Minute-Level Speed Identification and Assessment of Bacteria/Cells Using Electrokinetic Assistance

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Abstract

Conventional techniques for detection and analysis of cells/bacteria use Western blot and ELISA kits that are high cost and long time consuming. An ideal advanced biosensor (molecular or whole cells detections) unit must have several important features: rapid detection time (<15 minutes), high sensitivity (10^2 cells/ml for whole cell detection or sub-nM concentration for molecular detection), high specificity, small, and inexpensive instrumentation/configuration. Two novel platforms will be introduced here, including an optofluidic system for the rapid on-chip detection of bacterial infection and a cell-based biochip for the label-free assessment of drug susceptibility on cancer cells. Rapid identification of rare pathogen from a very dense human blood sample is realized through combining the hybrid electrokinetic concentration with on-chip surface-enhanced Raman spectroscopy (SERS) identification of bacteria based on their detected SERS spectra. Compared to the current method in the hospital, this simple and rapid platform accelerated the detection time from 2 days to a few minutes. The cell-based biochip uses a novel, rapid, and label-free approach- AC electric field induced electro-rotation (eROT) to evaluate the drug susceptibility of cancer cells. The isolated lung cancer cells were successfully analyzed using eROT approach for the rapid and label-free assessment of the drug susceptibility of cancer cells. eROT spectra for different drug-treated cancer cells was successfully determined to the drug resistance and susceptibilities through their frequency-dependent rotation speeds. The relationship and trend between eROT method and conventional method are very agreement.

Keywords: identification, bacteria, surface-enhanced Raman spectroscopy, drug susceptibility

1. Introduction

Rapid separation, concentration, and detection of pathogens in clinical diagnosis of bacteremia and sepsis infections have become increasingly important. Conventional bacteria identification assays typically require several hours for DNA amplification and hybridization, hours for antibody-antigen reaction, or even days for bacteria culture. The time consuming on process of incubating bacteria to a high concentration of 10^9 CFU/ml is required. It is even more difficult to detect pathogens from a mixed sample, such as the detection of bacteria in a blood sample, which is very important for diagnosing bacteremia and sepsis [1]. Therefore, micro-particle separation plays an important role in several microfluidic devices. Density gradient centrifugation method and flow cytometry fluoresce-based or magnetic bead-based cell sorting systems are the commonly used approach to isolate the target cells form a heterogeneous medical sample [2]. However, the low purity and cell health cannot meet requirements for further analysis and assessments. Furthermore, the huge and expensive equipments and fluorescence labeling also limit its generality and the further applications. In biomolecular detection fields, conventional antibody-antigen

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reaction such as enzyme-linked immunosorbent assay (ELISA) that at low concentrations, the diffusion-dominated kinetics are too slow and difficult to carry target molecules to the detection surface, thus resulting in low sensitivity (~100 nM) and long hybridization time (4–6 hr), which limits their practical applications [3]. ELISA and the Western blot are typically limited by their portability and high cost because they need elaborate fluorescent/enzyme tagging and sophisticated optical instruments. Therefore, pre-concentration of detection targets and label-free analysis are necessary to accelerate the detection speed and sensitivity and to reduce the size of the required equipment for in-field rapid diagnostics [4]. The successful targeted therapy is depended on specific targeted drug. For example, epidermal growth factor receptor (EGFR) inhibitors which respond rate of the patient with mutation of epidermal growth factor is from 60% to 85%, but the respond rate of the patient without mutation of epidermal growth factor is just only from 10% to 15%. Hence, founding suitable therapy in numerous targeted drugs is a challenge to clinicians. If the clinicians want to choose the suitable targeted drug, first, it is must to confirm the specific drug targets of cancer cell (ex. mutation of epidermal growth factor) or matched biomarker. Morphological methods such as SEM and TEM are used to examine when the cell morphology has been changed after drug treatments with a long-winded time. Biochemistry analysis methods such as Enzyme-linked immunosorbent assay (ELISA), Western blot, Immunocytochemistry and MTT assay are commonly used for analysis cancer cells [5]. However, neither animal experiments nor biochemistry methods require several time-consuming steps or need a professional user to conduct them. In addition, large number of cell and several hours detection time are needed by those approaches. There is a timeliness and urgent need to develop to reach rapid, low cost and highly sensitive cancer assessment platforms for acceleration of the cancer determination and new drug development/test.

We introduce a novel biochip for rapid separation and concentration of the target bacteria/molecules for further label-free and highly sensitive on-chip detection/analysis. Our proposed approach combines the biased AC electroosmosis (ACEO) and dielectrophoresis (DEP) with a 3D electric field design. In hybrid electrokinetics, ACEO provides long-range transportation, and 3D DEP provides a separation mechanism [6, 7], thus, resulting in a long-range and selective concentration of detection targets from a heterogeneous sample. These two mechanisms result in high-purity selective concentration of target nanocolloids/bacteria into the centre electrode, as shown in Fig. 1. Our study reports a novel microfluidic platform for rapid and long-ranged concentration of rare-pathogen from human blood for subsequent on-chip surface-enhanced Raman spectroscopy. The bacteria identification from blood sample can be finished within 5 minutes. The platform demonstrated excellent separation and concentration capabilities by AC electrokinetics and high bacteria discriminability by their measured SERS signatures.

The cell-based biochip uses a novel, rapid, and label-free approach- AC electric field induced electro-rotation (eROT) to evaluate the drug susceptibility of cancer cells. Due to different dielectric properties of cells which are induced various toque magnitudes, resulting in different rotation speeds on different cells. After cancer cell isolation [8], eROT spectra for different drug-treated cancer cells could be used to determine the drug resistance and susceptibilities through their frequency-dependent rotation speeds, as shown in Fig. 2. Lysing cells and biochemical analysis are not required before and during eROT monitoring.

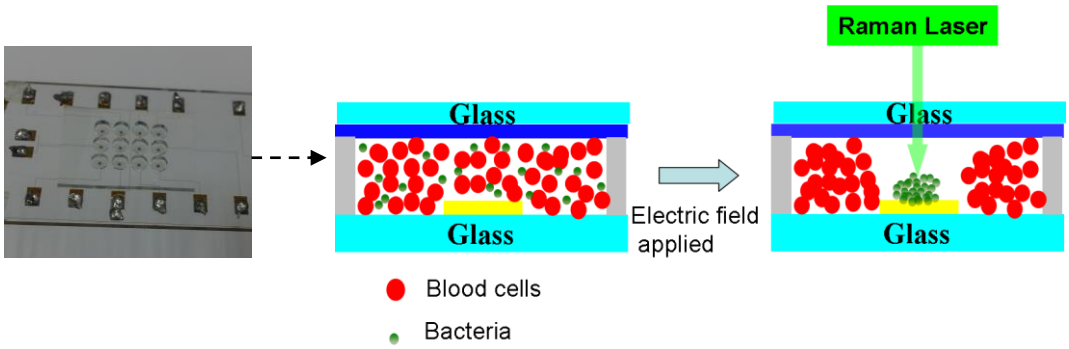


Fig. 1 Schematic illustration of the proposed biochip and its selective concentration mechanism

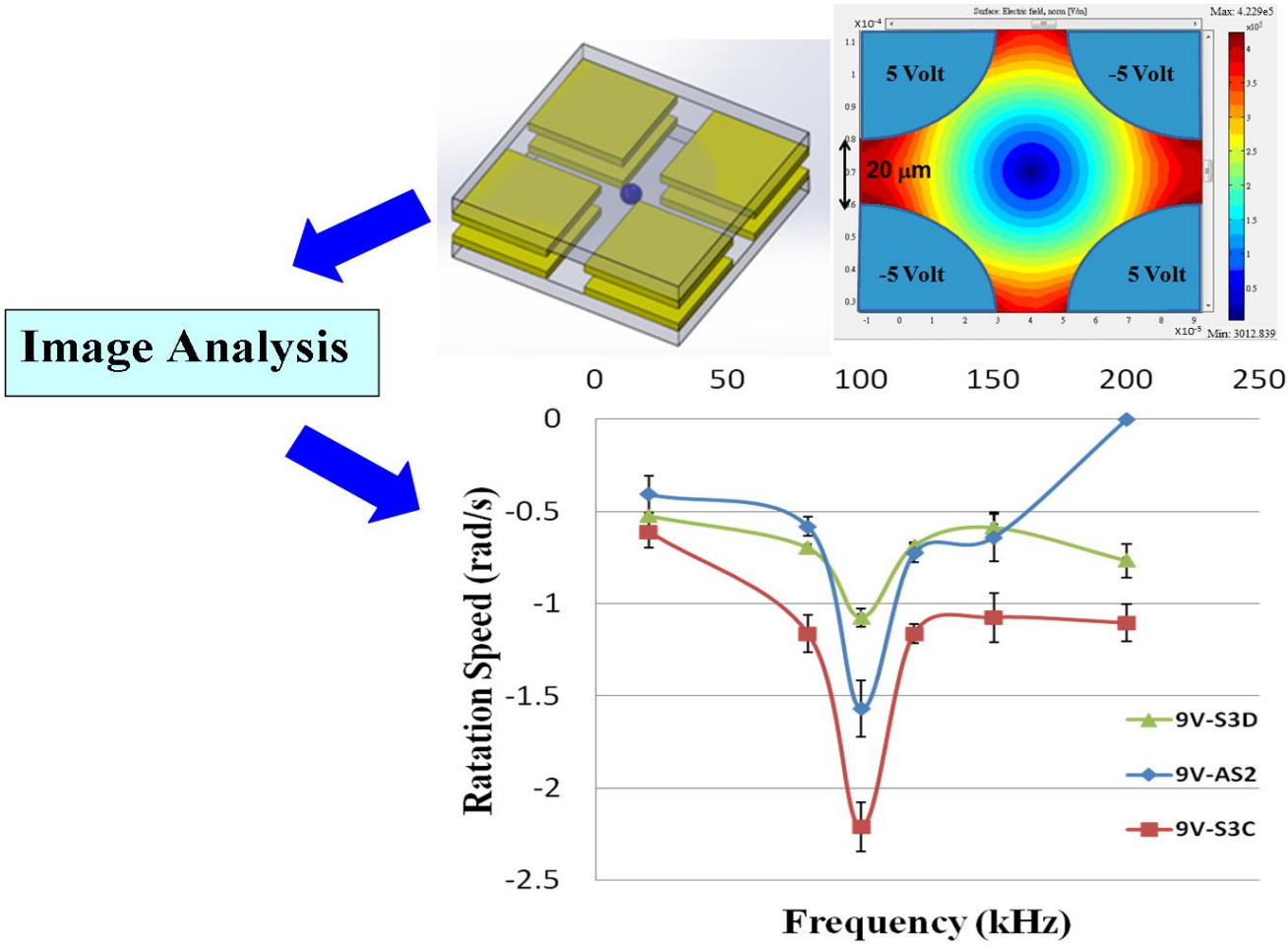


Fig. 2 Schematic illustration of eROT assay for rapid assessment of cancer cells

2. Method

2.1 Theory and Chip Designs

(a) Dielectrophoresis

The dielectrophoretic force is defined by $F_{DEP} = 2\pi r^3 \varepsilon_m \mathrm{Re}[f_{CM}(\omega)] \nabla E^2$. The DEP force is dependent on the permittivity of the medium ε_m, the radius of the particle r^3, the effective polarizability- Clausius-Mossotti (CM) factor f_{CM}, and the magnitude of the electric field gradient $\nabla \mathbf{E}^2$. If particles are more polarizable than the surrounding medium, they will be attracted to the region of relatively strong electrical field gradient (positive DEP, pDEP); if particles are less polarizable than the surrounding medium, they will be pushed to the region of relative weak electrical field gradient (negative DEP, nDEP).

(b) AC Electroosmosis

AC electroosmosis (ACEO) is caused by the interaction between a non-uniform AC electric field and the electric double layer that is induced via accumulating charges near the surface of the polarized electrode. The ion migration drags the fluid to generate a surface-driven bulk flow over the electrodes. This effect is capable of generating a long-range convection flow without selectivity for the particles with proper designs. The magnitude of ACEO velocity is dependent on the applied AC voltage V_0, the characteristic length of the electrode separation x, and the conductivity and permittivity of the medium and Debye length k. Biased ACEO combines a DC bias with AC signals to generate two electrode charging effects, capacitive charging, and Faradaic charging on different electrodes. This asymmetric polarization is capable of inducing fluid convection more effectively and could be used in a wider range of buffer conductivities to induce sufficiently high convections [6].

(c) AC Electroroation

The electro-rotation is generated from the phase gradient polarization between the electric field and its induced dipole moment. The particle could be induced through a rotation along the co-filed/anti-field direction based on the induced dipole moment lags/leads to the electric field. Electrorotation is a non-invasive tool that can be used to analyze the dielectric/physic properties of individual living cells. For a rotating electric field, the induced electric torque acting on a spherical particle is given by [9]

$$\Gamma_{ROT} = -4\pi\varepsilon_m a^3 \,\mathrm{Im}\left[f_{CM}(\omega)\right]|E|^2 \quad (1)$$

The limiting values of the real and imaginary parts of the CM factor are -0.5 to 1 and -0.75 to 0.75, respectively. Im[$f_{CM}(\omega)$] approaches zero at the high and low frequencies (approaches to the maxima values of the real part), giving rise to a sharp maximum at a resonant frequency.

Different dielectric properties of cells are induced various toque magnitudes, resulting in different rotation speeds on different cells (Fig. 2 and 3). The different dielectric properties caused various spin rates/directions which could be employed to rapid discriminate the viability of the cell without any labelling or lysing.

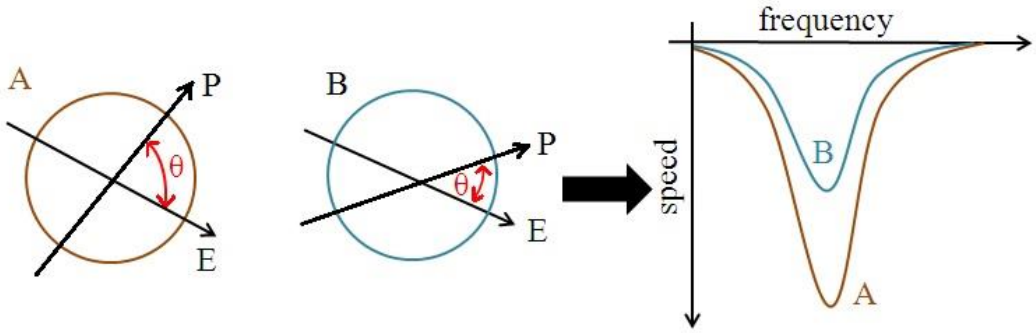


Fig. 3 The working principle of eROT for discriminating different cells

2.2 Sample Preparation

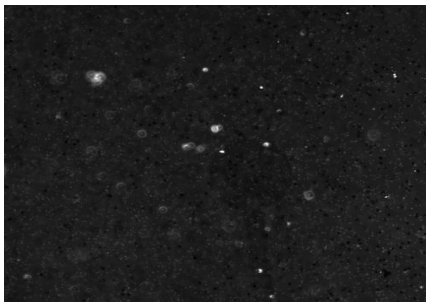
Bacteria and 30 nm-fluorescent colloids were used to investigate the selective concentrations of the micro- and nano-scale particles from the diluted blood, respectively. *E. coli* (BCRC 15922, Gram negative) was cultured on tryptic soy agar (TSA) at 35 ^{o}C for over 18 hr. 30 nm fluorescent colloids were purchased from *Sigma* company (2.5 %, solids-latex (w/w). A mixed sample including nanocolloids at a low concentration of 50 ng/mL and blood cells at a concentration of 5×10^7 cells/mL was prepared to investigate optimal voltage, frequency and the detection limit of the chip. The bacteria-blood cell mixture suspended in the experimental buffer was used for the purpose of investigating the behavior of the bacteria-blood cell separation mechanism. Bacteria and blood cell concentrations were adjusted to 2×10^5 CFU/mL and 2.5×10^8 cells/mL, respectively.

Human lung adenocarcinoma cell line PC14PE6/AS2 (AS2) was used to study the effects of P6/Jak/Stat3-siganling on the eROT response of cancer cells. These cells were maintained in MEM-α (Invitrogen, Carlsbad, CA, USA) with 10% fetal calf serum (FCS; Invitrogen) and were incubated at 37℃ in a humidified atmosphere containing 5% CO_2. For testing drug susceptibility, AS2 cells were seeded for 24 hrs before treatment and then the cells were treated with P6 (500 nM) in the indicated duration. The cells were collected by trypsin.

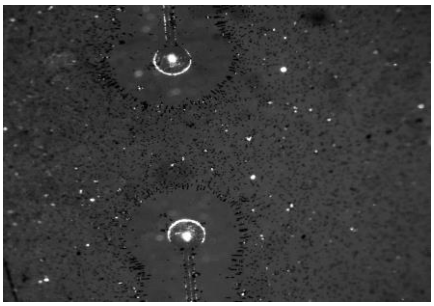
3. Results and Discussion

The mixture of RBCs-nanocolloids was dropped into the 3D chip with a wide-range electrode configuration of Fig. 1. Figure 4(a) shows that the blood cells and nanocolloids were distributed randomly when no electric field was applied to the

electrodes. The fluorescent nanocolloids were difficult to see due to the fact that the diffusion length of each molecule was longer than the length of the fluorescence scattering. Under an optimal frequency range, as shown in Fig. 4(b) for 2 kHz and after only one minute, the nanocolloids were effectively transported and concentrated to a small area of the central electrode, while blood cells were excluded from electrode edge and strongly pushed away from the centre electrode.



(a) Random distribution of nanocolloids and blood cells before electric field applied



(b) Rapid selective concentration of nanocolloids from diluted blood after electric field applied

Fig. 4 Electrokinetic concentration of nanocolloids from diluted blood

The platform demonstrated excellent separation and concentration capabilities by AC electrokinetics. The 3D electric field configuration generates an effective ACEO convection and 3D negative DEP force in the fluidic chamber. The proposed design is also used for concentrating bacteria from the diluted human blood sample and subsequent on-chip SERS identification of bacteria based on their detected bacteria fingerprints. Due to the difference in the polarizability and size of the particles, the net force at a given locality can be in the direction toward the centre electrode for bacteria and the direction away from the centre electrode for blood cells (Fig. 1 and 5). Three genera of bacteria, *S. aureus*, *E. coli*, and *P. aeruginosa* that are found in most of the isolated infections in bacteremia can be rapidly identified within five minutes by selectively concentrating target bacteria and comparing their detected bacteria fingerprints, as shown in Fig. 5.

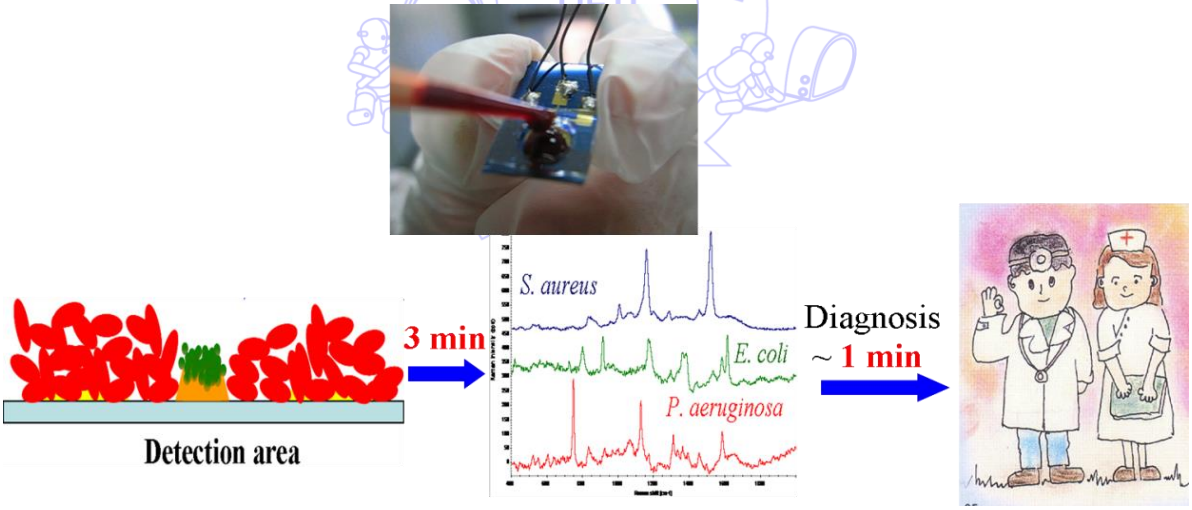
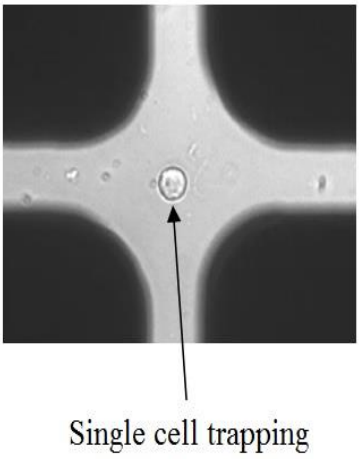
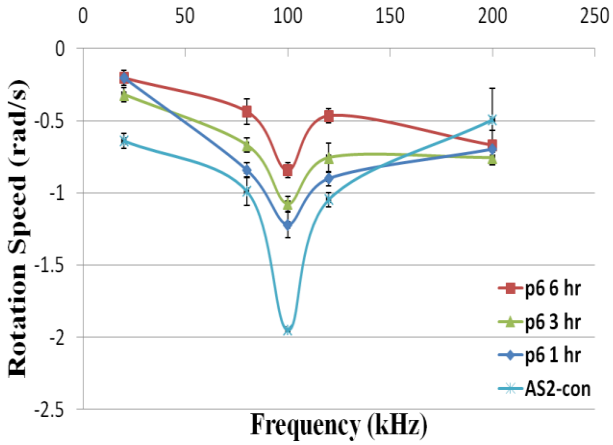


Fig. 5 The illustration of a novel biochip for rapid identification of pathogen from blood

P6 drug blocks off the Jak kinase (P6 receptor) that causes decreasing the kinase productions and inhibiting the activity of lung cancer cells. AS2 cells were treated with P6 by the dose of 500 nM for 0.5, 1, 3 and 6 hours, respectively. Firstly, the negative DEP force generated at low frequency was applied to trap single cell in the middle region (the minimum electric field region) between four phase-shift electrodes for single-cell eROT analysis, as shown in Fig. 6(a). The results of eROT spectra show that the rotation velocity has a significantly reduce from 2 rad/sec to 1.2 rad/sec after treating cancer cell with P6 for 1 hour, as shown in Fig. 6(b). Although the Stat3 is also phosphorylated, the phosphorylated Stat3 is not the function to generate the downstream productions. The tendency of the rotation velocity decreases when the treating time increases. The relationship and trend between eROT method and conventional method are very agreement. Even through the detailed correlation

mechanism between eROT and the biological is not clarified now; however, the accordance of the experimental results indicate that it is considerable to monitor cell viability with drug treatments in real time by means of the changes of rotation velocity.





(a) Dielectrophoretic single cell trapping for eROT detection (b) The eROT spectra of drug-treated cancer cells

Fig. 6 Rapid Assessment of drug response to cancer cells using eROT technology

4. Conclusions

In this paper, we demonstrate that a 3D hybrid AC electrokinetic strategy not only can separate bacteria/nano-molecues and blood cells but also it can concentrate the rare detection targets from manifold and dense blood cells. This strategy would allow an extremely high density of bacteria aggregate to be obtained for effective on-chip analysis. The appropriate situation occurred at a significant ACEO convection that was higher than the positive DEP force on the nanocolloids/bacteria and a lower ACEO drag force than the opposite DEP force on the blood cells. The couple effect allowed the selective concentration of nanocolloids/bacteria at specific frequencies of the applied voltage. The concentration factor of the proposed 3D hybrid electrokinetics device increased by several orders of local density and raised the local purity at least 6 orders (from 0.05% to greater than 99.9%). This novel microfluidic platform is also successfully used for rapid concentration of rare-pathogen from human blood for subsequent on-chip SERS identification of bacteria based on their detected Raman *fingerprints*. Three genera of bacteria, *S. aureus*, *E. coli*, and *P. aeruginosa* that are found in most of the isolated infections in bacteremia can be rapid identified by comparing their detected bacteria fingerprints. The total detection and identification can be finished within 5 minutes. Sample dilution takes 1 min, electrokinetic selective concentration takes only 3 min, and Raman measurement is quite fast (~10 sec).

After cancer cell isolation, the isolated sample can be further analyzed the drug susceptibility of cancer cells using the eROT chip. When applying an optimal frequency, cancer cell can be moved to the detection zone by nDEP force, and furthermore, it has a highest rotation speed in this frequency window. The P6 drug treated lung cancer cell line, AS2 were successfully be determined by 1 hr. This approach can also estimate the drug susceptibility of AS2 lung cancer cell treated with different P6 medicine. The correlation between ROT behaviors and drug responses has been demonstrated in agreement. Comparing to traditional laboratory detection methods, the detection time is significantly reduced from days to a few hours, because both of DEP behaviors and the speed of eROT are very sensitive to dielectric properties of cell. This assessment platform enables to significantly reduce the analyzed time and cost of cancer cell.

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