

Supplementary Information

On-chip stool liquefaction via acoustofluidics

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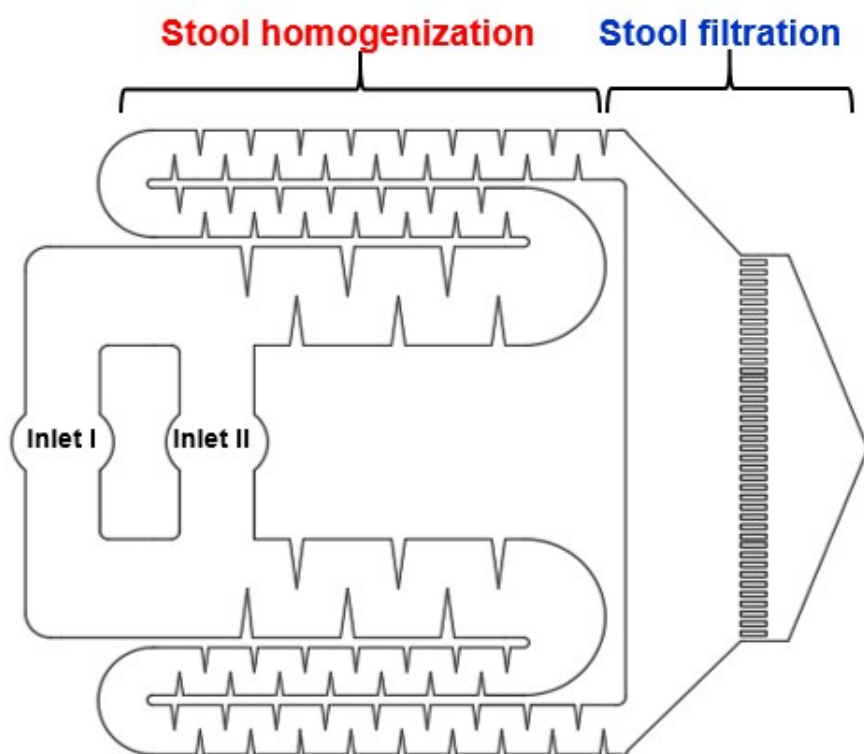


Fig. S1 Detailed design of the acoustofluidic-based stool liquefaction device. In order to prevent clogging due to the thick stool sample, the stool homogenization region was designed with two parallel channels where width of large channel and small channel sections were 2 and 1 cm, respectively. Additionally, a 2.5 cm diameter puncher (Kai, Japan) was used to punch the inlets and outlet. In the stool filtration region, the gap between the microstructures is 100 μm .

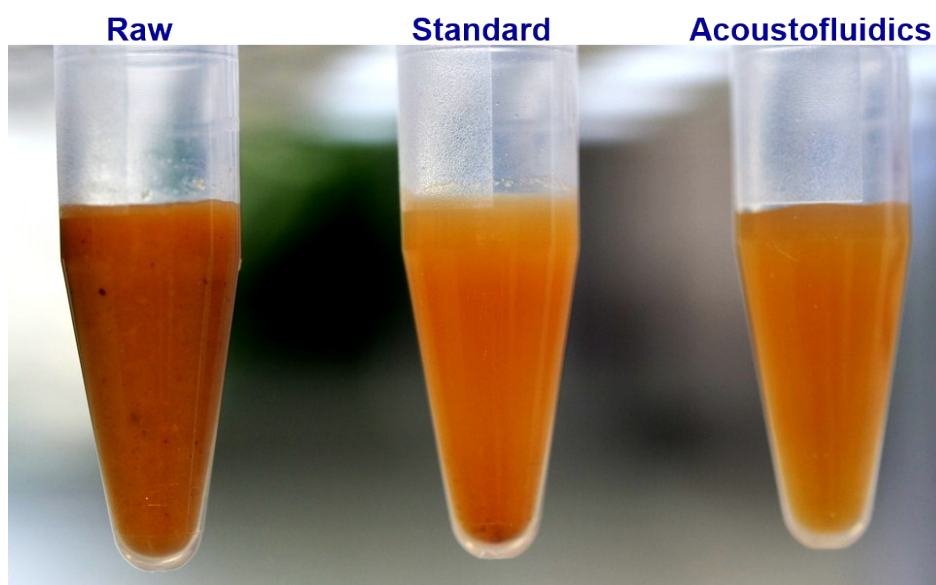


Fig. S2 Visual observation of watery human stool samples: “Raw”: an un-liquefied raw stool sample; “Standard”: a liquefied stool sample prepared using the standard method; “Acoustofluidics”: a liquefied stool sample prepared using our acoustofluidic device.

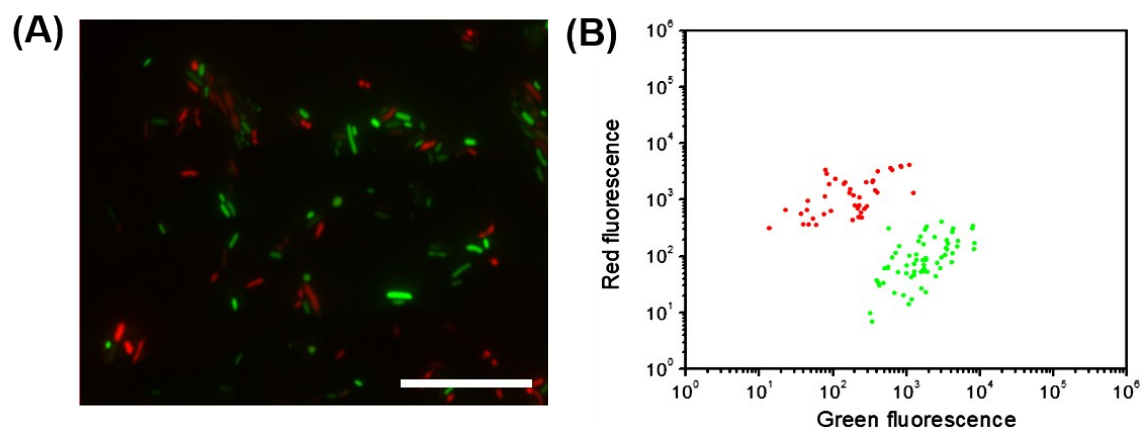


Fig. S3 Comparison of live (green)/dead (red) bacteria using (A) manual counting: red 44 and green 64 and (B) Matlab counting: red 48 and green 64. The mismatch of viability is only 3.5 %, demonstrating the reliability of the Matlab program. For a complete viability evaluation, over 2500 bacteria were counted using the Matlab program, and results of this analysis were shown in Table S1. Scale bar: 50 μm .

Table S1 The number of live/dead bacteria in the stool sample on different days; results were calculated using a fluorescence microscope image and a Matlab counting program.

Methods	Green	Red	Viability	Average
Standard	154	632	19.3%	39.2 $\pm$ 17.5%
	519	478	52.0%	
	488	560	46.5%	
Acoustofluidics	769	780	49.6%	39.1 $\pm$ 10.6%
	207	524	28.3%	
	581	883	39.6%	

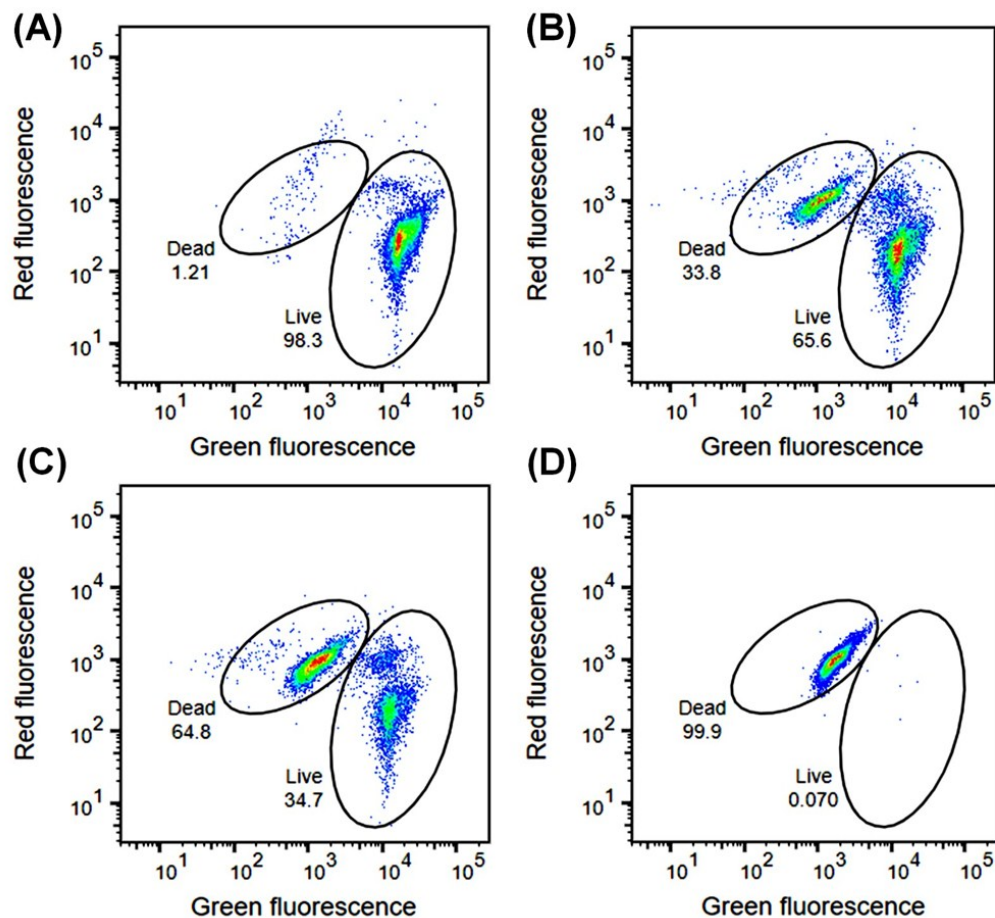


Fig. S4 Green fluorescence (SYTO 9) and red fluorescence (PI) plot of cultured *E. coli* mixtures at different viability: (A) 100%, (B) 70%, (C) 40%, and (D) 0% after stained with BacLight kit. Clear separation and obvious clusters for dead and live bacteria are observed. A correlation coefficient of $R^2=0.997$ was calculated between the viability measured by flow cytometry and the viability added in *E. coli* mixture, demonstrating an accurate and optimal condition for bacterial viability measurement.