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Dual Channel Detection of Ultra Low Concentration of Bacteria in Real Time and via Scanning Fcs

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The rapid quantification and identification of infectious disease agents is of primary importance for medical diagnosis, public health, food safety and environment monitoring. Here we describe an alternative, simple and rapid method to detect very low concentrations of bacteria in water. Our device consists of a small confocal microscope with a horizontal geometry with large pinhole and a holder for cylindrical cuvettes. Two motors provide a rotational and slower vertical inversion motion of the cuvette, so as to scan a total volume of 1ml/min. The device looks like a simplified flow cytometer without flow. Bacteria are stained by two nucleic acid dyes that fluoresce green and red and excited with two lasers. When a bacterium passes through the observation volume emits both red and green fluorescence. The light emitted from the sample is directed by a system of lens toward a dichroic beam splitter and then separated into two light paths for red and green fluorescence detection respectively. Data are analyzed with a correlation filter program based on particle passage pattern recognition. The passage of a particle trough the illumination volume is mimicked with a Gaussian pattern in both channels. The width of the Gaussian correlates with the time of passage of the particle. When the program finds a match with a Gaussian in both channels one particle is counted. The concentration of particles in the sample is deduced from the total number of coincident hits and the total volume scanned. This portable setup provides higher sensitivity (up to few bacteria per ml), rapid results, low cost and a wide use ranging from clinical applications to pollution monitors and water and air quality control.

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