Supplementary Information for:

FAST: Rapid Determinations of Antibiotic Susceptibility Phenotypes using Label-Free Cytometry

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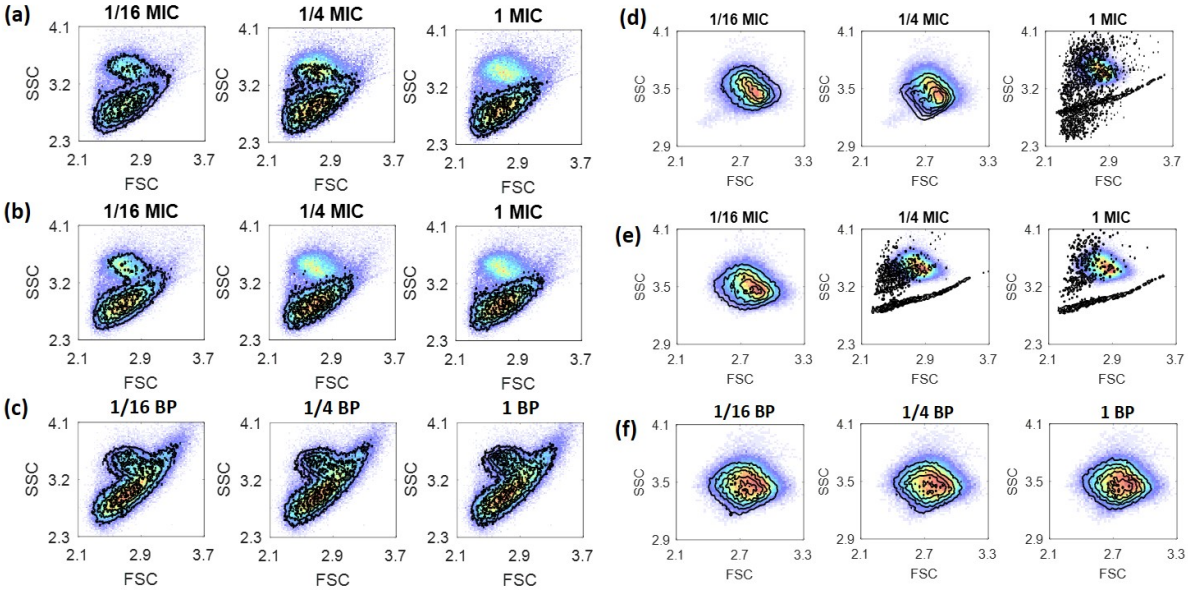
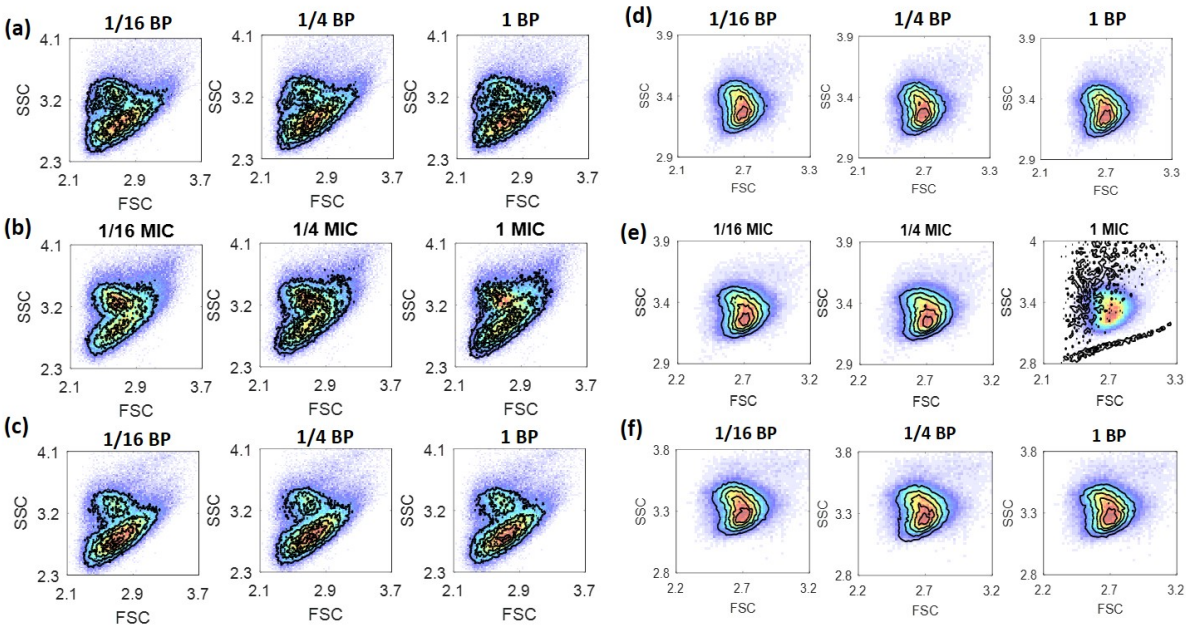
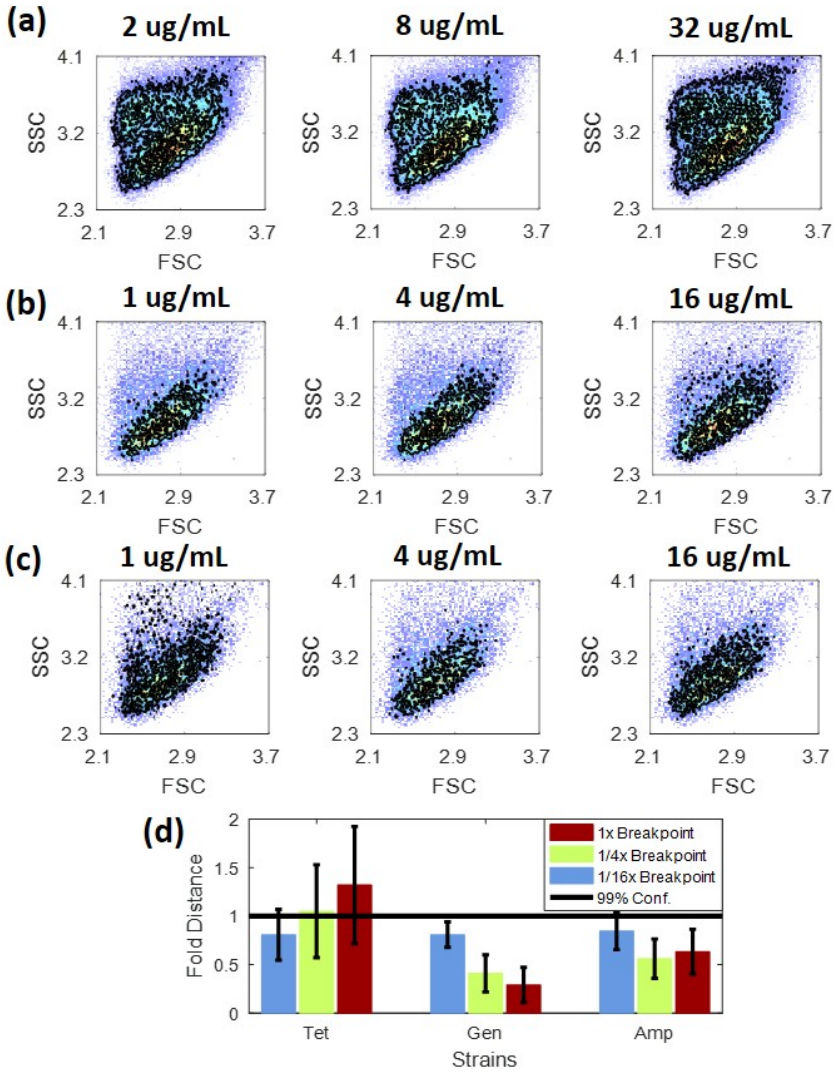


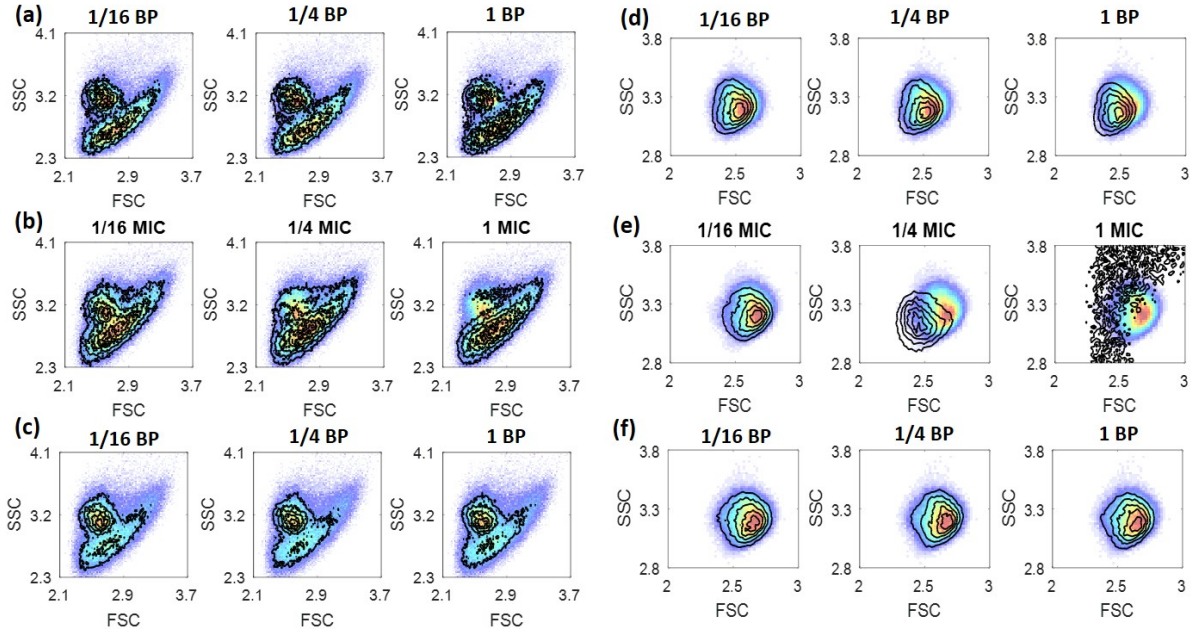
Supplementary Figure S1. Bactericidal Antibiotic-induced scatter changes for *E. coli* strain Mu890. For all data, pseudocolor plot: no-antibiotic, paired control. Black contour: antibiotic-treated data. (a) to (c) Mu890 recovered from 10% human blood. Complementary to Figure 2. (a) Tetracycline (b) Gentamicin (c) Ampicillin. (d) to (f) Mu890 pure culture started from around 1000 CFU/mL and incubated for 5 hours. (d) Tetracycline (e) Gentamicin (f) Ampicillin. Comparing the data with human blood (a to c) to the pure culture data (d to f), the bacteria signals appear at similar positions and disappeared at the same antibiotic concentration. In addition to the bacteria signals, the human blood data (a to c) also contain blood debris signals, appearing in the lower right corner. The blood debris signals are unchanged with different antibiotic treatments at various concentrations. The 1x MIC for tetracycline is 2 µg/mL and 8 µg/mL for gentamicin. For ampicillin, the resistant breakpoint for *Enterobacteriaceae*, 32 µg/mL, was used.



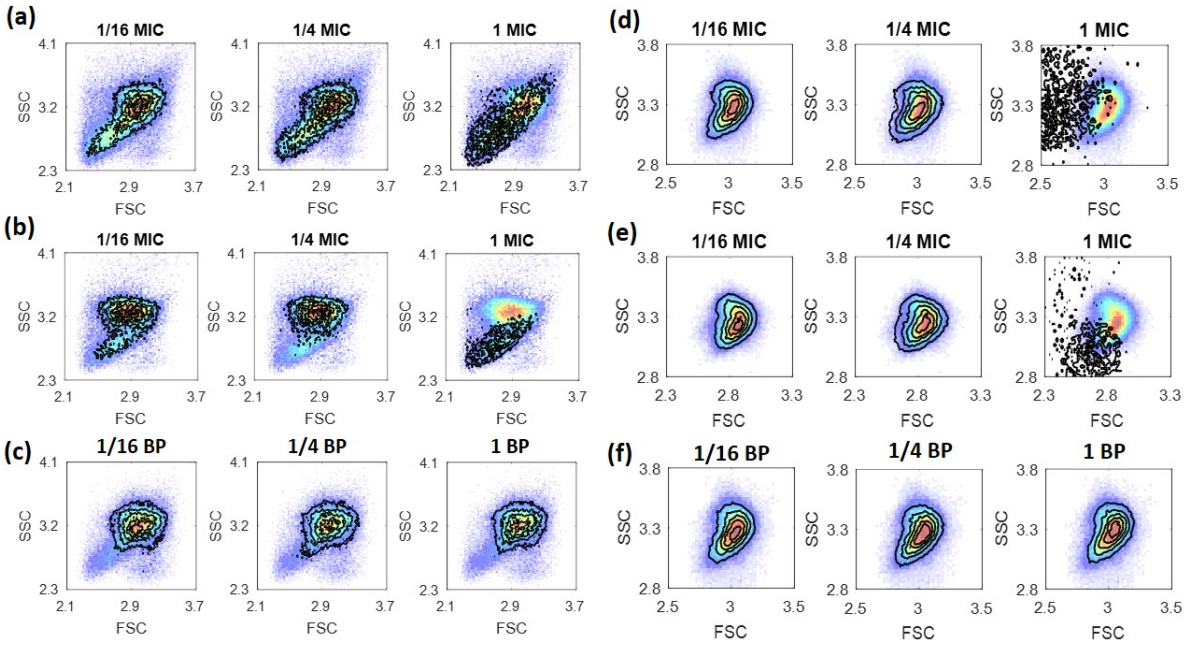
Supplementary Figure S2. Bactericidal Antibiotic-induced scatter changes for *E. coli* strain Mu14S. For all data, pseudocolor plot: no-antibiotic, paired control. Black contour: antibiotic-treated data. (a) to (c) Mu14S recovered from 10% human blood. Complementary to Figure 2. (a) Tetracycline (b) Gentamicin (c) Ampicillin. (d) to (f) Mu14S pure culture started from around 1000 CFU/mL and incubated for 5 hours. (d) Tetracycline (e) Gentamicin (f) Ampicillin. The 1x MIC for gentamicin is 8 µg/mL. For ampicillin and tetracycline, 32 µg/mL and 16 µg/mL were used. Both are the resistant breakpoint for *Enterobacteriaceae*.



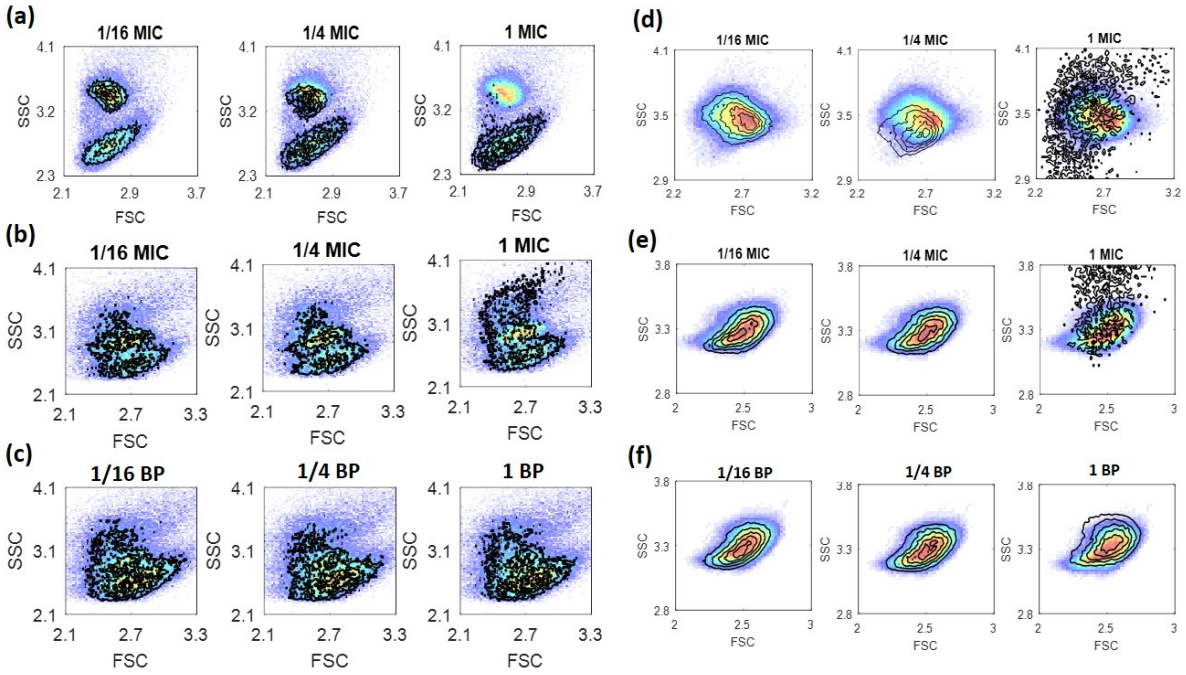
Supplementary Figure S3. Antibiotic-treated 10% human blood only results. Cytometric data with (a) Ampicillin (b) Tetracycline (c) Gentamicin. The pseudo-color plots are the no-antibiotic controls and the black contour plots are the antibiotic-treated data with the antibiotic concentration indicated at each plot. (d) PB-sQF results for (a), (b), and (c). Because the fold distance (y-axis) is calculated by dividing the test statistic between the histogram for each dataset and its paired control, by the 99% confidence level distance, any fold distance (with error bars) encompassing or smaller than unity means that the corresponding conditions are not significantly different from the control. For each antibiotic, the resistant breakpoints of *Enterobacteriaceae* are 16 µg/mL for tetracycline and gentamicin and 32 µg/mL for ampicillin.



Supplementary Figure S4. Bactericidal Antibiotic-induced scatter changes for *K. pneumoniae* strain Mu55. For all data, pseudocolor plot: no-antibiotic, paired control. Black contour: antibiotic-treated data. (a) to (c) Mu55 recovered from 10% human blood. Complementary to Figure 3. (a) Tetracycline (b) Gentamicin (c) Ampicillin. (d) to (f) Mu55 pure culture started from around 1000 CFU/mL and incubated for 5 hours. (d) Tetracycline (e) Gentamicin (f) Ampicillin. The 1x MIC for gentamicin is 1 µg/mL. For ampicillin and tetracycline, 32 µg/mL and 16 µg/mL were used. Both are the resistant breakpoint for *Enterobacteriaceae*.



Supplementary Figure S5. Bactericidal Antibiotic-induced scatter changes for *K. pneumoniae* strain Mu670. For all data, pseudocolor plot: no-antibiotic, paired control. Black contour: antibiotic-treated data. (a) to (c) Mu670 recovered from 10% human blood. Complementary to Figure 3. (a) Tetracycline (b) Gentamicin (c) Ampicillin. (d) to (f) Mu670 pure culture started from around 1000 CFU/mL and incubated for 5 hours. (d) Tetracycline (e) Gentamicin (f) Ampicillin. The 1x MIC for tetracycline is 2 µg/mL and for gentamicin is 4 µg/mL. For ampicillin, the resistant breakpoint for *Enterobacteriaceae*, 32 µg/mL, was used.



Supplementary Figure S6. Bactericidal Antibiotic-induced scatter changes for *A. nosocomialis strain* M2. For all data, pseudocolor plot: no-antibiotic, paired control. Black contour: antibiotic-treated data. (a) to (c) M2 recovered from 10% human blood. Complementary to Figure 3. (a) Tetracycline (b) Gentamicin (c) Ampicillin. (d) to (f) M2 pure culture started from around 1000 CFU/mL and incubated for 5 hours. (d) Tetracycline (e) Gentamicin (f) Ampicillin. The 1x MIC for tetracycline is 1/4 µg/mL and for gentamicin is 2 µg/mL. For ampicillin, the resistant breakpoint for penicillin-typed antibiotics for *Acinetobacter* spp., 128 µg/mL, was used.

Table S1. Overnight plate colony counts (CFU/mL) for Mu14S

Antibiotics	No Antibiotic	1/16x	1/4x	1x
Tetracycline (R)	*2.44 $x10^5$	0.96 $x10^5$	*2.04 $x10^5$	3.23 $x10^5$
Gentamicin (I)	$7.07x10^4$	6.07 $x10^4$	*5.28 $x10^4$	0
Penicillin g (R)	1.94 $x10^5$	0.98 $x10^5$	0.97 $x10^5$	1.29 $x10^5$

Fewer than 10 CFU/mL of Mu14S were spiked into 10% human blood and underwent the same process as described in Materials and Methods. The samples were treated with tetracycline, gentamicin, or penicillin. The 1x concentration for tetracycline is 1 μg/mL (MIC > 64 μg/mL), for gentamicin is 8 μg/mL (MIC = 8 μg/mL), and for penicillin g is 32 μg/mL (MIC > 5000 μg/mL). The plates were streaked from the sample after 5 hours incubation (2 hours enrichment and 3 hours AST). Although all experiments were done in triplicate, some of the overnight plates were not countable due to not enough of dilution before plate streaking. As a result, some of the numbers reported here are the average of duplicate data (labeled with *) while the rest are the average of triplicate data. R: resistant. I: intermediate. No bacteria were recovered from blood that was not spiked with blood-stable bacteria.