Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Detection of Sepsis in Patient Blood Samples Using CD64 Expression in a Microfluidic Cell Separation Device

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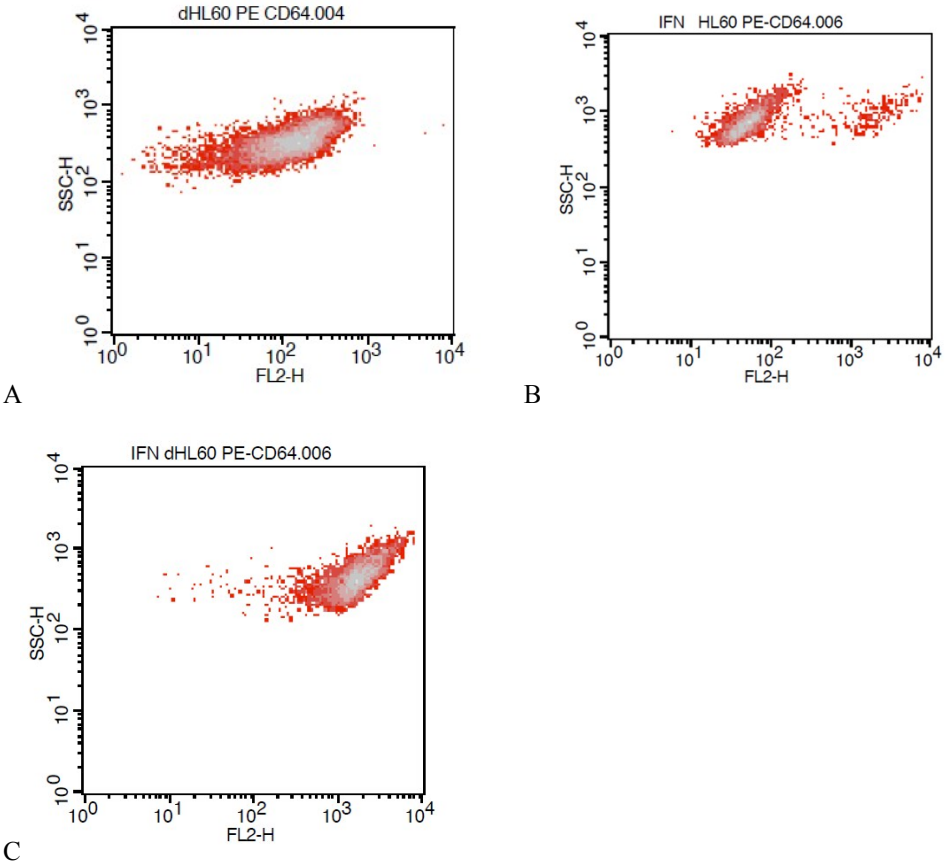


Figure S1. Cytograms of CD64 expression (FL2-H) and side scatter (SSC-H) measured by flow cytometry in the HL-60 expression model. HL-60 cells were differentiated (dHL-60) and treated with INF-γ. Differentiated cells (dHL-60) showed moderate fluorescence (A) when stained with phycoerythrin (PE) conjugated anti-CD64. Undifferentiated HL-60 precursor cells (B) had a small fraction of cells with upregulated CD64 after exposure to INF-γ. dHL-60 cells treated with INF-γ (C) exhibited a larger change in CD64 in the majority of cells. The differentiation of neutrophil-like cells and subsequent modulation in CD64 expression served as a controllable model for capture of cells based on changes in CD64 expression.

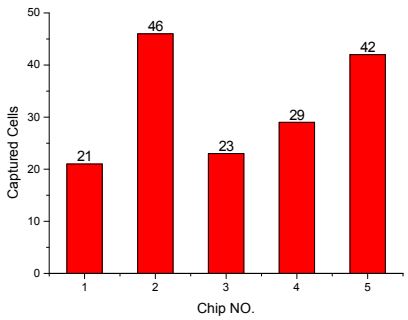
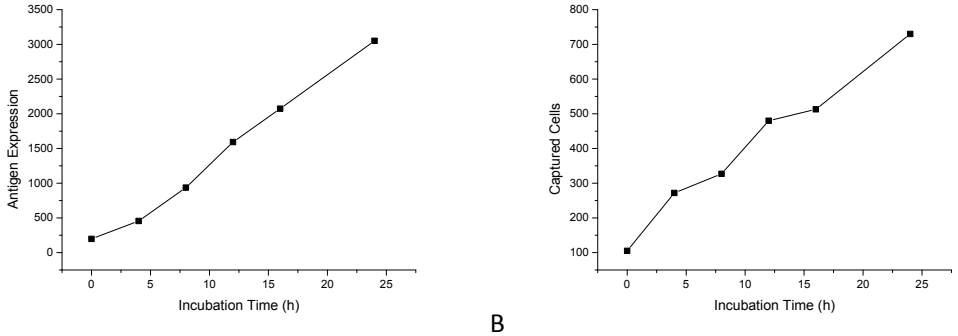


Figure S2. Evaluation of chip-to-chip variation. Captured cell number of CD64+ blood cells on herringbone chip. Aseptic blood samples were measured to generate a baseline cell capture number.

A threshold for cell capture that was 3 times larger than the standard deviation between chips was chosen. A capture number larger than 65 was therefore consider to be a positive result, indicating activated neutrophils were present in the sample.



A B

Figure S3. CD64 expression on dHL-60 cells was tested every 4 h after introducing IFN-γ via flow cytometry from 0 h to 24 h (A). Meanwhile, cell capture was conducted with the same sample on the sepsis detection chip. The absolute captured cell number (B) was measured. At 4 hours, the captured cell number has already exceeded the detection threshold for neutrophil activation, indicating that the device was capable of providing early information for CD64 expression.

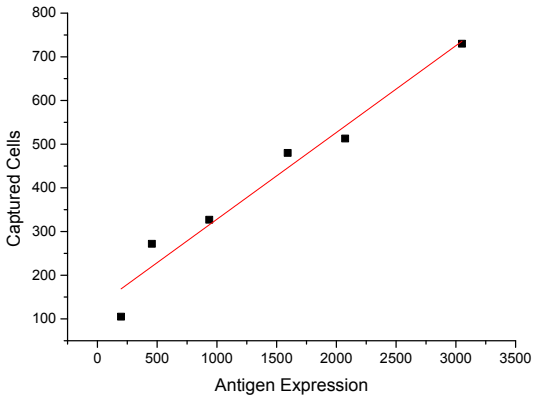


Figure S4. The relationship between total number of cell capture (chip) and CD64 expression (flow cytometry) in CD64+ expression model is linear. The linear regression of antigen expression vs. capture number was $y=0.1987x+129.38$, $R^2=0.96$.

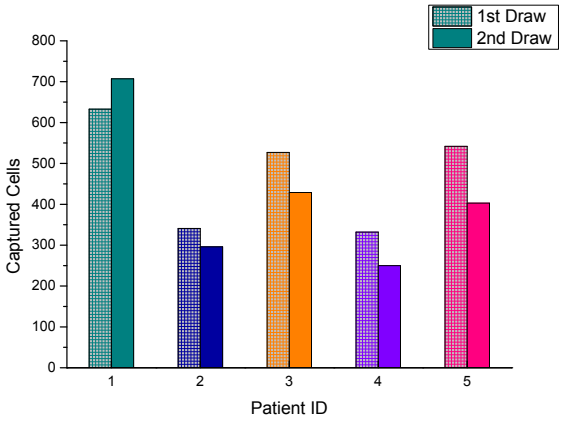


Figure S5. CD64+ cell capture from sepsis patient blood samples. Septic patients were identified by qSOFA score = 2, and the first blood draw was obtained within 24 hours of diagnosis. The second blood draw occurred 48 hours after diagnosis. The patterned columns represent on-chip cell capture of 1st blood draw and solid columns represent cell capture of 2nd blood draw. The decrease in CD64+ cells between the first and second blood draw may be due to response to antibiotic treatment.

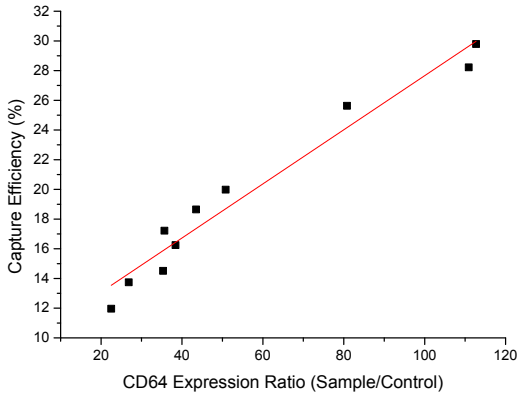


Figure S6. Relationship between on-chip cell capture of septic patient samples and corresponding CD64 expression is linear. Capture efficiency was presented to reduce variance caused by different initial cell concentration while loading on chip. Intensity ratio was presented to reduce variations introduced from sample preparation, experimental setting, and operation. Linear regression of CD64 expression ratio vs. capture efficiency were $y = 0.182x + 9.44$, $R^2 = 0.952$.