The goal of this research is to develop a microfluidic flow cytometer for deployment for environmental monitoring and point-of-care diagnostics. The key components of this device include the following:

1) microfluidic channels that use a novel, groove-based design to direct the sheath stream completely around the core (sample) stream and also to separate the sheath from the core for recapTURE and reuse.

2) Integrated waveguides for the detection of fluorescence emission at three different wavelengths, as well as 90º light scatter.

We have fabricated the microflow cytometer and demonstrated multi-analyte detection.

Advantages of groove-based sheath flow

- The sheath stream is directed completely around the core stream
- The core is prevented from touching the channel walls in the interrogation region
- The core is focused entirely within the interrogation beam.
- The potential risk of clogging is minimized

Chevrons and sheath flow generation.

Core fluid is surrounded by sheath fluid by first sandwiching the core stream between the two sheath streams via fluid focusing. The chevrons perform the final sheathing function by moving sheath fluid to the top and bottom of the channels.

The size of the core is proportional to the number of chevrons and the relative flow rates of the core and sheath.

Conical microscopy images of the sheathing process of the microflow cytometer. The core, with fluorescent dye, is initially sandwiched and focused between two sheath streams (0 to 3.5 mm). Upon entry into the chevron structures (3.5 to 5.0 mm), the core height is dramatically reduced as the sheath fluid fully surrounds the core. Finally, a tight core stream enters the interrogation region (6.0 mm). The core to sheath velocity is 10 to 400 µL/min.

Alignment of fibers in the interrogation region.

To observe the alignment of the optical fibers with the center of the flow channel, each fiber was connected to a laser while the channel was filled with CY5 dye solution and photographed. Three images were combined to show the overlap of the output of the single mode 635 nm excitation fiber (bottom), the phycoerythrin excitation fiber (bottom right) and the acceptance angle of the microsphere identification fiber (top right).

Microfluidic Flow Cytometer

Confocal microscopy images showing fluorescence and light scatter at 670 ± 10 nm and ≥ 700 nm, normalized to light scatter intensities at 635 ± 5 nm, to identify Luminex coded microspheres containing variable amounts of two fluorescent dyes. The emission is a result of excitation by 635 nm wavelength laser.

Dose-response curves from a 6-plex assay for 3 bacteria and 3 toxins. Data from the microflow cytometer is compared to analyses using a commercial flow cytometer. Limits of sensitivity are comparable.

For biohazard detection:

- Portable flow cytometer
- Automated system for continuous monitoring of air and drinking water
- Testing of food, clinical, and environmental samples for pathogens and toxins
- Identification of marine algae in situ.

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