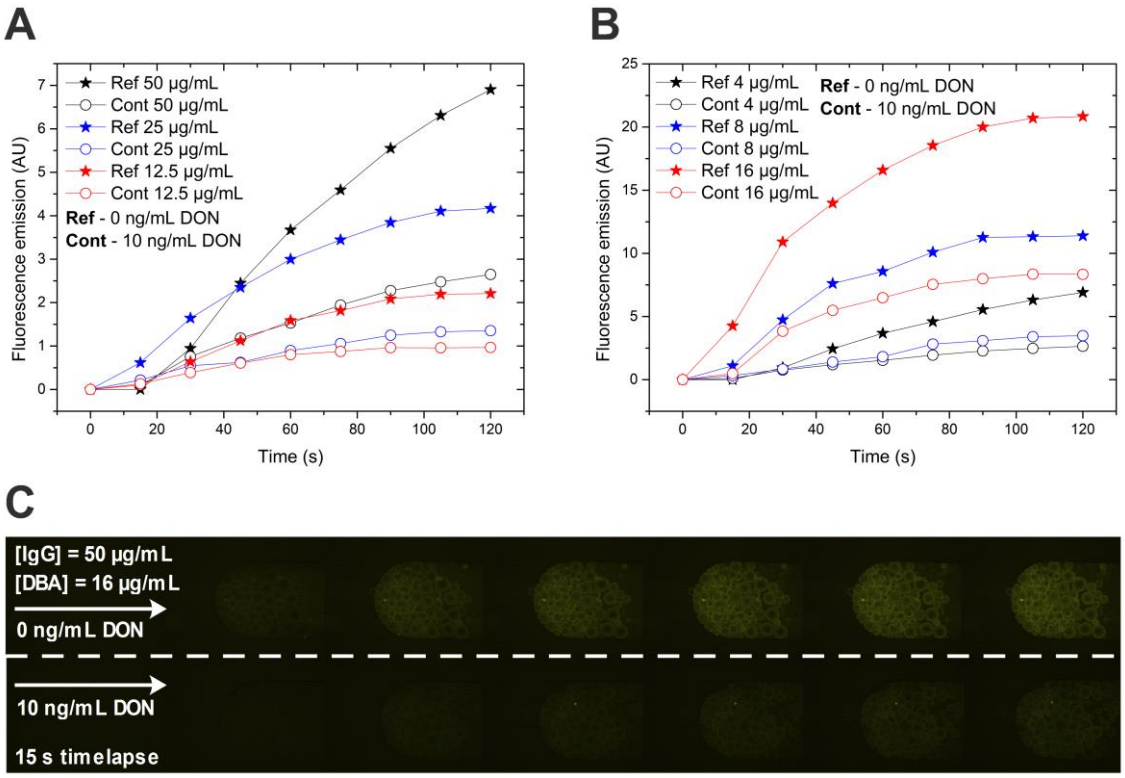

Figure S1 A- Optimization of anti-DON concentration to functionalize the agarose protein-G beads. **B-** Optimization of the concentration of DON-BSA-Alexa 430 spiked in the SUA solution. **C-** Time-lapse of the packed beads measured under the fluorescence microscope while flowing the SUA at 17.5 µL/min in the presence or absence of 10 ng/mL of DON. The raw microscopy photos shown were acquired every 15 s using an exposure time of 1 s.

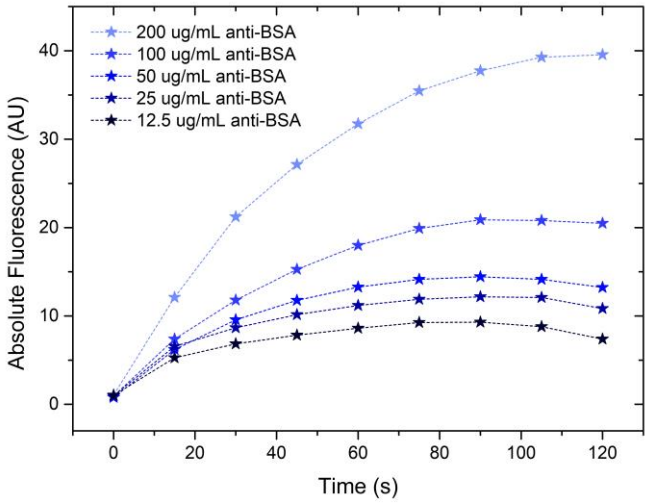

Figure S2 Optimization of anti-BSA concentration used to functionalize the agarose protein-G beads. The curves were measured while flowing a blank SUA (0 ng/mL mycotoxins) containing 8 µg/mL AFB1-BSA-Alexa 430, 8 µg/mL DON-BSA-Alexa 430 and 10 µg/mL OTA-BSA Alexa 430.

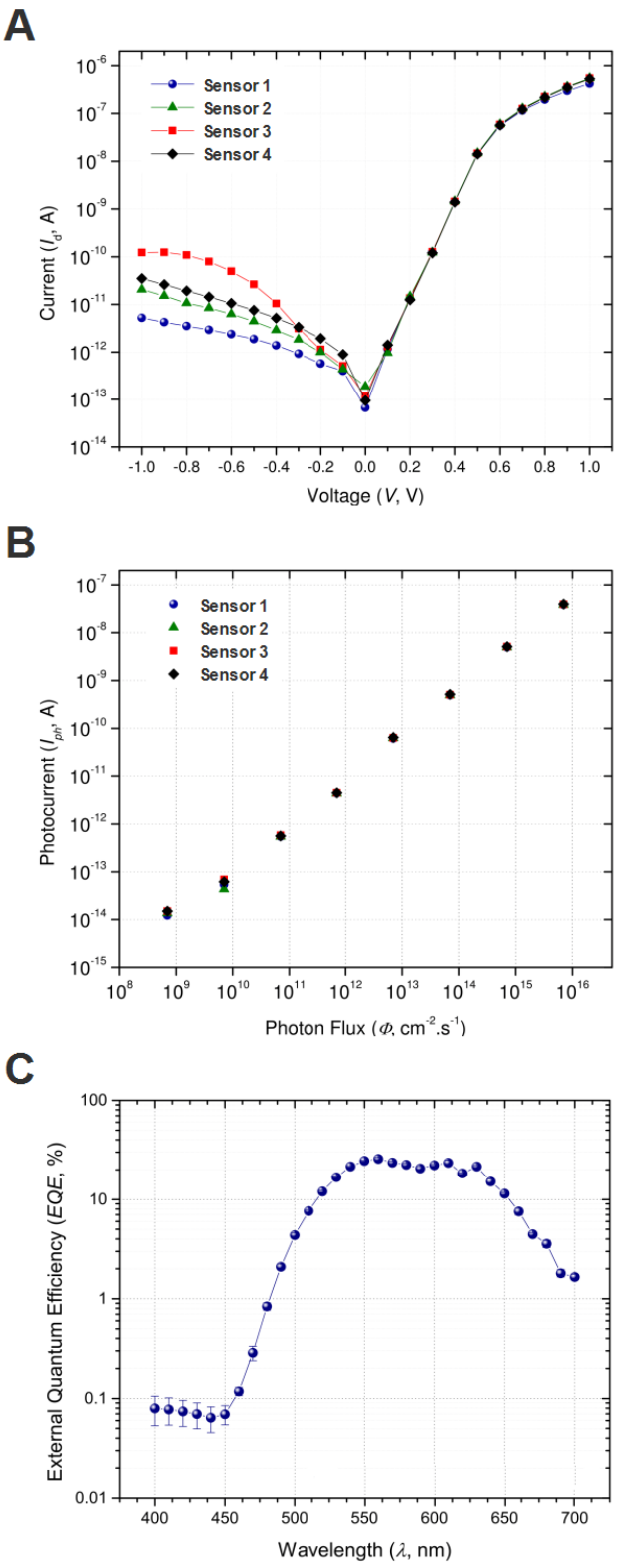

Figure S3 Photosensor characterization plots for each sensor in the array. The photosensors 1 to 4 correspond to those used to measure the anti-AFB1, anti-OTA, anti-DON and anti-BSA chambers, respectively. A- *I-V* curves measured in dark conditions; B- Photoresponse (*I-Φ*) at 540 nm and; C- Average external quantum efficiency measured for the 4 sensors (±SD).

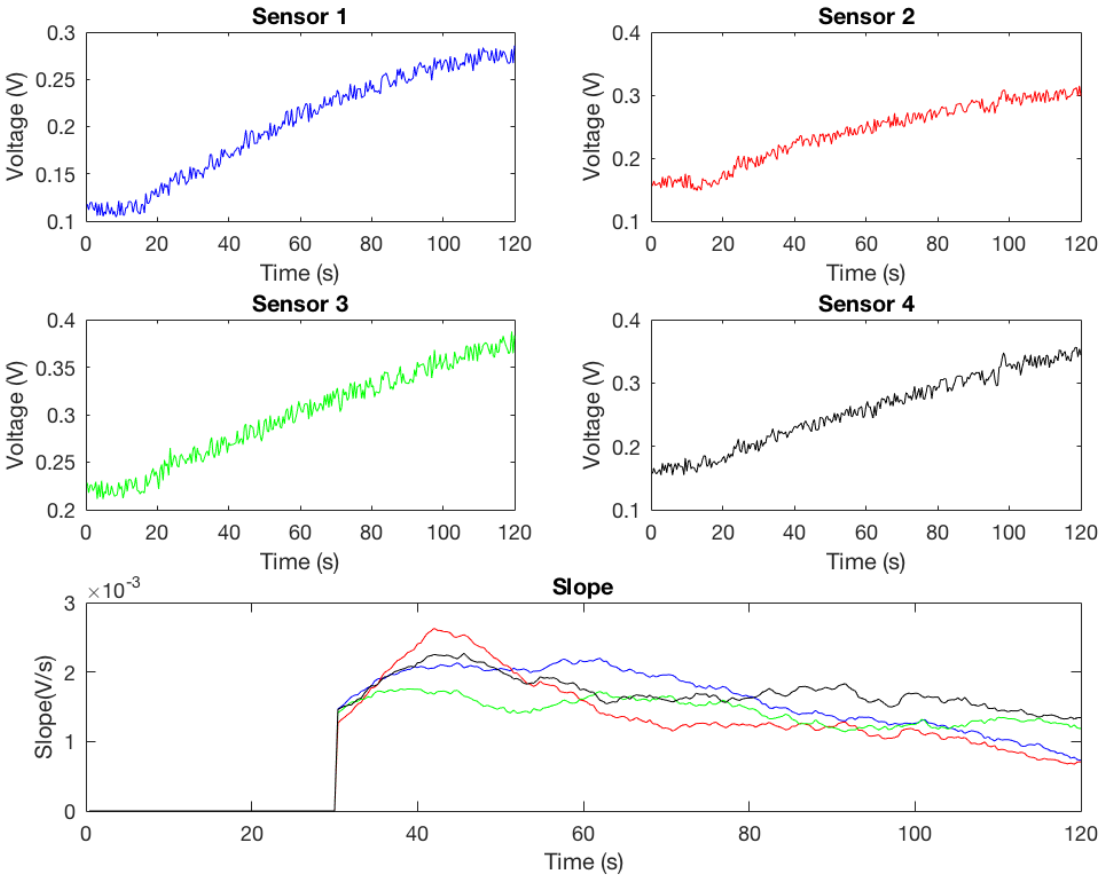

Figure S4 Example of the raw voltage measurements acquired in real-time (2.5 points per second) for each photosensor and respective 30 s linear slopes, plotted after the first 30 s. The figure corresponds to a screenshot of the data as acquired and processed using the MATLAB program. The results in the figure were acquired by flowing a non-contaminated sample (syringe pump turned-on at t = 10 s) in a device with anti-BSA antibodies immobilized in all chambers (same assay as plotted in Figure 5-A).

Section S3. Extraction of mycotoxins from artificially spiked corn samples

Corn samples were processed, spiked with mycotoxins and extracted as previously described in detail [2]. Briefly, corn samples were first manually ground to a fine powder using a mortar and pestle and 400 ± 1 mg were transferred to individual microtubes. Then, 1 µL of absolute ethanol containing each mycotoxin to the intended final concentration (in mass of mycotoxin per mass of corn) were added to the feed. Each microtube was vigorously agitated in a vortex mixer and subsequently left open for 5 min to allow the ethanol to evaporate. The mycotoxins were subsequently extracted by performing an aqueous two-phase extraction with a solution composed of 1,200 µL sodium citrate (15% w/w) and 75 µL of PEG 8,000 (50% w/w). After adding the extraction solution to the

microtubes, these were vigorously agitated for 3 min at 2,400 rpm in a vortex mixer and subsequently centrifuged for 15 min at 2,000 g in a compact Labnet (Edison, NJ, USA) Spectrafuge™ mini-centrifuge. To perform the immunoassay, 4 µL of the top PEG-rich phase were collected and mixed with 36 µL of mycotoxin-BSA-Alexa 430 conjugate solution in PBS to the final concentrations described in section 2.5. The corn samples were kindly supplied by Cargill Aqua Nutrition within the scope of the European project DEMOTOX (FP7-SME-2013- 604752).

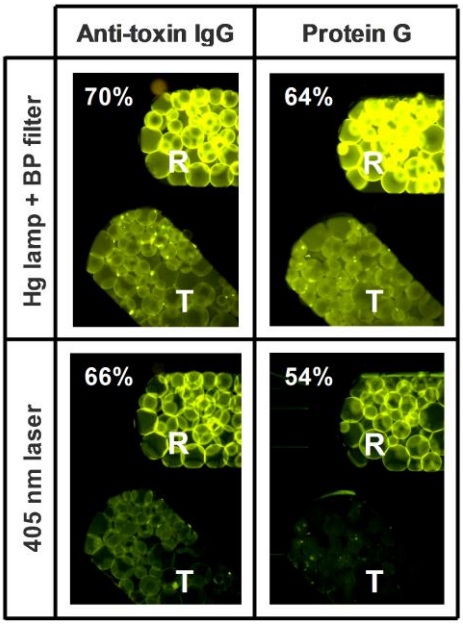

Figure S5 Fluorescence microscopy photos of the reference (R) and test (T) chambers of the multiplexing device, after flowing a non-contaminated corn extract spiked with the optimized mycotoxin-BSA-Alexa 430 mixture. The device was imaged having either anti-DON immobilized on the test chamber (left) or plain protein G beads (right). The light excitation source used to measure the fluorescence emission was either the 100 W Hg vapor lamp of the Leica DMLM microscope coupled with a blue excitation filter (450-490 nm band pass, model I3) or the 405 nm laser. The percentage values were calculated as the fluorescence signal of the T chamber relative to the R chamber in each case. All images were contrast enhanced for visualization purposes.

References

1. R. R. G. Soares, D. R. Santos, V. Chu, A. M. Azevedo, M. R. Aires-Barros and J. P. Conde, *Biosens. Bioelectron.*, 2017, **87**, 823-831.
2. R. R. G. Soares, A. M. Azevedo, P. Fernandes, V. Chu, J. P. Conde and M. R. Aires-Barros, *J. Chromatogr. A*, 2017, **1511**, 15-24.