A microflow cytometer for microsphere-based immunoassays using integrated optics and inertial particle focussing

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We present work towards a microflow cytometer for performing multiplex immunoassays using commercially available fluorescently-labelled microspheres. The device consists of a silica chip with integrated GeO₂:SiO₂ channel waveguides which deliver excitation light orthogonally to an etched flow channel [1], [2]. The rectangular cross section, 2:1 aspect ratio flow channel and flow rate create an inertial focussing effect on the microspheres [3] which ensures they flow through the plane of maximum optical excitation, halfway up the height of the channel, with minimal positional variation.

The optical waveguide core is fabricated by magnetron sputtering of GeO₂:SiO₂ films which are then etched to form channel waveguides by ICP etching. The silica cladding, up to 13.5 µm thick, is deposited by either flame hydrolysis deposition or a combination of magnetron sputtering followed by PECVD. Fluidic channels are etched with ICP etching. Channels with the dimensions of 14.1 µm x 27.5 µm and near vertical sidewalls (91°±4°) have been produced in silica as shown in the cross section in Figure 1A. Figure 1B shows a device with the fluidic channel etched through waveguides clad with PECVD silica.

![Figure 1](image_url)

Design parameters were established with PDMS test channels 25.5 µm deep by 12.2 µm wide. Figure 2A and Figure 2B show transmission fluorescence imaging of streaks from multiple 5.6µm diameter microspheres flowing at 0.49 ms⁻¹ down the fluidic channel. The microspheres are shown to be focused into a tight stream at 15 mm from the channel entrance in Figure 2C, indicating the minimum channel length required for the final devices.
Future work will include dual channel quantification of microsphere fluorescence and development of an assay for TNFα and later multiplex measurements. Collection of fluorescence with channel waveguides and also characterisation of transmission measurements from flowing microspheres will also be studied.

