Supporting Information

Multiplexed Nanoplasmonic Temporal Profiling of T-Cell Response under Immunomodulatory Agent Exposure

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1) Detailed device overview and explanation of signal detection

Figure S1. a) Photo image of LSPR nanoplasmonic biosensor microarray chip constructed with 120 sensing spots for cytokine detection. The device consists of a PDMS-based sample-loading/detection channel layer and a glass substrate with four meandering parallel sensor stripe patterns of gold nanorods (AuNRs). The surfaces of the AuNRs were conjugated with antibodies targeting four different types of cytokines (L-2, INF-γ, TNF-α, and IL-10). b) Top view of the device. Ten sample loading microfluidic channels were covered by the glass substrate with its AuNR sensor patterns orthogonal to the microfluidic channels. c) Magnified view of AuNR biosensor patterns, which have three repeats of four parallel arrays, each functionalized with antibodies targeting one of the four cytokines above. This arrangement allowed for triplicate measurements across the four cytokines with each sample, which minimized measurement error.
2) Dark-field imaging optical setup

Figure S2. Schematic of the dark-field microscopy setup for LSPR biosensor microarray imaging. In the dark-field LSPR imaging process, light-source illumination from the top is first introduced to the dark-field condenser lens. The illumination light hits the sensor surfaces of the underneath chip with its central light beam blocked. This only allows the scattered light from the sensor image to be collected by the objective lens and subsequently filtered by the optical bandpass filter. In our study, the chip device was tightly mounted on a motorized stage and placed in the microscopy system for the entire measurement. The sample was both loaded to the device and washed using a syringe pump.
3) ELISA vs. LSPR nanoplasmonic biosensor assay

The LSPR nanoplasmonic biosensor assay provides several advantages over the conventional ELISA immunoassay. First, the total assay time required for the LSPR measurement after the sample loading is 30 min, which is 8 times shorter than that of ELISA (4 hrs). The LSPR assay is label-free and able to eliminate many process steps that involve tagging with secondary antibodies carrying labels and washing unbound agents. Second, the LSPR biosensor microarray chip only requires a small sample volume of ~1 μL for the measurement. This enables us to perform multi-time-point measurements through repeated sampling of small-volume cell-culture supernatants without significantly altering the analyte concentration in the original sample. Moreover, our LSPR biosensors have a large dynamic range (10-10000 pg/mL), which is suitable for multiplexed analysis of analytes coexisting in a single sample with large concentration variations.

The multiplexed LSPR nanoplasmonic biosensor assay requires less sample volume compared to ELISA. We calculated the volume required for the LSPR nanoplasmonic biosensor assay and ELISA. In LSPR nanoplasmonic biosensor assay, we obtained 12 data points with 10 μL of cell culture medium from triplicate measurements of the four target cytokines. To obtain the same amount of data with the standard singleplexed ELISA technique, the total volume of 50 μL × 12 = 600 μL would be needed.

Furthermore, Multiplexed LSPR nanoplasmonic biosensor is expected to be more cost effective than ELISA. Leng et al.\(^1\) estimated the cost of commercial kit (R&D systems)-based ELISA measurement to be $16.53/analyte. Our LSPR assay costs $1.06/analyte in a multiplexed setting, where multiple analyte species are detected in parallel within the same chip device. This cost is estimated as follows:
Each LSPR biosensor microarray chip requires 10 µL of gold-nanoparticles (stock price; 10 mL for $200), thus the AuNR material cost is $0.2/chip. The glass substrate costs $43.7/72 = $0.6/chip. We use 2µL of each antibody out of 1 mL stock solution ($229), which is diluted 10 times for agent loading with ease. The total cost for the four antibodies is $229× 4 × \frac{2}{1000} = $1.832/chip. We estimate the labor charge for the entire 2-hour process, which includes device preparation, sample loading, sample detection, and data collection, to be $20/hr × 2 hour = $40/chip. Thus, the total cost of our LSPR biosensor assay involving 8 different samples, each having 4 target analytes, is only $1.06/chip for analysis of each analyte.

Figure S3. a) Correlation of LSPR biosensor assay data vs. ELISA data obtained from identical samples. b) Comparison of LSPR biosensor assay and ELISA.
We prepared unknown concentration of purified samples of IL-2, TNF-α, INF-γ, and IL-10 and took measurements for identical samples using both the LSPR biosensor assay and ELISA. Total eight samples (two unknown concentrations prepared from each cytokine) were divided into two vials, one for the ELISA measurement and the other for the LSPR measurement. The data from both the ELISA and LSPR measurements were compared to each other and plotted in the Figure S3a. Our study obtained an excellent correlation between the two groups of data (Figure S3a). The table shown in Figure S3b summarizes key features of the two assay methods for comparison.

4) Real-time dynamic analyte binding curve from LSPR multiplexed assay

Our LSPR nanoplasmonic biosensors allowed us to monitor analyte surface binding events in real time. This gave us an idea how soon each binding event reached equilibrium. In our study, we prepared a mixture of four purified target cytokine samples in phosphate buffered saline (PBS) solution and loaded it into the LSPR nanoplasmonic biosensor microfluidic chip. The cytokine mixture sample has the concentrations of 100, 500, 1000, and 2500 pg/mL for IL-10, TNF-α, IFN-γ, and IL-2, respectively. The analyte binding process for each cytokine was recorded for 30 min right after loading the sample into the chip (Figure S4). From the obtained real-time binding curves, we verified that all the analyte binding processes reached equilibrium within nearly 30 min as shown by the signals reaching the plateaus. As a result, our assay protocol only required the 30 min sample incubation time to fully complete the analyte binding on the sensors. After the incubation process, we washed the sensor surfaces to remove nonspecifically bound agents from them and performed the LSPR biosensor intensity
measurement. An alternative method would be to quantify the analyte concentrations from the initial slopes of the binding curves. This could even shorten the assay time down to a few min.

Figure S4. Real-time binding curves obtained from LSPR nanoplasmonic biosensor assay for four different cytokines (Red line – 2500 pg/mL of IFN-γ, Blue line – 1000 pg/mL of IL-2, Green line – 500 pg/mL of TNF-α, and orange line – 100 pg/mL of IL-10). A mixture of purified IL-2, INF-γ, TNF-α, and IL-10 at the different concentrations was loaded into the device and incubated for 30 min.
5) Cell viability test

![Image of live and dead cells](image)

| Condition      | Total cell # | Dead cell # | Viability (%) |
|----------------|--------------|-------------|---------------|
| a) Control     | 235          | 10          | 95.75         |
| b) TAC 0.1 ng/mL | 234          | 8           | 96.59         |
| c) TAC 1 ng/mL  | 235          | 8           | 96.6          |
| d) TAC 10 ng/mL | 229          | 7           | 96.95         |

Figure S5. Cell viability test using trypan blue solution. The images were taken using a hemocytometer after adding the trypan blue solution to the Jurkat T-cells after all the assay experiments under the conditions in a)-d). All cases resulted in cell viability over 95%, which indicates that the cytokine secretion suppression was not primarily due to cell death.
1. Leng, S.X.; McElhaney, J. E.; Walston, J. D.; Xie, D.; Fedarko, N. S.; Kuchel, G. A.

ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences* 2008, 63, 879-884.