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AC electroosmosis-enhanced nano-plasmo-fluidic detection of ultra-low-concentration cytokine

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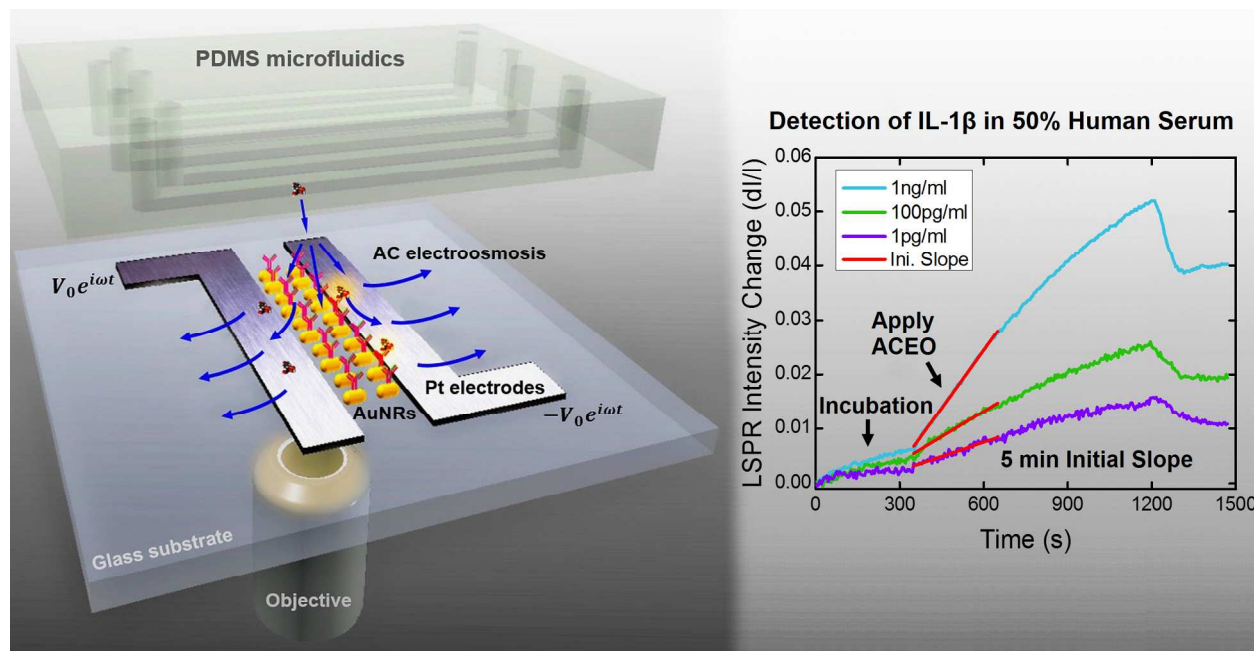
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KEYWORDS: Nanorod biosensor, AC electroosmosis, localized surface plasmon resonance, immunoassay, cytokine analysis

TOC Image



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ABSTRACT

Label-free, nanoparticle-based plasmonic optical biosensing, combined with device miniaturization and microarray integration, has emerged as a promising approach for rapid, multiplexed biomolecular analysis. However, limited sensitivity prevents the wide use of such integrated label-free nanoplasmonic biosensors in clinical and life science applications where low-abundance biomolecule detection is needed. Here, we present a nano-plasmoﬂuidic device integrated with microelectrodes for rapid, label-free analysis of a low-abundance cell signaling protein, detected by AC electroosmosis-enhanced localized surface plasmon resonance (ACE-LSPR) biofunctional nanoparticle imaging. The ACE-LSPR device is constructed using both bottom-up and top-down sensor fabrication methods, allowing the seamless integration of antibody-conjugated gold nanorod (AuNR) biosensor arrays with microelectrodes on the same microﬂuidic platform. Applying an AC voltage to microelectrodes while scanning the scattering light intensity variation of the AuNR biosensors results in signiﬁcantly enhanced biosensing performance. The AC electroosmosis (ACEO) based enhancement of the biosensor performance enables rapid (5-15 min) quantiﬁcation of IL-1 β , a pro-inﬂammatory cytokine biomarker, with a sensitivity down to 158.5 fg/mL (9.1 fM) for spiked samples in PBS and 1 pg/mL (58 fM) for diluted human serum. Together with the optimized detection sensitivity and speed, our study presents the ﬁrst critical step towards the application of nanoplasmonic biosensing technology to immune status monitoring guided by low-abundance cytokine measurement.

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3 The ability to detect the dynamic surface binding of biomolecules without using labeling
4 agents provides a critical basis for emerging biochemical analysis. Plasmonic biosensors based
5 on biologically functionalized noble metal nanoparticles enable rapid, label-free detection of
6 proteins, DNA, and mRNA with ease of signal acquisition and robustness in complex biological
7 solutions.¹⁻⁴ These biosensors transduce biomolecular surface binding events into optical signals
8 that reflect changes in the resonant behavior of conduction band electrons near the surface of
9 optically excited metallic nanoparticles. Among them, gold nanorod-based plasmonic biosensors
10 have been demonstrated to achieve single-molecular sensitivity⁵⁻⁷ and its microarray enables
11 practical protein detection at high-throughput and multiplexity.⁸⁻¹⁰ Despite their practicality and
12 the intrinsic sensitivity of the individual nanoparticles, these biosensors exhibit performances
13 limited by poor diffusion of the target molecules, exemplified by the presence of a large
14 depletion zone near the sensor surface, especially for high-density microarrays. The diffusion-
15 limited molecular binding kinetics and the resulting poor LOD prohibit the wide use of the
16 nanoplasmonic biosensors in clinical diagnostics and biochemical assays.

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18 A miniaturized microfluidic design with effective sample mixing techniques¹¹⁻¹⁴ plays a key
19 role in the detection of a wide variety of chemical/biological species. In particular, AC
20 electrokinetics, including dielectrophoresis (DEP), ACEO, and electrothermal effects, have been
21 widely used to preconcentrate biomolecules and enhance the analyte capture efficiency in
22 microscale heterogeneous assays.¹⁵⁻¹⁷ For example, Gong¹⁸ et al. monolithically integrated
23 silicon nanowire field-effect transistor (FET) biosensors with micro-fabricated electrodes and
24 achieved 10^4 times sensitivity improvement for detection of prostate specific antigen (PSA) in
25 PBS buffer. Cheng¹⁹ et al. integrated ACEO and positive DEP with an electrochemical
26 impedance spectroscopy (EIS) biosensor in the detection of IgG-protein A. More recently, DEP
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was utilized to enhance surface plasmon resonance (SPR) sensing with gold nanohole arrays,²⁰ and ACEO was coupled with surface-enhanced Raman spectroscopy (SERS),²¹ in both studies, unprecedented levels of detection limit and assay speed were demonstrated. Among these sensing enhancement mechanisms, ACEO coupled with label-free optical biosensing logically offers a promising approach because it can generate strong three-dimensional (3D) vortices for microfluidic mixing at relatively low voltage;¹⁶ does not require any invasive, agitating mechanical components interfering with far-field plasmonic detection optics, and can be readily tuned by voltage and frequency using microfabricated electrodes of simple design which is integrated in a microfluidic system. The ACEO approach can improve mass transfer-enhanced analyte-receptor interactions, thus enhancing biosensing performance.

In this letter, we report synergistic integration of plasmonics and microfluidics to develop a label-free plasmonic gold nanorod (AuNR) optofluidic device coupled with ACEO analyte flow to overcome the barrier for diffusion-limited nanoparticle biosensing performance. The plasmofluidic integration has recently attracted much attention as an emerging approach to advancing plasmonic device technology.²² Our device consists of a glass substrate coated with gold nanorods between two coplanar rectangular Pt microelectrodes and another polydimethylsiloxane (PDMS) microfluidic channel layer (Figure 1a). The ACE-LSPR biofunctional nanoparticle imaging process starts by applying an 180° out-of-phase AC voltage across the microelectrodes of the device. The movement of ion charges in the electrical double layer induced on the electrode surfaces (Figure 1a) generates a rotational fluid motion. This fluid motion causes microfluidic agitation, facilitating the transport of analytes down to the sensing surface of AuNR to break the depletion zone (depicted by the blue arrows in Figure 1b, c). The sensor response is therefore enhanced by the increased molecular collision events. The binding

of target analytes onto the antibody-conjugated AuNRs causes a spectral shift of the scattering light from the AuNR surfaces, which translates into a far-field intensity increase of the optical signal detected by optics using a bandpass filter (Figure 1d). Patterned AuNR arrays on the device were imaged by darkfield microscopy coupled with an Electron Multiplying Charge Coupled Device (EMCCD) with high sensitivity. As shown by the darkfield image (Figure 1e), the glass substrate contains a 25 μm -wide patterned area of AuNRs between 50 μm -wide microelectrodes and two additional reference AuNR sensor patterns of the same shape placed sufficiently (200 μm) away from the microelectrodes. Three sets of such patterns were constructed on the same chip for triplicate measurements. The PDMS layer was attached to the glass substrate with the sample detection channels (400 μm in width and 80 μm in height) running orthogonal to the line-shaped AuNR sensor patterns and the microelectrodes (depicted by the dotted line in orange). A scanning electron microscope (SEM) image (Figure 1f) shows the distribution of nanorods and the formation of a thin dielectric layer (from the binding of antibody/antigen) after assay. Details of the device layout, fabrication process, and AuNR functionalization are provided in Supporting Information (Figure S1, S2).

Figure 2a provides a schematic illustration of the theoretical model for the ACEO-enhanced analyte transport and surface reaction during the ACE-LSPR optofluidic device operation. Using a simplified two-dimensional model, AC electroosmosis velocity u_{ACEO} was first calculated from the movement of the electrical double layer in the presence of an alternating tangential electric field at the frequency f near the electrode surface as²³

$$u_{ACEO} = \frac{1}{8} \frac{\epsilon V_0^2 \Omega^2}{\mu \chi (1 + \Omega^2)^2} \frac{C_s}{C_s + C_D} \quad (1),$$

where Ω is the non-dimensional frequency defined as $\Omega = \pi^2 \kappa \chi (\epsilon_m / \sigma_m) f$, in which κ is the reciprocal of the Debye length, and ϵ_m and σ_m are medium electrical permittivity and

conductivity, respectively, μ is the dynamic viscosity of the solution, C_S is the capacitance of the Stern layer, or the thin layer of tightly associated counter ions near the electrode surface, C_D is the capacitance of the Diffuse layer, or the layer behind the Stern layer in which the electric potential exponentially decays, and V_0 is the peak value of the applied potential. The AC electroosmosis velocity u_{ACEO} was then included into the slip boundary condition when solving the incompressible Navier-Stokes (N-S) equation to obtain the velocity field (u, v) of the solution. The transient local analyte concentration $c = c(x, y, t)$ was then calculated by solving the mass transport equation that accounts for both diffusion and convection, given by

$$\frac{\partial c}{\partial t} = D \left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) - (u \frac{\partial c}{\partial x} + v \frac{\partial c}{\partial y}) \quad (2)$$

where D is the diffusion coefficient. At the sensor surface ($y = 0$), the boundary condition was set up such that the analyte mass flux is balanced with the surface binding reaction rate based on the first-order Langmuir absorption model as

$$-D \frac{\partial c}{\partial y} = k_{on} c|_{y=0} (B_0 - B) - k_{off} B \quad (3),$$

where k_{on} and k_{off} are association/dissociation constants, B_0 is the initial binding site density, and B is the immobilized antigen concentration. Here, the key parameters to be optimized are the applied voltage, frequency, and microfluidic chamber height under different ionic concentrations in solution. Both the applied voltage and frequency determine u_{ACEO} while the microfluidic chamber height affects the entire mass transport process. The inlet and outlet were set as an “open boundary”, where the local concentration is equal to the bulk solution concentration under zero normal stress. Further details of modeling the electrical double layer and of selecting the parameters can be found in the Supporting Information.

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3 In order to accurately predict the enhancement of the ACEO-facilitated analyte transport and
4 surface reaction, we experimentally verified the ACEO velocity calculated above using a flow
5 visualization technique developed in this study (see Supporting Information, Flow visualization
6 & video 1). This technique employed AuNRs suspended in a testing buffer solution as tracing
7 particles. Real-time imaging of particle trajectories with a darkfield microscopy setup allowed
8 us to measure the flow velocity field within the device under ACEO operation by two-
9 dimensional (2D) micron resolution particle image velocimetry (μ -PIV) analysis (see Supporting
10 Information for details). We quantitatively observed a good match between our theory and
11 experiment for frequency-dependent ACEO flow behaviors and determined the best operating
12 frequency to be $f = 200\text{Hz}$ for the testing buffer (conductivity: 1.59 mS/m), as shown in Figure
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29 Using the experimentally verified model, we calculated the binding ratio $BR = B/B_0$ (the
30 fraction of binding sites occupied by analyte molecules over the total sensor surface area) after
31 an incubation period of 20 min for three assay conditions: (1) ACEO agitated incubation without
32 forced convection at $V_0 = 2\text{V}$ and $f = 200\text{Hz}$ (Figure 2b), (2) static incubation driven by pure
33 diffusion without forced convection or ACEO (Figure 2c), and (3) forced convection without
34 ACEO (Figure 2d). The ACEO operation at $2\text{V}/200\text{Hz}$ was later determined to yield the optimal
35 biosensing condition. The value of BR was assumed to be proportional to the LSPR scattering
36 light intensity. Figure 2b shows that the circular flow generated by ACEO can significantly
37 suppress the depletion zone as compared to the static incubation condition in which sample
38 solution is not replenished at the sensor surface. Figure 2e shows numerical results of BR over
39 time for human cytokine IL-1 β at 1 ng/mL in 1000 times diluted ($0.001\times$) PBS (conductivity:
40 1.59 mS/m) with an ionic strength of 0.2105 mol/m^3 . Here, we chose IL-1 β , a proinflammatory
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cytokine (cell signaling protein) produced by monocytes and macrophages, as the model analyte in our study. IL-1 β has a relatively low molecular weight (17 kDa) and a small dissociation constant (10^{-10} - 10^{-11} M), and is responsible for systemic inflammatory responses of the host, activation of phagocytes, and production of acute phase proteins by the liver.²⁴

With the optimum frequency fixed at $f = 200$ Hz, we further varied the applied voltage and channel heights. Since u_{ACEO} scales with V_0^2 (see Equation (2)), our simulation in Figure 2e shows that BR is significantly enhanced as V_0 increases from 1V to 4V. Now, we define the enhancement factor as BR_{ACEO}/BR_{Diff} , where BR_{ACEO} and BR_{Diff} are the binding ratios under ACEO agitated incubation (Figure 2b) and the diffusion-driven static incubation (Figure 2c), respectively. The enhancement factor thus quantifies the ACEO-driven enhancement of the analyte binding at the sensor surface. Figure 2f shows the enhancement factor as a function of time and channel height, with its maximum (5 - 7) reached within the first 3 - 5 min of device operation. When the channel height is small, the confinement of fluid by the channel ceiling suppresses ACEO-induced fluid mixing. Without forced convection of the sample fluid, the analyte depletion zone quickly grows over time since no analytes are replenished at the sensor surface. A larger channel height prevents the depletion region from reaching the channel ceiling, effectively increasing the enhancement factor. However, the enhancement factor starts to taper off with the channel height exceeding ~ 140 μ m as ACEO-induced fluid mixing decreases with the distance from the electrode. To keep our device fabrication practically easy, we selected a channel height of 80 μ m.

We next experimentally demonstrated detection of low-concentration IL-1 β using the AuNR optofluidic device and ACEO-induced fluid mixing operation. To this end, we first conjugated anti-IL-1 β to AuNR surfaces using a two-step EDC/sulfo-NHS protocol (see Supporting

Information, Device fabrication). The use of a low conductivity medium, 0.001x PBS buffer (1.59 mS/m), was expected to achieve high ACEO velocity without undesired heat generation and electrolysis. To monitor the real-time binding of IL-1 β on the sensor surface, we recorded the scattering intensity change of the line-shaped AuNR sensor patterns every 5s. Figure 3a shows the time-resolved intensity change upon analyte binding, corresponding to the IL-1 β concentration from 50 fg/mL to 100 pg/mL in PBS buffer along with a negative control (0.001x PBS). The assay started with sample loading for 5min and incubation for another 5-6 min, followed by applying an AC bias of 180° phase difference for around 15 min. We observed a significantly enhanced intensity shift and a much faster binding speed towards equilibrium after introducing the ACEO flow (indicated by the dashed blue line in Figure 3a).

As a result, we were able to distinguish the signal indicating the presence of IL-1 β in PBS down to 50 fg/mL (2.9 fM, dark blue line in Figure 3a) from the signal of the negative control (purple line, Figure 3a) within 10 min after turning on ACEO. In Figure 3b, the end-point intensity increases of the triplicated AuNR line-shaped sensor patterns are mapped at different analyte concentrations with and without ACEO. Figure 3c shows a close comparison of sensor responses as a function of IL-1 β concentration with and without ACEO. Here, we define the LOD as the analyte concentration which generates a signal corresponding to 3 standard deviations above the mean of the noise level in the negative control.²⁵ The LOD of the ACE-LSPR device is thus 158.5 fg/mL (9.1 fM) in 0.001x PBS, 100 fold more sensitive than that without ACEO (Figure 3c). In addition, enhanced analyte-antibody interaction by ACEO yields a larger number of binding events occurring at the sensor surface, as indicated by the ~2.5 times increase of scattering signal with ACEO (Figure 3c). This is the key reason for the ACE-LSPR device to achieve such a low LOD value and short assay time. It should be noted that the signal

enhancement ratio is around 40% smaller than the theoretical predictions. This discrepancy is acceptable, considering (1) the assumption made to simplify our model, in which the ion charge density on the microelectrode surface induced by the applied AC bias is linearly proportional a potential drop in the electrical double layer, and (2) uncertainties in model parameters (see Supporting Information for simulation details).

To explore the practical utility of the ACE-LSPR device for clinical diagnosis, we further sought to detect human IL-1 β in complex human serum, which has a greater ionic strength and contains a wide spectrum of background proteins. From Equation (1), it is known that ACEO flow velocity decreases with ion concentration, which causes the optimum operating frequency to shift to a higher value. A higher ion concentration also potentially introduces other undesirable effects, such as Joule heating, DEP concentrating, and electrolysis. These additional effects can adversely affect the stability of the sensor performance¹² and are difficult to predict by theory. Therefore, we empirically chose a 50% DI water diluted, cytokine-free heat inactivated and charcoal stripped human serum matrix (HSM, EMD Millipore) as the testing buffer. And we determined the optimum device operation condition to be $V_{p-p} = 1.5V$ at $f = 1kHz$ based on the flow visualization (Supporting Information, video 1). Following the same protocol described previously, we obtained real-time analyte binding curves from 1 pg/mL to 10 ng/mL of IL-1 β spiked-in human serum and the corresponding calibration curves, as shown in Figure 4a and 4b, respectively. A strong binding enhancement was observed immediately at 6 min, which is the time point when the bias was applied. This strong binding enhancement is similar to what we observed in the test using PBS buffer but may not entirely due to the ACEO fluid transport. It should be noted that even for the negative control (human serum without IL-1 β spiked), we observed a noticeable enhancement of binding by implementing ACEO. This could be attributed

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3 to the non-specific adsorption of free background proteins in serum promoted by the positive
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5 DEP effect at the electrode edges, as discussed in previous studies.¹⁹ Therefore, we incorporated
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7 a washing step with deionized (DI) water at 20 min after the assay, in order to remove the non-
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9 specifically adsorbed proteins and to eliminate false signals. Based on the calibration curves, we
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11 determined the LOD of the ACE-LSPR device for human serum samples to be <1 pg/mL (58
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13 fM), which is about one order of magnitude higher than the LOD in PBS buffer. This higher
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15 LOD for human serum samples is likely due to the nonspecific binding of the complex human
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17 serum background that still partially remains after the washing step.
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22 Finally, we were able to extract the initial slope values of the binding curves within the first 5
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24 min of sample incubation in the presence of ACEO. As shown by red bold lines in Figure 4a, a
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26 linear regression was utilized to fit the initial real-time binding curves. Here, we found good
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28 linear fits to the initial 5-min regions of the binding curves for IL-1 β concentrations from 10
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30 pg/mL to 1000 pg/mL ($R^2 > 0.95$) as the Langmuir model predicts. By plotting the initial slopes as
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32 a function of IL-1 β concentration, a calibration curve with a similar trend to the scattering
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34 intensity-based analysis was obtained (Figure 4b inset). The selection of the incubation time for
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36 initial slope fitting would undoubtedly affect the shape of the calibration curve and the
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38 subsequent analysis of the biosensing performance. In this study, we decided to use the first 5
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40 min real-time binding data based on a variance analysis after carefully comparing the fitted data
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42 at different incubation time periods over the entire real-time dataset. (See Supporting
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44 Information for t-test). By correlating the analyte concentration with the initial slope of the
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46 sensor response curve, we could reduce the assay time down to ~5 min while most of other
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48 analyte diffusion-limited biosensor operations require much longer time for the system to reach
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Comparing biosensor signals against the calibration curves obtained above, we further measured IL-1 β concentrations in serum samples extracted from six sepsis patients. Three of these patients were stratified into a hyper-inflammatory subgroup, and the other three are into a mild-inflammatory subgroup.²⁶ All studies were approved by the University of Michigan Institutional Review Board (IRB) and conducted with informed parental consent. Blood samples were collected from patients younger than 18 years old, meeting criteria for septic shock admitted to the pediatric intensive care unit at the C.S. Mott Children’s Hospital within 24 hours of admission. The tests were simultaneously performed using both ELISA and the ACE-LSPR device with 3 to 4 repeats in the same freeze-thaw cycle. Using the ACE-LSPR device, we measured serum IL-1 β across the patient samples from both the scattering intensity images obtained after the entire assay process and the 5-min initial binding curve slopes. These intensity and slope values were converted to IL-1 β concentration values by 5-parameter logistic fitting,²⁷ which is a standard fitting method in commercialized immunoassay²⁸ for plotting a sigmoidal shape calibration curve on the semi-log axes, as shown in Figure 4b. Figure 4c shows that the data obtained from the three different assay methods are consistent for all the patient samples. An excellent linear correlation ($R^2 = 0.947$) was obtained between the results from ACE-LSPR intensity imaging-based analysis and ELISA measurements across both the spiked-in and patient samples (Figure S4). We performed one-tailed paired difference t-tests between the two patient subgroups using the 5-min initial slope data. The result shows a significantly higher level of IL-1 β ($P < 0.01$) for the hyper-inflammatory subgroup as compared to the mild-inflammatory subgroup. This proves that the 5 min initial-slope assay can reliably distinguish the sepsis patients with either hyper-inflammatory or mild-inflammatory conditions. Although this initial-slope method could potentially introduce some errors, the ultrafast assay time within 5 min offers

unprecedented opportunities in clinical diagnosis to quickly determine the patients' immune conditions and to make timely stratification decisions.

In summary, we have uniquely integrated AC electroosmosis with nanoplasmonic microfluidic biosensors and demonstrated that effective electrohydrodynamic agitation in bulk solution can significantly improve the label-free sensing performance of the nanoplasmonic biosensors. The ACE-LSPR biofunctional nanoparticle imaging technique applied to our device has achieved femtomolar-level detection of IL-1 β , a pro-inflammatory cytokine, in PBS buffer with assay time less than 15 min using a sample volume as small as 5 μ L. To the best of our knowledge, it is the most sensitive label-free nanoparticle-based plasmonic cytokine sensor reported so far. A compromised sensing performance is observed for the ACE-LSPR device when operating with high ion-concentration biological medium due to the augmented electrical screening effect. Nonetheless, we have successfully shown that 50% serum dilution still enables the ACE-LSPR device to retain its capability of enhancing the sensing speed and sensitivity even for complex human serum samples. Analyzing initial analyte binding curve slopes obtained from the ACE-LSPR measurements, we have successfully demonstrated a 5-min cytokine immunoassay for stratifying sepsis patients into statistically distinct inflammatory status groups. The significantly improved sensitivity and shortened assay time clearly indicate the promising potential of the ACE-LSPR device for rapid screening and stratification of acute inflammatory diseases. Using micro-inkjet printing and 3D-stage controlled micro-brush patterning for AuNR patterning and antibody conjugation, we may potentially scale up the manufacturing throughput of the current device. Our future development of a faster, cheaper and smaller CMOS photon detector together with a high NA air condenser would allow our ACE-LSPR devices to be integrated in a handheld

system, which would enable point-of-care personalized treatment with timely, precise immunomodulatory drug delivery.

ASSOCIATED CONTENT
Supporting Information

This material is available free of charge via the Internet at <http://pubs.acs.org>.

Material, methods and supplementary figures. (PDF)

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Author Contributions

*These authors equally contributed to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ACE-LSPR, AC electroosmosis-enhanced localized surface plasmon resonance; ACEO, AC electroosmosis; AuNRs, gold nanorods; LOD, limit of detection; SEM, scanning electron microscope; EMCCD, electron multiplying charge coupled device.

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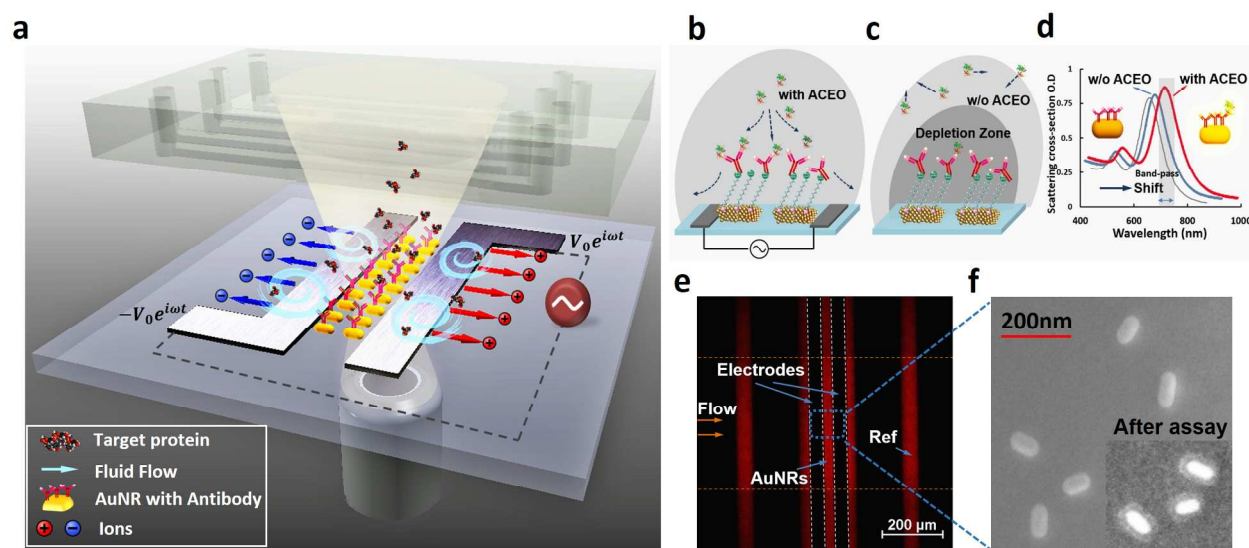


Figure 1. (a) Schematic and principle of AC electroosmosis-enhanced localized surface plasmon resonance (ACE-LSPR) biofunctional nanoparticle imaging. Pt microelectrodes were first patterned on a glass substrate by photolithography and metal lift-off. AuNRs were then deposited to form line-shaped sensor pattern between the microelectrodes using a microfluidic patterning technique. With 180° out-of-phase AC bias applied to the Pt electrodes, the electrical double layer horizontally moves along the electrode surfaces. The electrical double layer movement generates a hydrodynamic rotational flow within the microfluidic channel. The hydrodynamic flow facilitates the transport of the target biomolecules down to the sensing surface and their surface binding reaction. (b)-(c) Close views of the analyte-receptor interactions with/without ACEO. A large depletion zone is formed and slows down the surface reaction under diffusion-limited operation. (d) The binding results in an increase of scattering cross-section and a red shift of the plasmon resonance which is converted into the scattering intensity increase by a band-pass filter (680/13 nm, shown by the shade). (e) Darkfield image of AuNR line-shaped patterns (shown in red) and microelectrodes (black strips in the middle) with a microfluidic flow channel perpendicular to them (400 μm , orange dotted line). (f) SEM image showing the zoomed-in view of the isolated AuNRs patterned on the glass substrate between the microelectrodes. The size of the AuNRs was measured to be ~ 40 nm in diameter and ~ 84 nm in length. After the assay, the formation of a dense dielectric layer surrounding each AuNR was observed, suggesting the binding of antibodies and analytes onto the sensor surface (Inset).

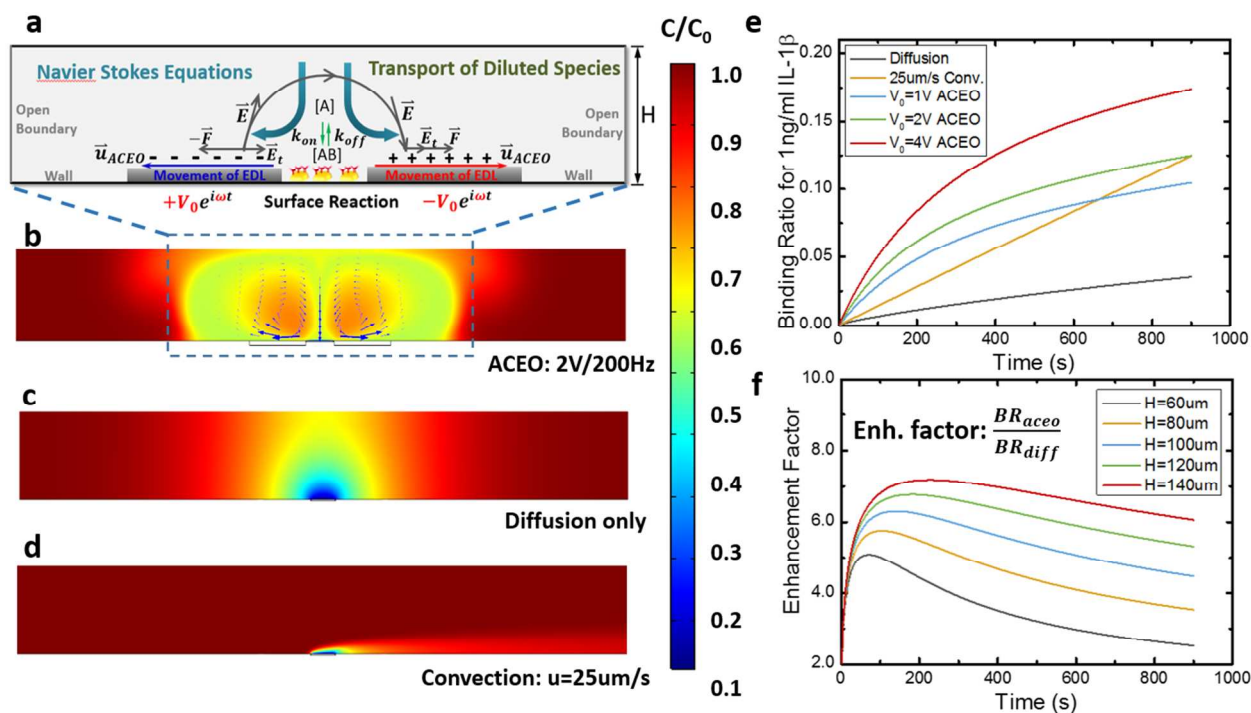


Figure 2. 2D Finite-element analysis (FEA) simulations on the mass transport and surface reaction in the ACEO-LSPR optofluidic device. (a) Simulation setup and boundary conditions. The ACEO velocity was first characterized by calculating the movement of the electrical double layer driven by the tangential component of the electric field using Coulomb's law. This velocity was then coupled with incompressible N-S equation to solve for the flow field, which was subsequently integrated with Fick's law to derive the concentration profile. Finally, the first order Langmuir Absorption was adopted as a boundary condition accounting for the surface reaction at the sensor surface. (b)-(d) Analyte concentration profiles within microfluidic channel of the device operated under ACEO at $V_0 = 2\text{V}$ $f = 200\text{Hz}$, with diffusion only, and with convection flow at $25\mu\text{m/s}$, respectively. The ACEO velocity direction and magnitude are shown by the blue arrows. The initial concentration for all three cases was set to be $c/c_0 = 1$. The inlet (left edges) and the outlet (right edges) for ACEO and diffusion only cases were defined as open boundaries with no analyte replenishment ($c/c_0 = 1$ at $x = \infty$). (e) Time-course change of binding ratio (BR) for different operating conditions. The BR is defined as the ratio of analyte occupations over the entire available binding sites. (f) Time-course change of enhancement factor for different channel heights under ACEO operation at $V_{p-p} = 2\text{V}$ $f = 200\text{Hz}$. Here, the enhancement factor is defined as the BR under ACEO over the BR under the condition of diffusion only.

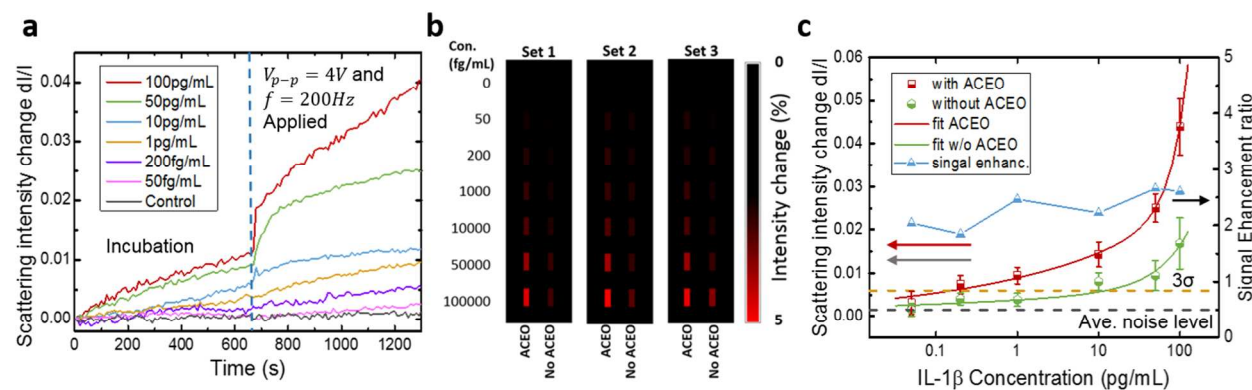


Figure 3. Detection of purified IL-1 β in 0.001x PBS buffer. (a) Real-time scattering light intensity profiles of the line-shaped ACE-LSPR AuNR sensor patterns at various IL-1 β concentrations (50 fg/mL to 100 pg/mL). Here, the operation condition at $V_{p-p} = 4V$ $f = 200Hz$ was applied after 10-11 min of incubation. (b) Darkfield images showing the mapping of the scattering intensities of triplicated sensing patterns under conditions with/without ACEO. (c) Calibration curves obtained from the intensity mapping in b (left y axis). The LOD was determined by the intersection of the fitted calibration curve with 3 standard deviations (3σ) above the mean of the background noise. The LOD was calculated to be 158.5 fg/mL (9.1 fM) for “with ACEO” and 15.2pg/mL for “without ACEO”. A reduction of LOD by two orders of magnitude was achieved. The signal enhancement ratio (right y axis) was calculated by the scattering intensity change for the “with ACEO” condition over the “without ACEO” condition. The “without ACEO” condition data were obtained from the intensity change of the Ref bar shown in Figure 1c.

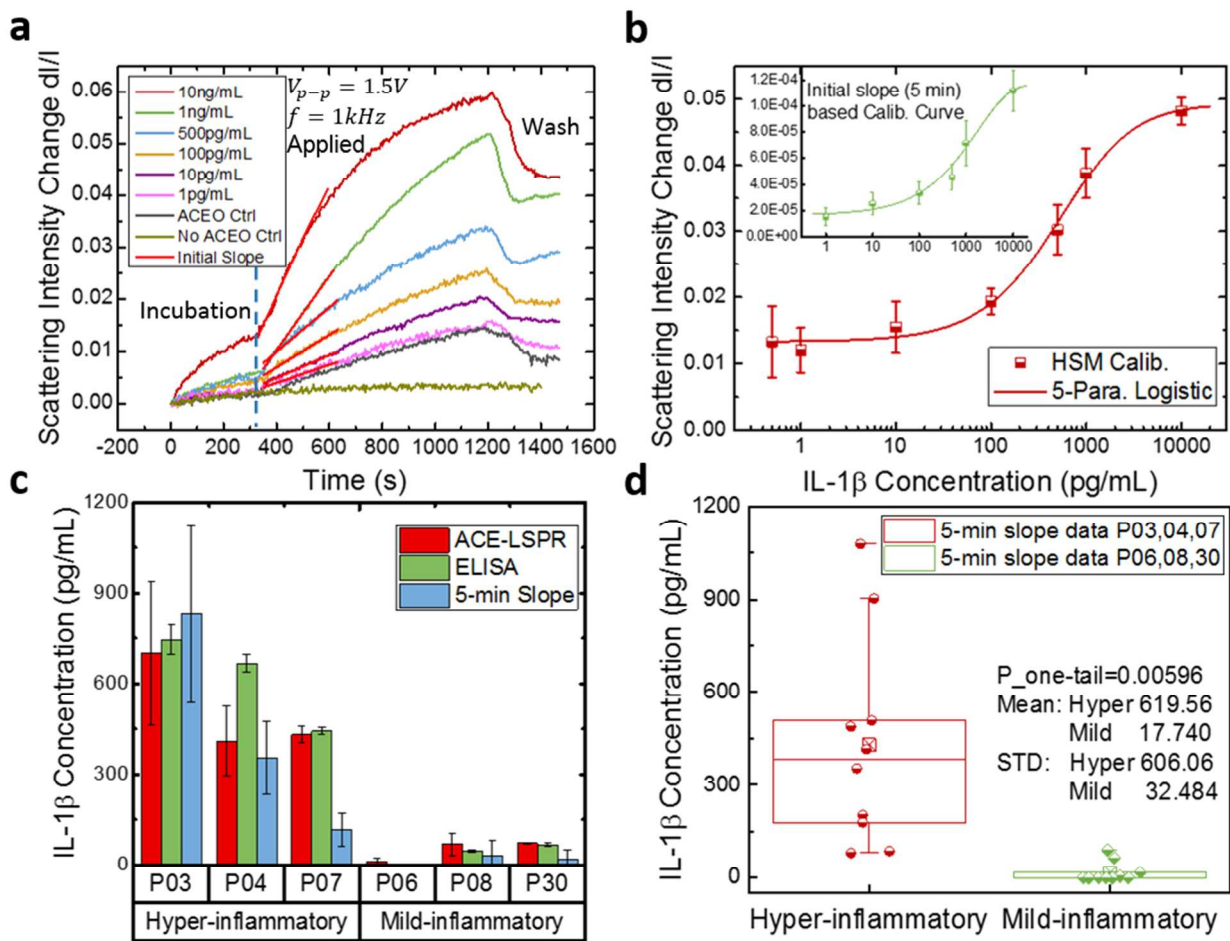


Figure 4. Detection of IL-1β in human serum. (a) Real-time scattering light intensity profiles of the line-shaped ACE-LSPR AuNR sensor patterns presenting enhanced signals for 50% human serum matrix (HSM) spiked by IL-1β at concentrations of 1 pg/mL to 10 ng/mL. Here, the operation condition at $V_{p-p} = 1.5V$ $f = 1kHz$ was applied after an incubation period of 5 min. (b) Calibration curves obtained from intensity mapping and 5 min initial slope analysis (shown by the inset). The raw data were fitted by a 5-parameter logistic function. (c) Serum IL-1β concentrations measured by three different methods for 6 pediatric sepsis patients stratified into hyper-inflammatory and mild-inflammatory subgroups. The labels of “ACE-LSPR,” “ELISA,” and “5-min Slope” indicate data obtained for the clinical samples by ACE-LSPR signal intensity analysis after an incubation period of ~15 min, conventional 96-well plate ELISA, and 5-min initial slope-based analysis, respectively. (d) One-tailed paired difference t -test performed for 5-min initial slope measurement between the two patient subgroups. The p -value calculated between the two patient subgroups is < 0.01 .