List of Supplementary Figures



Fig. S 1 Micrographs (fluorescence on the top, bright field on the bottom) corresponding to ~5.6 nL spots of 67 nM anti-goat IgG– FITC aqueous solutions spotted on a glass surface using different glycerol concentrations in the spotted solution. The use of glycerol slows the droplet evaporation leading to improved spot homogeneity.



Fig. S 2 Study of the surface density of immobilized antibodies as a function of their concentration in the spotting solution. Each spot corresponds to \sim 8.4 nL of anti-goat IgG–FITC solution with the indicated concentrations. The number of probes adsorbed is quantified by measuring the fluorescence after washing with PBS using as calibration the fluorescence of the spots prior to washing. For clarity, the microchannel walls are highlighted in white.



Fig. S 3 Multiplexed immunodetection using spotted antibody probes and fluorescence detection. Two antibody probes are spotted inside the same microchannel (Mouse and Goat IgG) and sequential immunodetection is performed and detected using fluorescence microscopy: Flow 1) use of anti-mouse IgG–FITC as target; Flow 2) use of anti-goat IgG – FITC as target. (Inset) Specific vs. non-specific immunodetection (performed in different microchannels): fluorescence of the labeled target as a function of the concentration of the mouse IgG solution spotted, using a constant concentration of 67 nM of the target antibody solution. The line is a fit to the experimental results using the Langmuir model.



Fig. S 4 Testing the HRP activity, via chemiluminescence, of anti-mouse IgG–HRP antibodies immobilized on PDMS microchannels. Intensity of the luminescence as a function of the average velocity of the luminol solution in the microchannel - the intensity of the signal saturates for $v_{in} \ge 1.38$ mm/s ($v_{max} = 2$ mm/s). Chemiluminescence resulting from enzyme activity; (inset, left) a series of sequential steps of turning on and off luminol injection; (inset, right) a test of the stability of the enzyme activity including both a decrease and an interruption of luminol injection. The line is a guide to the eye.



Fig. S 5 Multiplexed chemiluminescence-detection of antibody-antigen molecular recognition. Two separate probe antibody spots of \sim 14 nL of mouse IgG and rabbit IgG were generated in a PDMS microchannel. Anti-mouse IgG-HRP was then used as target. The line is a fit to the experimental results using the Langmuir model. Microchannel walls are highlighted in white in the fluorescence micrograph that shows the chemiluminescence signal of the specific molecular recognition event.



Fig. S 6 Normalized immunoassay signal as a function of the concentration of the probe mouse IgG in the spotted solution. The results obtained during the immunoassay optimization using fluorescence microscopy and the results obtained by chemiluminescence and colorimetry using the integrated detection by a-Si:H photodiodes are consistent. The line is a fit to the experimental results using the Langmuir model.



Fig. S 7 Chemiluminescence (current density, right) and colorimetric (initial velocity, left) signal captured by the integrated a-Si:H photodiode after molecular recognition plotted as a function of the number of the immobilized probe antibodies in the spot. The response signal due to the enzymatic reaction is linear with respect to the number of the antibodies for both detection strategies.



Fig. S 8 System modeling. Calculated target antibody concentration measurable for a constant surface density of antibody probes (θ_{max}) and for a given surface area (A). IgG and anti-IgG were used as model antibody-antigen pair with the adsorption and desorption constants (from fig. 5). The red line indicates the minimum level of probe antibody density on the microchannel surface currently measurable (from fig. 13). The black line corresponds to the modeling values of the current setup. Green and blue dashed lines were obtained by multiplying the value of the surface area, used for the black line, by 2 and 10 respectively.