PocketDX—Compact Flow Cytometer for Industrial Water Quality Monitoring

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Monitoring the quality of water is a constant concern for public health and particularly for the water treatment industry. By law water quality has to be guaranteed until the entry of buildings. Therefore, there is a growing demand for rapid and reliable instruments for in-line monitoring the microbiological quality of drinking water at low costs per data point. We demonstrate here an automated and compact sample preparation module with zero dead volume. A ten-fold reduction of the dye consumption for lower manufacturing costs could be achieved thanks to a novel microfluidic rotary valve concept.

Fluorescent staining coupled with flow cytometry (FCM) is used for quantification and characterization of bacteria in both natural and engineered environmental water sample [1]. This method allows precise and rapid detection of the total microbial cell count of a water sample. Water samples are incubated and stained with an asymmetrical cyanine dye (SYBR® Green I) that binds to nucleic acids and results in the formation of a DNA-dye complex that emits green and red light when excited by a 488-nm blue laser [2]. The ratio of the green to red fluorescence levels is used to differentiate bacteria with high and low nucleic acid content (HNA and LNA).

Most commercially available flow cytometers are full-sized laboratory bench-top instruments, which are too sensitive and bulky to be operated in water distribution facilities. In addition, sample preparation is usually conducted manually by a trained laboratory expert using costly proprietary dye solution. Consequently, there is a great potential in developing in-line flow cytometers that adopt miniaturization and automation of the fluidic sample preparation.

A novel sample preparation module combining fluid distribution and mixing in one microfluidic device has been developped for automated operation with lower reagent consumption and waste generation per measurement, reduced manufacturing costs, lower power consumption and lower weight. This, in turn enables the rapid, precise and fully automated analysis of bacteria quantity in water at relatively low costs. Further details can be found in another report [3].

The sample preparation was validated using a commercial optical readout module which was calibrated with calibration beads before running the experiment. Using the automated sample preparation module, 90 µL of water sample containing known concentrations of e-coli bacteria were stained with a fluorescent stain (SYBR Green I). Samples were pre-warmed to 37°C (± 2°C) followed by staining for 13 minutes with 10 µL of SYBR® Green I (sigma-Aldrich, Switzerland) at a concentration of 1x (which is a 10,000x final dilution of DMSO stock solution). The prepared solution was then directed to the capillary inside the optical read-out module at a constant flow rate of 20 µL/min. SYBR® Green I solution fluorescence was visualized using a two-dimensional FL1 (emission filter 533/30) vs FL2 (emission filter 715 LP) log-scale density plot.

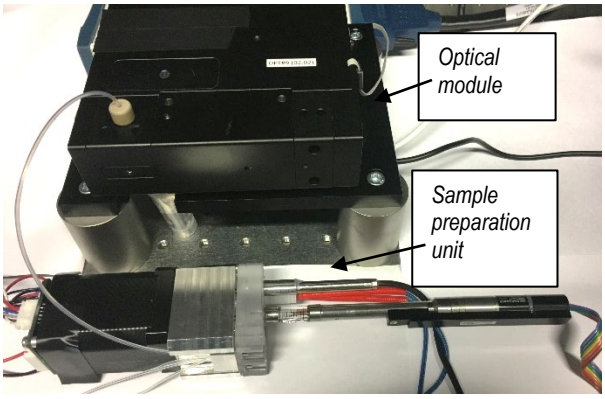

Figure 1: Configuration of miniaturized flow cytometer consisting of a fluidic sample preparation unit, a multi-wavelength optical readout module (scattering and fluorescence) and real-time data processing algorithm.

The results of the measurement with an e-coli concentrations of 10^5 and 10^6 CFU/ml are shown in Figure 2. The results confirm the correct functioning of the sample preparation module. Further quantitative investigation are on-going.

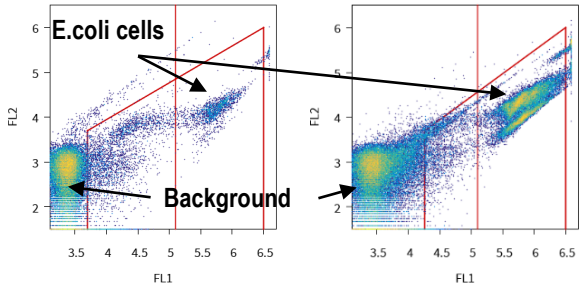

Figure 2: Flow cytometric analysis of a water sample contaminated with E. coli cells (10^5 and10^6 CFU/ml).

In order to further reduce the cost of this FCM platform, a novel optical readout module is under development. Silicon photomultipliers will be used instead of the traditionally expensive photomultiplier tubes. Furthermore the fluorescent filters will be replaced by a diffractive optical system. Both factors will lead to an overall reduction of the manufacturing and assembly costs.

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[2] SLMB, 2012. Determining the Total Cell Count and Ratios of High and Low Nucleic Acid Content Cells in Freshwater Using Flow Cytometry. Analysis Method 333.1, the Swiss Food Book.(Schweizerisches Lebensmittelbuch). Federal Office of Public Health, Switzerland.

[3] Z. Halvorsen, N. Schmid, V. Revol, S. F. Graf, H. F. Knapp, "First microfluidic valve with integrated mixer for in-line sample preparation", CSEM Scientific and Technical Report (2018) 46.