

SUPPLEMENTARY MATERIALS

An Integrated Microfluidic Platform for In-situ Cellular Cytokine Secretion Immunophenotyping

Nien-Tsu Huang^{a#}, Weiqiang Chen^{a,b#}, Bo-Ram Oh^a, Timothy T. Cornell^c, Thomas P. Shanley^c,
Jianping Fu^{a,b,d*} and Katsuo Kurabayashi^{a,c*}

^aDepartment of Mechanical Engineering, University of Michigan, Ann Arbor, Michigan 48109, USA; ^bIntegrated Biosystems and Biomechanics Laboratory, University of Michigan, Ann Arbor, Michigan 48109, USA; ^cDepartment of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, Michigan 48109, USA; ^dDepartment of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan 48109, USA; ^eDepartment of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, Michigan 48109, USA.

[#]These authors contributed equally to this work; ^{*}Corresponding authors

(E-mail: jpfu@umich.edu, katsuo@umich.edu).

This supplementary material includes

Supplementary Figures (Fig. S1-S4, Video S1)

Supplementary Figure Captions

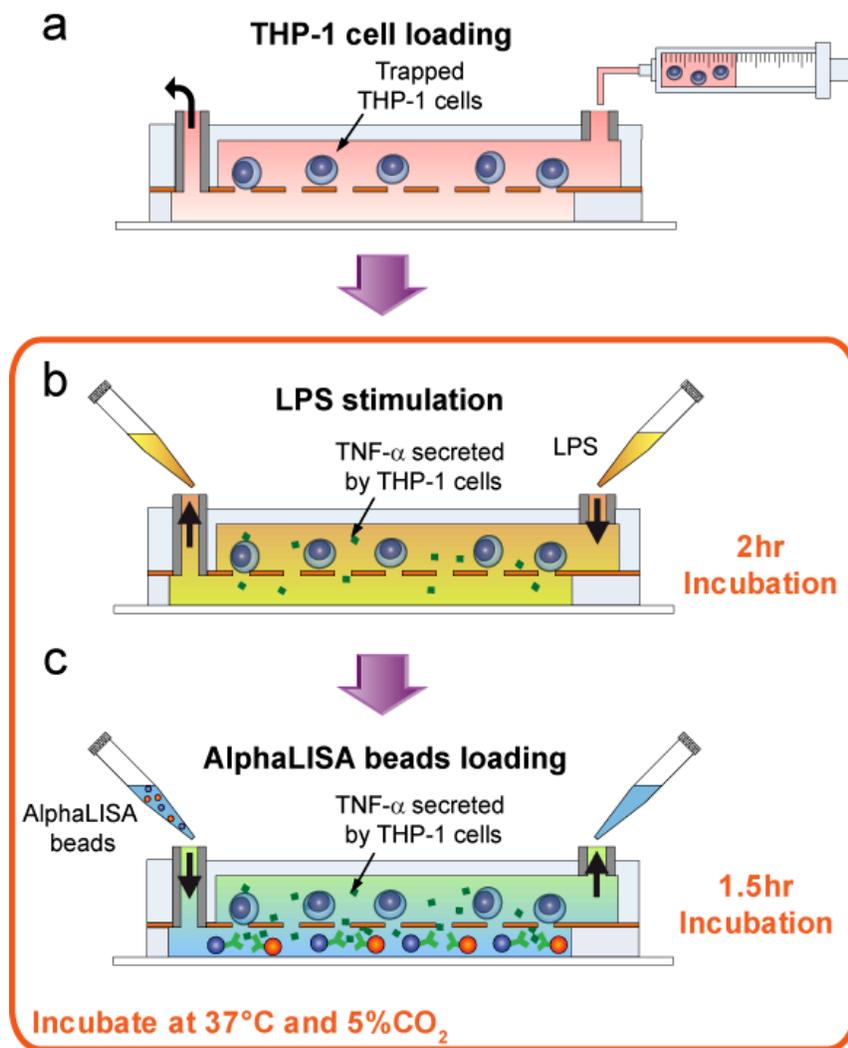


Figure S1 | Immunophenotyping assay loading protocol (a) THP-1 cells loading from the inlet of cell culture chamber. (b) Various LPS concentration (100, 50 and 10 ng/mL) loading from the inlet of cell culture chamber. (c) AlphaLISA bead loading from the inlet of immunoassay chamber. The MIPA device was incubated in the incubator at 37°C and 5% CO₂ during (b) and (c). Two pipette tips were attached on both inlets to prevent evaporation and provide a shear stress free microenvironment for cell stimulation and analyte diffusion.

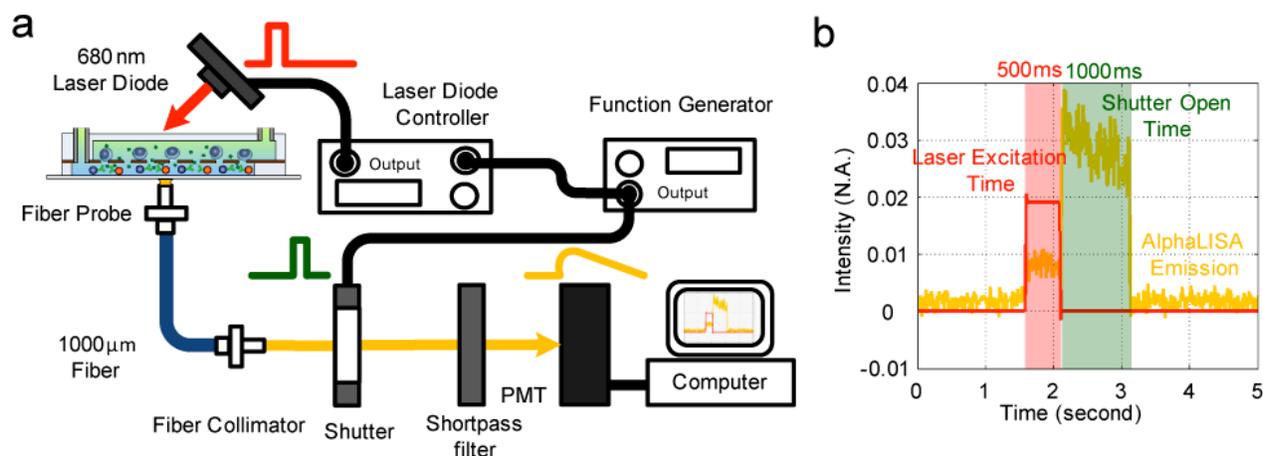


Figure S2 | Detection of TNF- α secreted from THP-1 cells using the AlphaLISA assay and the MIPA device (a) Schematic of a customized optical setup for the on-chip AlphaLISA signal detection. A laser diode was placed and aligned on the top of the MIPA device for AlphaLISA excitation. A fiber probe was attached under the MIPA device using immersion oil to achieve close to 100% optical coupling. For collection of AlphaLISA emission signal, an electronic shutter and a 660 nm shortpass filter were placed in front of a PMT to cut off unwanted scattering light. (b) Plot of time-sequenced laser excitation (red curve) and shutter opening (green box). A representative emission signal from the AlphaLISA (yellow curve) is also plotted for comparison. Note that laser excitation time was 0.5 sec and shutter opening time was 1 sec. The time gap between laser excitation and shutter opening was 20 msec.

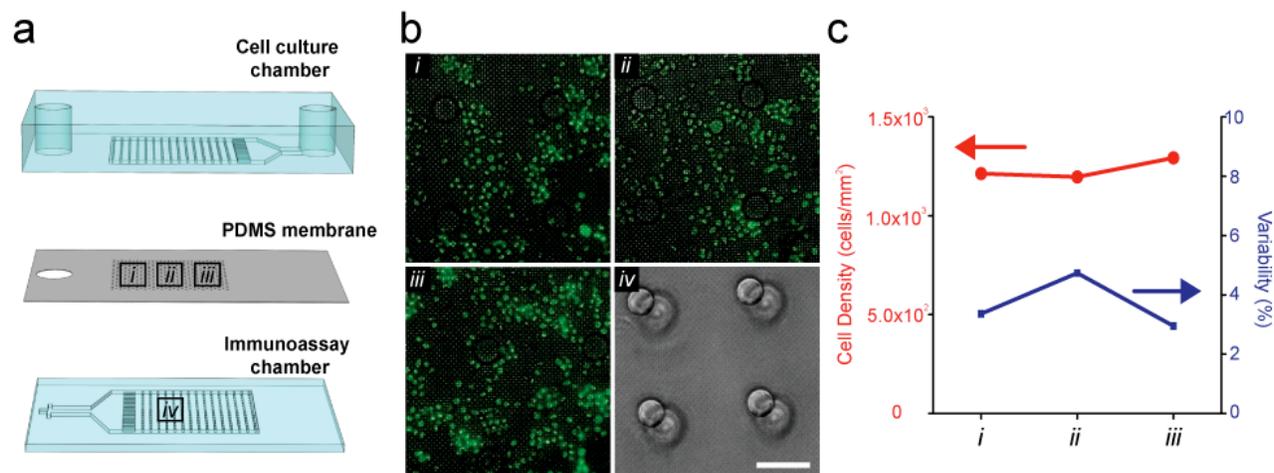


Figure S3 | Cell seeding and uniformity in the MIPA device (a) The schematic of three layer MIPA device. The dotted area is the focus area of objectives. (b) Optical images of THP-1 cells distribution in four different position of device. The optical images in (1)-(3) were taken above the PMM. The optical image in (4) was taken below the PMM. Scale bars, 100 μ m. No cells were observed in the bottom layer of device, which means high capture efficiency of PMM. (c) Plot of cell density and variability to the average cell density in three positions of device.

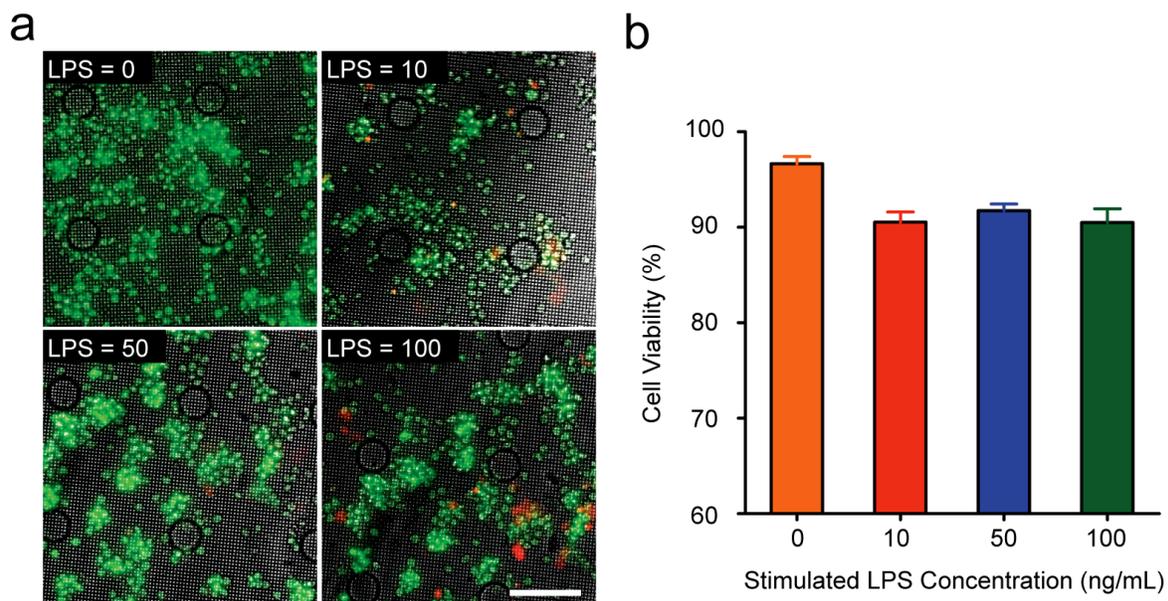


Figure S4 | Cell viability (a) Optical images of THP-1 cells distribution and corresponded live/dead cell image on PMM after 2 hr 0, 10, 50, 100ng/mL LPS stimulation. The picture is merged from brightfield, FITC and TexasRed image by the microscopic analysis software (Nikon NIS-Element BR, Nikon). Scale bars, 100 μ m. (b) Plot of the cell viability under different LPS concentration stimulation.

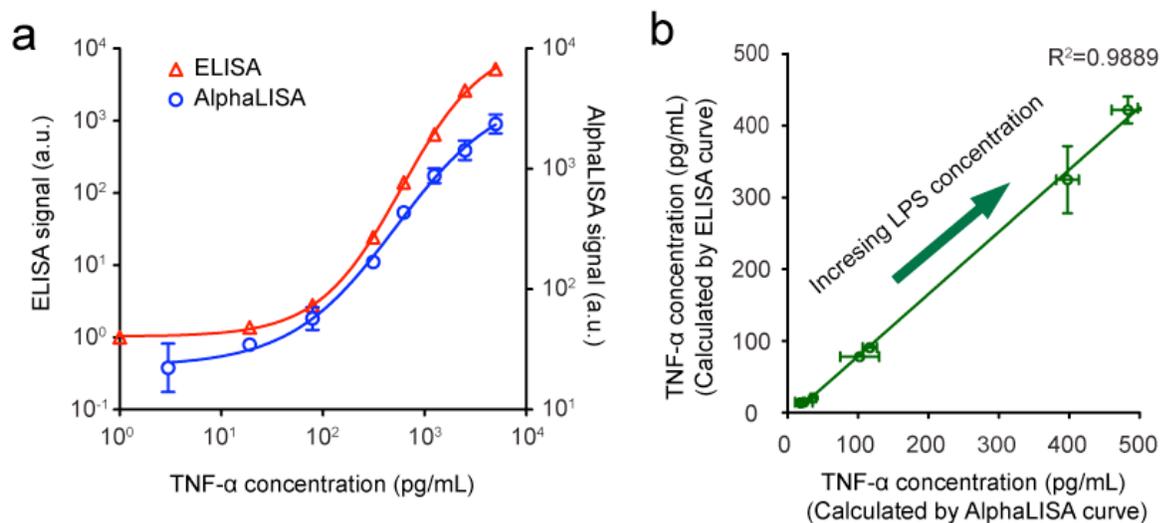


Figure S5 | Comparison between AlphaLISA and ELISA signals (a) Standard curves for TNF- α detection by AlphaLISA and ELISA. A TNF- α solution at a known concentration (0 – 5,000 pg/mL) was first spiked into a complete cell growth medium. Subsequently, TNF- α in the cell growth medium was detected by AlphaLISA using our customized optical setup and by ELISA using a commercial plate reader (SpectraMax M2e, Molecular Devices). (b) Correlation plot of ELISA and AlphaLISA signals for TNF- α secreted by 20,000 THP-1 cells as a function of LPS concentration (0-100ng/mL).

Video S1 | Cell loading video

cell loading.wmv

Video S1 | Cell loading video THP-1 cells were loading into the MIPA device at 5×10^5 cells/mL by syringe pump (5 μ L/min). THP-1 cells were seeded and enriched on the PMM.