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

Supplementary information for:

Single chip SPR and fluorescent ELISA assay of prostate specific antigen

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Materials

Glass (BK7) wafer of 4" in diameter were purchased from University Wafer. The dove prism were manufactured by Vy Optics (China). Resin LOR1A and remover PG were purchased from MicroChem Corp. (Westborough, MA). Resin OIR 674-11 was purchased from *Fujifilm* Electronic Materials Inc. (North Kingstown, RI). AZ726 MIF developer was purchased from MicroChemicals (Ulm, Germany). Microscope cover slips of 22 x 22 mm, dimethylformamide (DMF), sulfuric acid and 30 % hydrogen peroxide were purchased from Fisher Scientific (Pittsburgh, PA). 16-mercaptohexadecanoic acid (16-MHA), N-hydroxysuccinimide (NHS), ethyl-(dimethylaminopropyl)carbodiimide (EDC), ethanolamine hydrochloride, Ampliflu^{TM} Red and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Phosphate buffer saline (PBS) was purchased from Corning cellgro (Manassas, VA). AffiniPure goat anti-human IgG (H+L) and human gamma globulin were purchased from Jackson Immuno Research Labs (West Grove, PA). Human prostate specific antigen (PSA), unlabeled PSA antibodies (catalog # 10-P20A and 10-P21A) and PSA antibody modified with HRP were purchased from Fitzgerald Industries International (Acton, MA). Ethanol was purchased from Commercial alcohols (Brampton, ON).

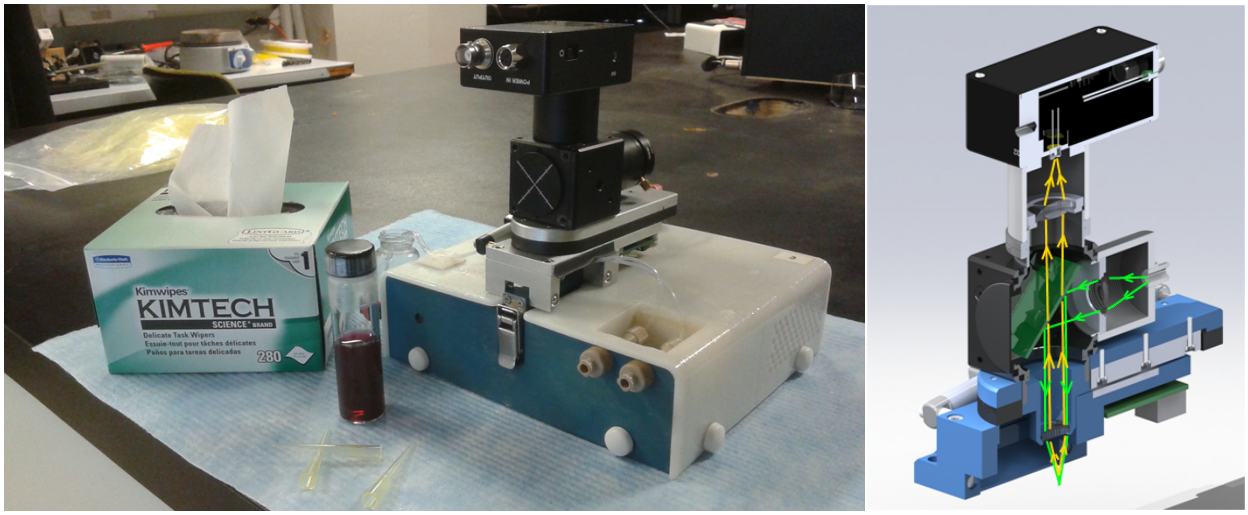

Figure SI1. SPR instrument with a fluorescence unit. Left) A photograph of the actual unit in the presence of common laboratory items to highlight the small size of the device. The black fluorescence unit was composed of the filter cube (See the white "x") and the avalanche photodiode at the top of the fluorescence unit. The linear actuator was mounted on the gray aluminum railing and the fluidic cell of the SPR system was underneath the fluorescence unit. Right) Ray tracing of the excitation laser in green and of the emitted light in yellow. Lenses focused the light on the SPR surface and on the avalanche photodiode. The filter cube was composed of a dichroic beam splitter and a long-pass fluorescence filter.

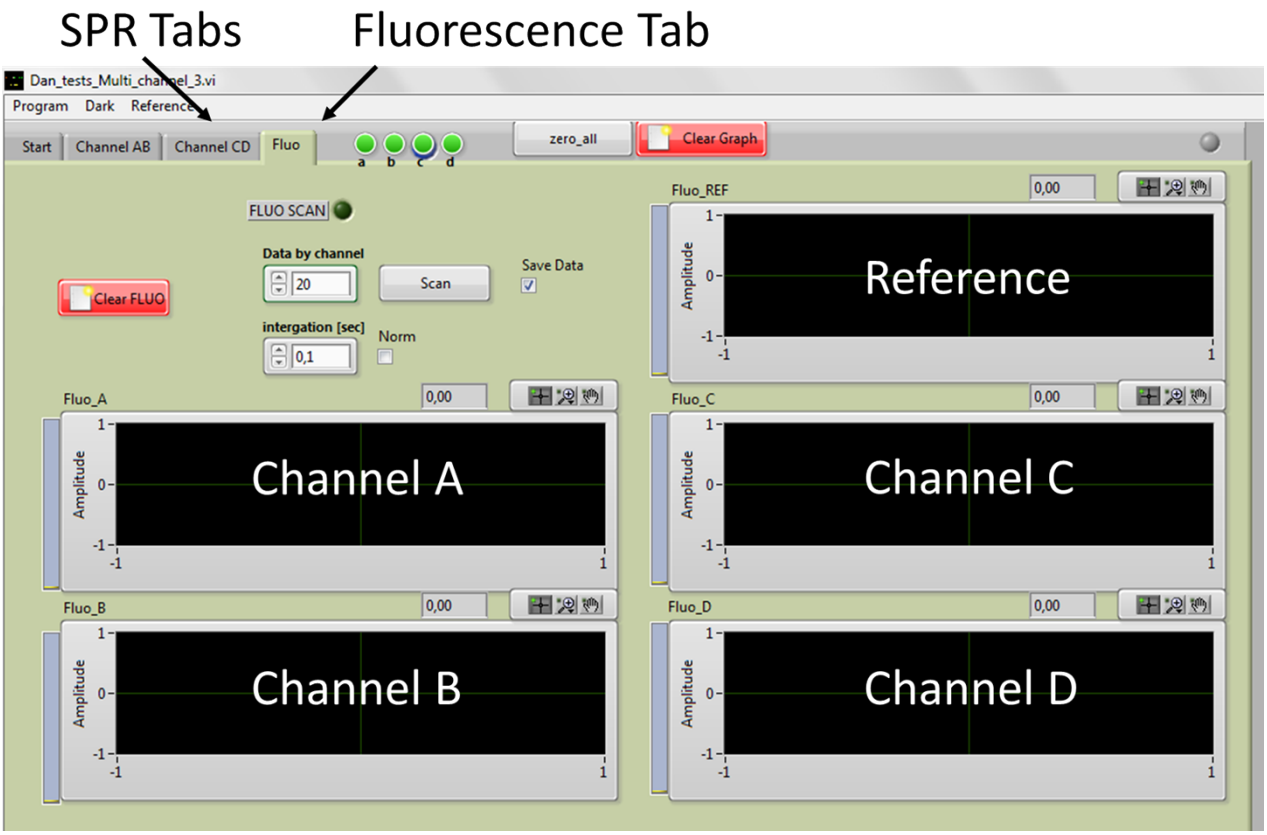

Figure SI2. Screenshot of the fluorescence tab of the data acquisition software.

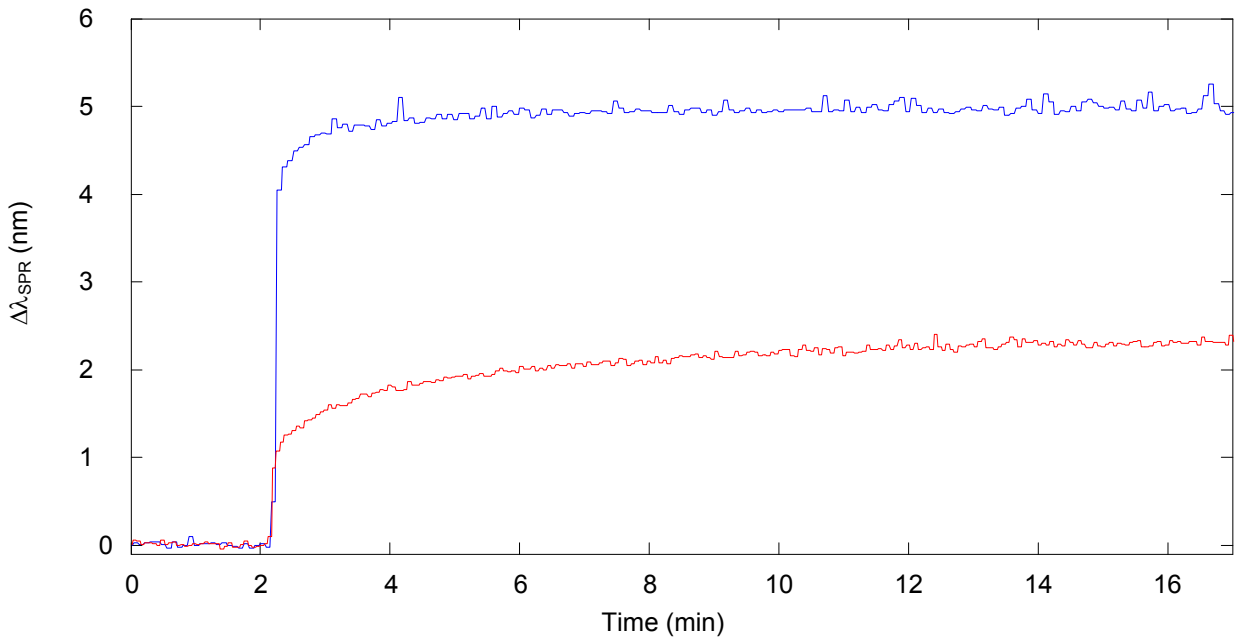

Figure SI3. SPR sensorgrams for the immobilization of 500 nM anti-PSA (capture antibody) on a surface of 3-MPA-HHHDD-OH for a continuous gold film (red) and a microhole array (blue). The SPR response was stabilized in PBS for 2 minutes before incubating anti-PSA with the activated SPR sensor.

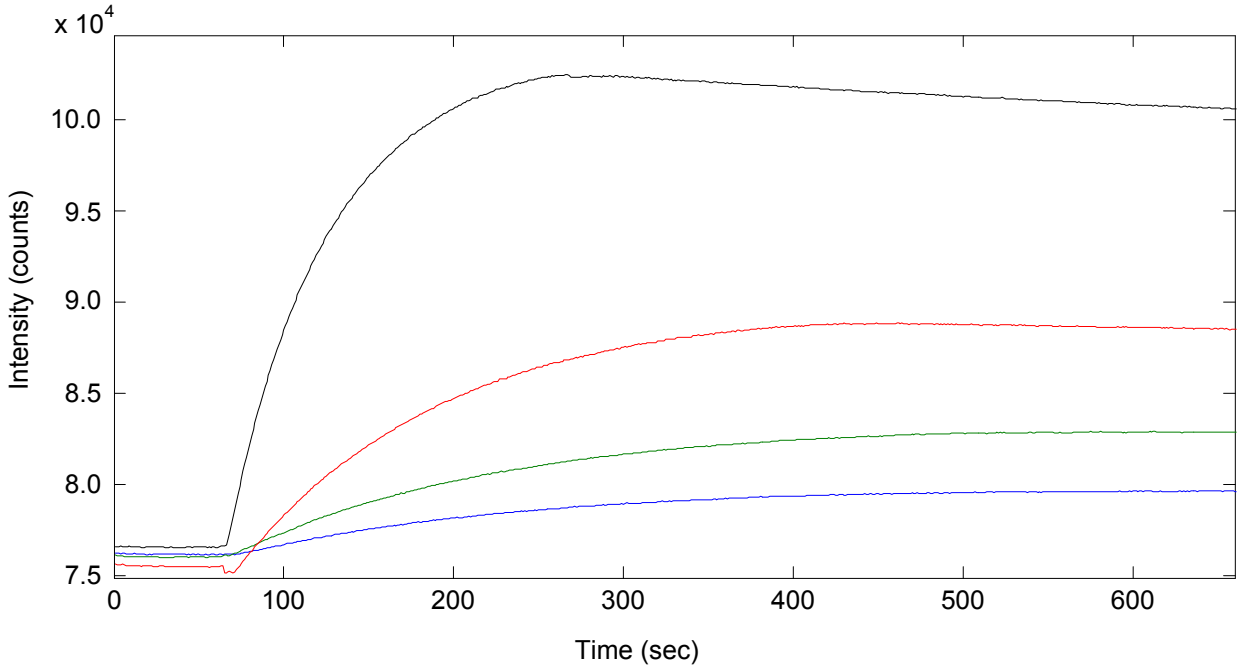

Figure SI4. Real time measurements of fluorescence emission with the SPR-fluorescence instrument for Ampliflu™ Red peroxidase substrate at 10 (blue line), 20 (green line), 50 (red line) and 100 µM (black line) on the SPR sensor. A concentration of 5 nM PSA was previously incubated with anti-PSA-HRP and the complex formed was captured with anti-PSA bound to the microhole array SPR sensor. The kinetic curve consists of a blank measurement in PBS from 0 to 60 seconds followed by the incubation with Ampliflu™ Red solution injected at 60 s. The decrease in fluorescence at nearly 260 s for 100 µM (black line) was due to self-quenching of the fluorophore.