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

An integrated microspectrometer for localised multiplexing measurements

Zhixiong Hu[a,b], Andrew Glidle[b], Charles Ironside[b], Jon Cooper[b], Huabing Yin[b*]

[a.] Division of Medical and Biological Measurements, National Institute of Metrology, Beijing, P.R.China.

[b.] Division of Biomedical Engineering, School of Engineering, University of Glasgow, Glasgow, UK.

*Corresponding authors: huzhixiong@nim.ac.cn and Huabing.Yin@glasgow.ac.uk

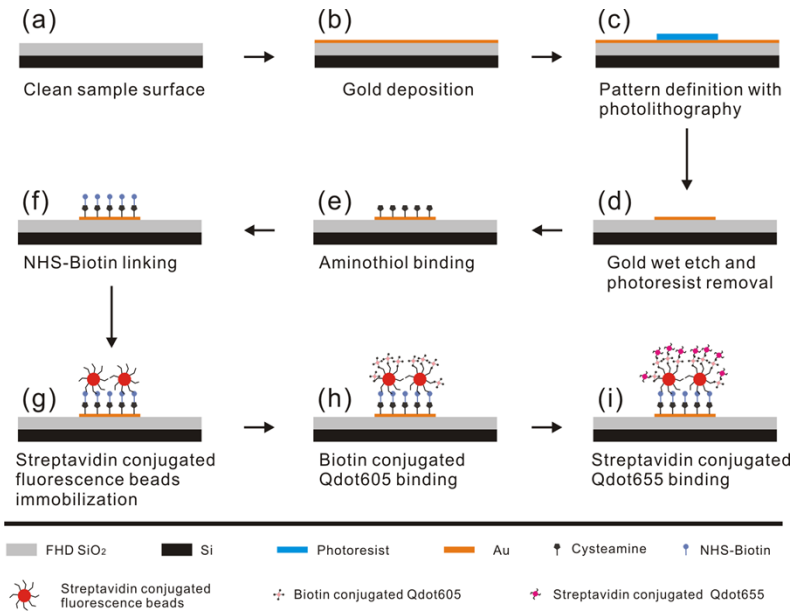
Supplementary Information:

Protocol for immobilization procedures

The process flow diagram is presented in Scheme 1. Following fabrication of the AWG chip, a metal deposition and a photolithography step were used to define a gold pattern in the centre of the sampling cuvette (Scheme 1a-c). After an Au wet etch process, a gold disk with diameter of 40μm was obtained (Scheme 1d). A thiol functionalization procedure was utilized to selectively bind recognition agents to the gold pattern for subsequent spectral multiplexing assay (Scheme 1e).

Before surface functionalisation of the gold pattern, the AWG chip was cleaned with organic solvent and piranha solution. 5 mM Cysteamine hydrochloride in DI water was used to create a NH_2-terminated self-assembled monolayer (SAM) on the gold disk overnight (Scheme 1e). Then the sample was thoroughly rinsed with PBS buffer to remove the excess reactive chemicals on the surface. NHS-ester activation was done by adding 2-3 mg Biotin (long arm) NHS to 100 μL EDC/NHS solution (a mixture of 5mM 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and 6mM N-hydroxysuccinimide (NHS) in 30 mM MES buffer at pH 6) and reacting for 15 minutes at room temperature. Then the buffer pH was increased to about 7.7 by adding 100 μL PBS buffer (100 mM, pH=8.5). Immediately, the solution was added onto surface of the aminothiol modified gold disk and allowed reaction to proceed for 2 hours at room temperature (Scheme 1f).

It is worth noting that streptavidin coated fluorescent beads need to be washed 2 or 3 times with a 10× volume of wash buffer through a centrifugation-resuspension process. 10 μL bead solution was used each time and a concentration of 0.1% solids was achieved by suspending the final pellet in wash buffer. Then a drop of the bead solution was placed onto the gold disk, allowing the streptavidin-biotin affinity interactions to occur over 30 minutes before washing (Scheme 1g). After immobilisation of streptavidin coated fluorescent beads, 2 μL solution of the biotin conjugated quantum dots (605 nm) was dispensed onto the gold disk followed by washing with buffer after 30 minutes (Scheme 1h). Likewise, the streptavidin conjugated quantum dots (655 nm) was added in order to perform a multiplexed Flash Red (682 nm), Qdot605 and Qdot655 spectral measurement (Scheme 1i).



Scheme 1. Process flow diagram of micro beads-based fluorescence assay. (a) The surface of flame hydrolysis deposited (FHD) silica based AWG device is cleaned with organic solvent and piranha solution. (b) Deposition of gold layer. (c) Pattern definition with photolithography. (d) Gold wet etch and photoresist removal. (e) Aminothiol binding on the gold surface. (f) NHS-Biotin linking. (g) Adsorption of streptavidin conjugated micro beads. (h) Immobilisation of biotin conjugated quantum dots (605 nm). (i) Immobilisation of streptavidin conjugated quantum dots (655 nm).

To reduce non-specific binding of beads on the device, the absorption time was optimized. For this purpose, two other samples with gold pattern and identical surface chemistry were tested using different times for the incubation. Biotin functionalization was restricted to the cross-shape gold pattern only. As such, the attachment of beads to the silica area between the gold pattern was largely due to non-specific adsorption. Figure s1 shows the comparative result with different lengths of incubation time for the beads adsorption. Non-specifically bound beads were obviously seen after the 60-minute incubation (Figure S1a) while 30 minutes offered a more satisfactory result (Figure S1b). Hence, a 30-minute incubation time was used for the real AWG sample to allow the streptavidin-biotin affinity interactions and prevent excessive non-specific binding.

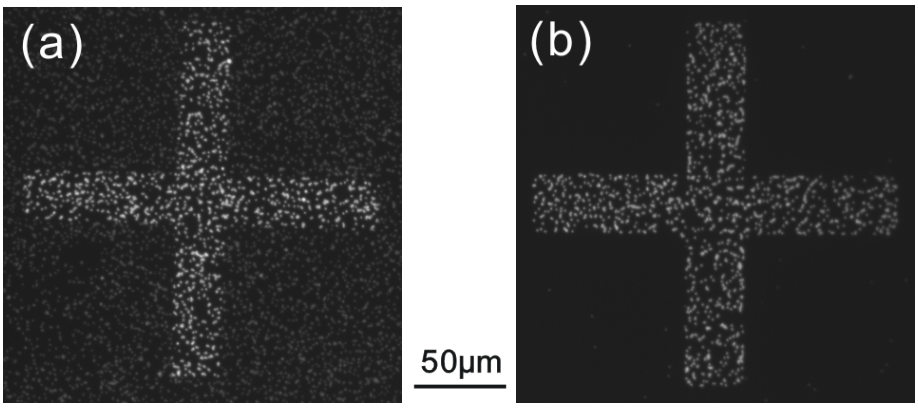


Figure S1. Fluorescence images of gold patterned samples after fluorescent beads adsorption for (a) 60 minutes and (b) 30 minutes. The brightness of the images is proportional to the amount of the attached beads. Beads attached to the area between the cross-shape gold patterns were due to non-specific adsorption.

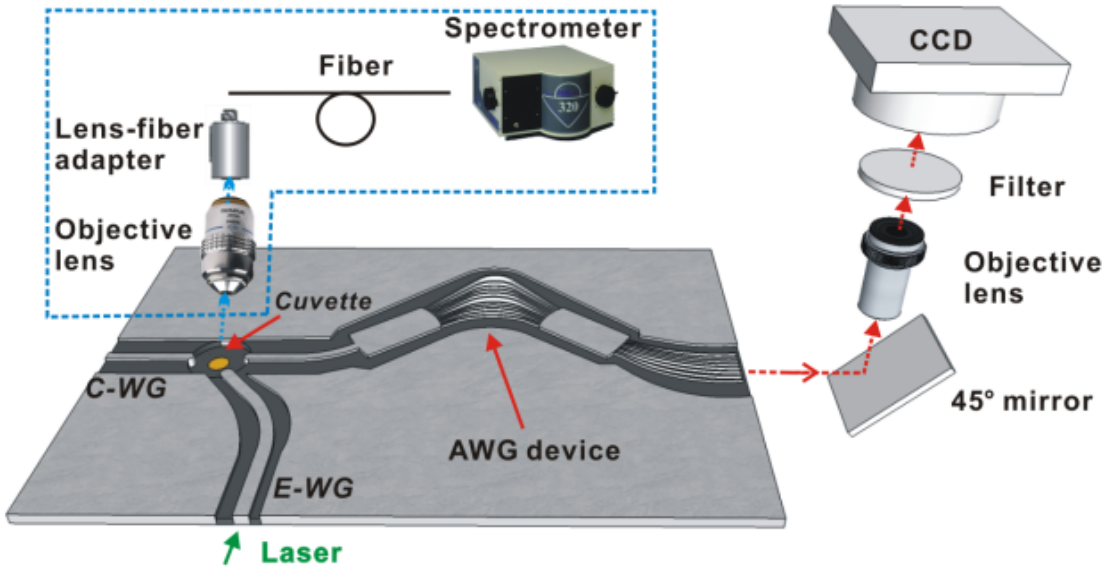


Figure S2. Schematic set-up of microbeads based multiplexing fluorescence assay. A laser light beam was fibre coupled into the planar excitation waveguide (E-WG). A 45° mirror and a ×4 objective lens were used to reflect AWG output signals onto a vertically mounted CCD camera or a photon detector. Prior to measurement with the CCD camera, fluorescence signal from the sampling cuvette was collected by an above ×50 objective lens and then fibre connected to a conventional spectrometer (inside the blue dashed area).