Smartphone-Imaged Microfluidic Biochip for Measuring CD64 Expression from Whole Blood

Tanmay Ghonge^{1,2,3}, Hatice Ceylan Koydemir⁶, Enrique Valera^{1,2,3}, Jacob Berger^{1,2,3}, Carlos Garcia^{1,2,3}, Noshin Nawar^{1,2,3}, Justin Tiao^{1,2,3}, Gregory L. Damhorst⁵, Anurup Ganguli^{1,2}, Umer Hassan⁹, Aydogan Ozcan^{6,7,8,*}, Rashid Bashir^{1,2,3,4,*}

- ¹ Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801 USA.
- ² Micro and Nanotechnology Lab, University of Illinois at Urbana-Champaign, Urbana, IL 61801 USA.
- ³ Biomedical Research Center, Carle Foundation Hospital, Urbana, IL, 61801 USA.
- ⁴ Carle Illinois College of Medicine, Urbana, IL, 61801 USA.
- ⁵ Emory University School of Medicine, Emory University, Atlanta, GA, 30322 USA.
- ⁶ Electrical and Computer Engineering, University of California, Los Angeles, CA, 90095 USA.
- ⁷ Bioengineering, University of California, Los Angeles, CA, 90095 USA.
- ⁸ California NanoSystems Institute, University of California, Los Angeles, CA, 90095 USA.
- ⁹ Department of Electrical and Computer Engineering, Rutgers University, Piscataway, NJ, 08854, USA.

*Corresponding Authors:

Rashid Bashir, email: rbashir@illinois.edu; Aydogan Ozcan, email: ozcan@ucla.edu

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S-1. Master mold fabrication protocol

SU8-50 was spun at 2200 rpm (30 s.) on the Si wafer. After soft-baking the wafer at the recommended temperature and time, SU-8 was exposed with UV light (400 mJ cm⁻²). Then, the wafer was bake. After baking (at the recommended temperature and time), unexposed SU-8 was washed away by developing the wafer in SU8 developer. The wafer was hard-baked (150 °C, 10 min.) on a hotplate. Finally, the surface of the mold was silanized by 3-mercaptopropyltrimethoxysilane.

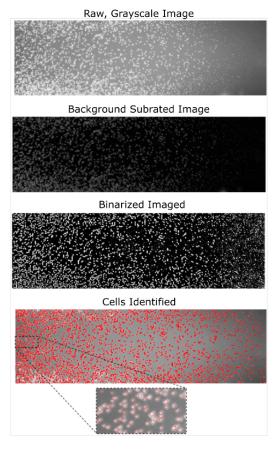
S-2. Biotin-beads conjugation protocol

COMPELTM beads solution (25 μ L) was incubated with 100 mM EDC and 50 mM NHS for 20 min. After the incubation, the beads were centrifuged, and the solution was aspirated. Then, a solution containing Amine-PEG2-Biotin (50 μ L) was added and incubated for 2 h. at RT.

After the incubation, the beads were centrifuged, and the solution was aspirated. The beads were blocked with a glycine solution (100 uL, 7500 µg mL⁻¹). The beads were centrifuged and resuspended in PBS (storage solution, 100 µL).

S-3. Sequence of operation for extracting cell-coordinates

In order to identify cells from the images acquired through the smartphone-based microscope, the following steps were taken; 1) the background was subtracted from raw, grayscale images; 2) the images were binarized by performing a thresholding operation, and 3) the cells in the binary image were identified by using a transform operation in the MATLAB library.



S-4. Relevant patient information

Table S-1 below summarizes relevant patient information provided by Carle Foundation Hospital

Patient	Gender	Age (years)	nCD64 Range	Neutrophil Count Range (μL-1)	Blood Culture Test	Sepsis Diagnosis	Remark
A	Male	73	0.55 – 1.23	2547 – 4933	Negative	Sepsis; due to unspecified organism	NA
В	Male	>89	2.60 - 4.52	1149 – 2533	Negative	NA	NA
С	Male	84	5.42 – 5.45	3177 – 5238	Negative	NA	Patient died 26.8 hours after admission to the hospital
D	Male	70	0.36 – 1.36	2954 – 5116	Positive; Enterococcus faecalis - (Group D)	Sepsis; due to Enterococcus	NA
Е	Male	81	1.54 – 6.6	831– 1748	Positive; Enterobacter Cloacae Complex & Coagulase negative Staphylococcus	Gram negative septicemia	NA
F	Male	64	2.96 - 4.09	1266 – 3360	Negative	Severe sepsis	NA
G	Female	77	0.41 - 0.84	444 – 2425	Negative	NA	NA
Н	Female	77	1.45	5470	Negative	Sepsis; due to unspecified organism	NA

S-5. Biochip measurement protocol

The measurement procedure included: 1) sample injection and cell immunocapture (1 μ L, 1 min); 2) wash step (1% BSA in PBS, 50 μ L, 5 min); 3) cells fixation (1-step fix/lyse solution, 50 μ L, 5 min, incubation time: 15 min); 4) cells staining (2 μ M SYTOTM 16, 50 μ L, 5 min, incubation time: 15 min); 5) imaging (30 s); and 6) counting analysis (30 s).

