Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPORTING INFORMATION

Rapid Detection and Enumeration of Total Bacteria in Drinking Water and Tea Beverages by a Laboratory-Built High-Sensitivity Flow Cytometer

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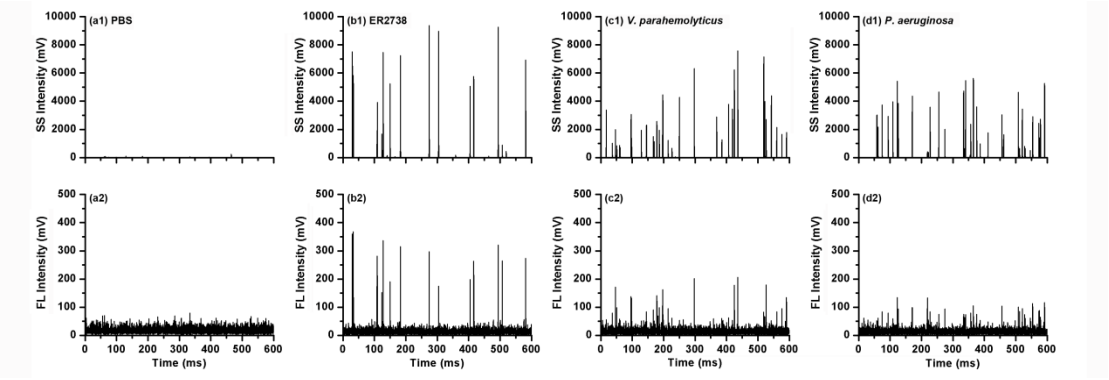

Figure S1. Representative side scatter (1) and green fluorescence (2) burst traces for PBS (a), and unstained *E. coli* ER2738 (b), *V. parahemolyticus* (c), and *P. aeruginosa* (d) detected on the HSFCM. The bacterial concentration was around 5×10^7 cells/mL. The PMT voltage used for the side scatter and the fluorescence detection was 270 V and 600 V, respectively.

Note: *V. parahaemolyticus* is a curved, rod-shaped, Gram-negative bacterium found in brackish saltwater, which, when ingested, causes gastrointestinal illness in humans. *P. aeruginosa* is a common bacterium that can cause disease in animals, including humans. It is found in soil, water, skin flora, and most man-made environments throughout the world. Both *V. parahaemolyticus* and *P. aeruginosa* are smaller than *E. coli* ER2738. The burst trace data shown in Figure S1 demonstrate that both the side scatter and autofluorescence signals of individual bacterial cells for these three bacterial species can be detected above the background on the HSFCM. Most importantly, due to the significantly reduced sheath flow rate and detection volume, only 5-8 impurity particles of very small sizes were detected in 1 second on the HSFCM. This provides a nearly background-free condition for the quantitative measurement and enumeration of bacterial samples.

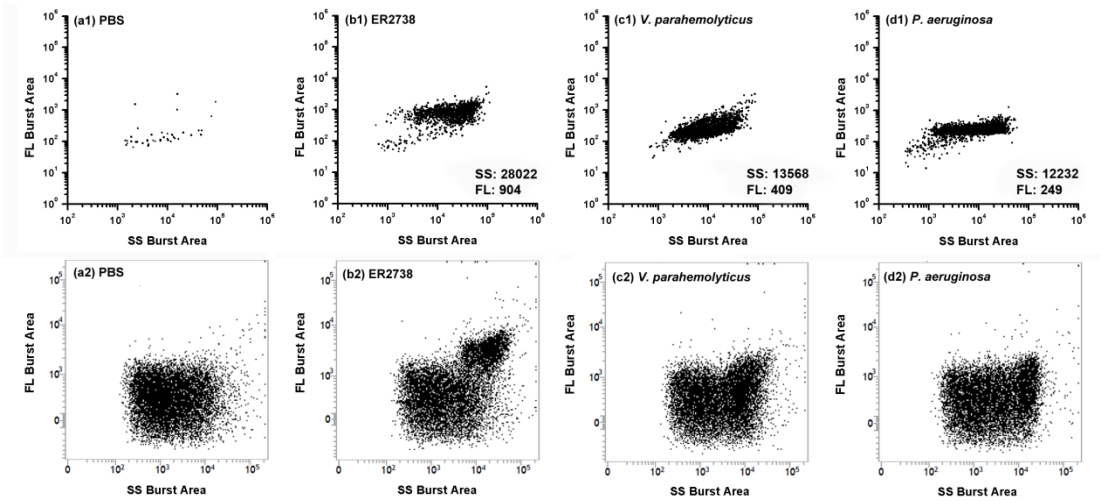

Figure S2. Sensitivity comparison between the HSFCM and the conventional flow cytometer for the detection of unstained bacteria. Bivariate dot plots of the fluorescence burst area *versus* the side scatter burst area for PBS and unstained *E. coli* ER2738, *V. parahemolyticus*, and *P. aeruginosa* detected on the HSFCM (a1−d1) and the BD FACSVerse (a2−d2). Median values of both the side scatter and the fluorescence detected on the HSFCM are provided in b1-d1 for each bacterial sample. Solid state 488-nm laser was used as the excitation source for both instruments. The bandpass filters used for fluorescence detection were 520/35 nm and 530/30 nm for the HSFCM and the BD FACSVerse, respectively. On the BD FACSVerse, the PMT voltages used for side scatter and FITC channel detection were 500 V and 750 V, respectively.

Note: Figure S2 (a2-d2) indicates that a large background signal was detected on the conventional flow cytometer BD FACSVerse, and unstained bacterial samples can hardly be discriminated against the background. This situation is particularly worse for bacteria of smaller sizes which in general also exhibit dimmer autofluorescence, such as *V. parahemolyticus* and *P. aeruginosa*. This background signal can mainly be attributed to impurity particles in the sheath fluids even after filtration using standard 0.22-μm filters. For the HSFCM, both the sheath-flow velocity (~20 mm/s for HSFCM *versus* ~20 m/s for the conventional FCM) and the probe volume (sub picoliter for HSFCM *versus* ~tens of picoliter for the conventional FCM) are much smaller, which result in a significant reduction in the detected event rate of impurity particles in the sheath fluid.

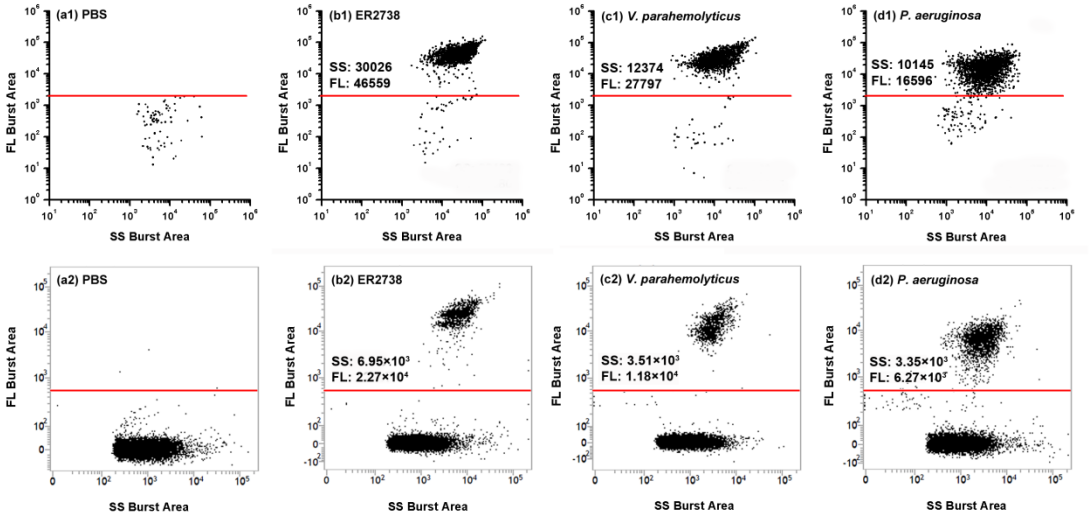

Figure S3. Sensitivity comparison between the HSFCM and the conventional flow cytometer for the detection of bacteria upon PicoGreen nucleic acid staining. Bivariate dot plots of the fluorescence burst area *versus* the side scatter burst area for PBS and unstained *E. coli* ER2738, *V. parahemolyticus*, and *P. aeruginosa* detected on the HSFCM (a1−d1) and the BD FACSVerse (a2−d2). On the BD FACSVerse, the PMT voltages used for side scatter and FITC channel detection were 400 V and 350 V, respectively. For the HSFCM, the PMT voltage used for the side scatter and the fluorescence detection was 270 V and 450 V, respectively. Note that PicoGreen staining via 10 min of heating treatment may alter slightly the bacterial structure and result in change in the detected side scattering signal before and after staining.

Note: When the bacterial samples were stained with PicoGreen, despite of the overlap of side scatter signals with impurity particles, all three bacterial species can be well discriminated from the background on the FITC channel by the BD FACSVerse. However, compared with megabasepairs of genomic DNA, most biological attributes of bacteria such as specific low abundance proteins cannot be brightly stained via fluorescence. In these cases, more distinct advantage of HSFCM in bacterial analysis versus conventional flow cytometry can be gained.