Plasmophore Enhancement in Fibroblast Green Fluorescent Protein-Positive Cells Excited by Smoke

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ABSTRACT: Considering the large consumption of nicotine and its sedative/stimulant effect on different organs of the body, the detection of low concentration of this material and its subsequent effect on live animals plays a significant role. Optical detection techniques such as plasmonics are the pioneers in highly sensitive detection techniques. However, for investigating the nicotine/smoke effect on live cells, not only the interaction between cell nicotine should be optimized but also the plasmonic interface should show a high sensitivity to the reception of nicotine by the cell receptors. In this study, the sensitivity of the plasmonic detection system was greatly increased using the coupling of plasmon and fluorophore. This coupling could enhance the main plasmonic signal several orders of magnitude besides improving Δ and Ψ ellipsometry parameters. Benefiting from the green fluorescence proteins, the phase shift and the amplitude ratio between the reflections under s- and p-polarized light enhance considerably which verifies the coupling of the dipole of the fluorescence emitter and the plasmons of the metal nanostructure. For 1 s increase of the maintenance time, we encountered a considerable increase in the Δ values that were 0.15° for $T_e = 1$ s and 0.24° for $T_e = 3$ s. Benefiting from extracted ellipsometry parameters, this study could open new avenues toward studying the effect of various types of drugs and stimulants on biological samples using a novel plasmophore platform.

1. INTRODUCTION

Nicotine, as the main component found in cigarette, has various sedative and stimulant effects on different organs of the human. The received nicotine by the brain is attracted to the nicotine receptors, which leads to dopamine release and can improve memory and concentration because of the increase in acetylcholine and norepinephrine. Norepinephrine itself increases the sensation of wakefulness or arousal. Therefore, it causes changes in the heart rate and rhythm, blood pressure, constrictions and diseases of the coronary artery, and the increased risk of stroke. There are many methods to detect nicotine from biological biofluids such as blood, urine, or saliva tests. One of the fast, precise methods is using optical methods such as the surface plasmon resonance (SPR) technique. The SPR technique, as a label-free and real-time technique, can detect the interaction between the biological samples and the metallic interface with high resolution. If a fluorophore is placed close to the metallic surface, the resonant coupling of the fluorescence signal and the surface plasmon (SP) mode increases the interaction and amplifies the fluorescence signal because of the enhancement in intensity of the electromagnetic field on a metallic surface and subsequent increase in the excitation rate of the fluorophore. Because of this coupling, plasmon-induced new peaks would appear at plasmonic resonance wavelength besides the intrinsic fluorescent peak. The coupling of the fluorophore and the plasmonic mode makes it difficult to distinguish which phenomenon causes the emission, so the coupled system has found a combinatorial name called “plasmophore” or “fluoron” firstly proposed by Lakowicz et al. The plasmon-enhanced fluorescence spectroscopy (SPFS) finds application in detection of very low concentration of the analytes and the small molecules. Attridge et al. reported the first SPFS-based biosensor, and its simplified version was then introduced by Liebermann and Knoll. Stranik et al. have reported plasmophore enhancement using adsorption and emission spectroscopies of ordered arrays of metallic nano-islands adjacent to fluorophore Cy5 dye. Using the fluorescence image pattern, Kong et al. proposed protein discrimination and plasmophore enhancement about 20 fold by employing fluorescent gold nanoparticles on plasmonic substrates. In 2018, this approach was further investigated by Sajade et al. and successfully applied for various applications. Sajade et al. reported the plasmophore enhancement using adsorption and emission spectroscopies of ordered arrays of metallic nano-islands adjacent to fluorophore Cy5 dye. Using the fluorescence image pattern, Kong et al. proposed protein discrimination and plasmophore enhancement about 20 fold by employing fluorescent gold nanoparticles on plasmonic substrates. In 2018, this approach was further investigated by Sajade et al. and successfully applied for various applications.
Zhao et al.\textsuperscript{10} have experimentally proved the enhanced light emission from the hybrid structure of gold nanoparticles and fluorescent nanodiamonds using photoluminescence spectroscopy and performing theoretical simulations using the three-dimensional FDTD method. Kreyeune et al.\textsuperscript{11} have employed the same phenomenon on individual plant light-harvesting complexes adjacent to Au nanorods. They accomplished large enhancement of fluorescence brightness up to 240 fold and emission enhancement up to 3.8 fold. Therefore, the integration of SPFS with SPR biosensors can enhance the sensitivity multiples of magnitude that leads to use of fluorescent molecules in sensing the disease biomarkers and bioimaging.\textsuperscript{3,12} While fluorescence from a molecule directly adsorbed on the surface of a metal is strongly suppressed, at a few nanometers from the metal, its fluorescence can be strongly increased.\textsuperscript{13} Interaction between SPs and fluorescent positive cells allows the enhancing fluorescence signal to attend molecular binding events by several orders of magnitude.\textsuperscript{14,15} The enhanced fluorescence intensity causes the affinity of metal nanostructures in a way that much lower concentrations of biomarkers marked with fluorescence molecules can be detected especially in sensing format or for tissue imaging.\textsuperscript{16,17} Many techniques in modern nanotechnology are explored for increasing molecular fluorescence in various applications from single-molecule sensing and biochemistry imaging to medical diagnostics and treatment.\textsuperscript{18,19}

In this paper, by benefiting from green fluorescent protein (GFP) in fibroblast cells, not only we had plasmonophere enhancement but also we detected the smoke effect on cells with different exposure \((T_e)\) and maintenance \((T_m)\) times in a highly-resolved platform. Previously, Cao et al.\textsuperscript{20} have applied graphene-enhanced microfiber resonators for high selective detection of dopamine, nicotine, and ssDNA molecules with a sensitivity of 10 mN, 0.7 and 0.2 mM, respectively. Yan et al.\textsuperscript{21} have detected nicotine in urine fluid and living cells with a sensitivity of 0.98 \(\mu\)M using fluorescence imaging and record of the luminescence intensity. To best of our knowledge, it is the first time that plasmonophere enhancement was applied for investigating the effect of smoke on cells. In this study, we have shown a good overlap between fluorescent excitation/emission of GFP and plasmonic resonance leading to plasmoaphore entity. In parallel, flow cytometry results showed the viability of the cells in the presence of the smoke. Using subsequent ellipsometry analysis, \(\Delta\) and \(\Psi\) parameters were extracted besides recorded reflections increasing the reported sensitivity. In our technique, the phase \((\Delta)\) sensitivity demonstrated that for 1 s increase of the smoke maintenance time, we encountered a considerable increase in the \(\Delta\) value that showed a bigger split between the phases under s- and p-polarized incident light. This study could open new avenues toward understanding the mechanism of smoke and drugs on the cells and organs using highly-resolved plasmosphere platforms.

2. EXPERIMENTAL SETUP AND MEASUREMENT

2.1. Fabrication of a 2D Plasmonic Sensor. In this study, a high-quality two-dimensional grating was extracted from commercial charged coupled devices of the cameras. These could be used as molds in the soft lithography procedure. We fixed these molds on glass slides, covered their four sides with hollow cubes, and sealed them with hot melt glue sticks. Poly-dimethyl siloxane (PDMS) and its curing agent with an aspect ratio of 10:1 were mixed and poured gently on the mold. For degassing, the mold was put in the vacuum for 15 min to remove the bubbles in the PDMS mixture. Afterward, the mold was heated for 1 h at an average temperature of 80 °C. After cooling down for about 24 h, we peeled off the patterned PDMS layer from its mold using a scalpel. Then, a 30 nm gold layer was sputtered on its surface. The fabricated 2D plasmonic chip had an average periodicity of 2.16 \(\mu\)m with hollow semicubic patterns. These hollow cubes had an average distance of 1.68 \(\mu\)m from each other. The schematic of the fabrication process flow is shown in Figure 1a. The scanning electron microscopy (SEM) image of the fabricated structure and the unit cell of the structure are shown in Figure 1b. This fabricated chip was ready for culturing GFP positive fibroblast cells.

2.2. Cell Culture and the Chamber. The C57BL/6-Tg (CAG-EGFP)131Osb/LeySop(J) was purchased from Jax company. For fibroblast isolation, the ear of the mouse (BALB/c transgenic mouse containing GFP genes in front of the actin genes promoter) was dissected. The pieces were rinsed with 70% methanol (Merck Co.) for 2 min following Dulbecco’s modified Eagle’s medium (DMEM) rinsing. In the next step, tissue slices were mechanically chopped with surgical blade and trypsinized at 37 C for 1 h. The solution was centrifuged and resuspended in 89% DMEM (Gibco Co.) containing 10% FBS (Invitrogen Co.) and 1% pen-strep (Invitrogen Co.). The cells were validated by hematoxylin and eosin staining. The cells were passaged after reaching 70–80% confluency. For seeding cells on chips, the chips were sterilized by UV and 75% ethanol. The chips were transferred to culture plate and immobilized. After trypsinizing and centrifugation, we aspirated the medium, added the fresh medium, and resuspended the cells. Cells were transferred directly to the chips with excess medium to cover the chips. After successful cell culture, we inserted the cultured chips in the designed chamber. The chamber was a hollow cube with closed bottom and some separate inlets/outlets for smoke and DMEM injections. The chip was placed into the open side. Then, the chip was embedded into the chamber in a way that its front face was inside the chamber in contact with the cell medium.

2.3. Theory. Spectroscopic ellipsometry is a non-invasive method for highly-resolved detection of changes in plasmonic resonance between the metal and the dielectric which provides information on both the amplitude ratio and phase difference between reflected light for s- and p-polarized incident light.\textsuperscript{22} The reflection from the back surface of the sample in the s- and p-polarization is strongly dependent on the absorption of biomolecular materials on the gold surface.\textsuperscript{23} A complex reflection coefficient of \(\rho = \frac{4 \tan \Psi e^{i\Delta}}{R_p + iR_s}\) can be obtained using Fresnel coefficients, where \(R_p, R_s\) and \(\Psi\) and \(\Delta\) are Fresnel coefficients, and ellipsometry parameters are defined as follows

\[
\Delta = \theta(p) - \theta(s) = -\frac{2\pi P}{\int_0^\infty \left[\ln\left(\sqrt{R_p(o'w') / R_s(o'w')}\right) - \ln\left(\sqrt{R_s(o'w') / R_p(o'w')}\right)\right] \, dw'}
\]

\[
r_p \text{ and } r_s \text{ refer to reflections for } p- \text{ and } s- \text{ polarizations. } \Psi \text{ and } \Delta \text{ define the amplitude ratio and the phase difference between } p- \text{ and } s- \text{polarized incident light, respectively.} \]
between a semi-infinite metal and a dielectric, SPs are propagated by the complex propagation constant \( \beta \) described as

\[
\beta = k_0 \sqrt{\frac{n_m^2 n_d^2}{n_m^2 + n_d^2}} + i \left( \frac{2 \pi}{\Lambda} \right)
\]

where \( k_0 = \frac{2 \pi}{\lambda} \) is the wave vector of light in vacuum, \( \lambda \) is the wavelength, \( n_d \) is the refractive index of the dielectric, and \( n_m \) is the metal complex refractive index. In grating coupling, diffraction on a periodically modulated surface is used to amplify the constant propagation of an optical beam to identity \( \text{Re}[\beta] \). The component of the scattered wave vector that is parallel to the grating surface changes is as follows

\[
k_{xp} = k_0 n_p \sin \theta + \frac{2 \pi}{\Lambda}
\]

where \( \theta \) is the incident angle of the light beam, \( \Lambda \) is the periodicity of the grating, and \( p \) is an integer. The parallel component of the \( k_{xp} \) can be displaced by the real constant of the SP propagation constant along the surface of the metallic grating.

\[
k_{xp} \sin \theta + \frac{2 \pi}{\Lambda} = \pm \text{Re}[\beta]
\]

Upon absorption, the fluorophore moves from basic \( S_0 \) state to the higher \( S_1 \) state and is spontaneously excited. In a free space, fluorophores can return to the ground state \( S_0 \) by releasing another photon in a higher wavelength subtraction channel with or without a single-photon emission, for example, because of accidental collision of the non-radiative decay channel. The amount of \( P_{em} \) fluorescence emission depends on the amount of \( P_e \) excitation, the amount of \( P_t \) radiation decay, and the amount of nonradiative decay of \( P_{nr} \).

\[
P_{em} \propto P_e^p P_t + P_{nr}
\]

Thus, the radiation rate \( P_{em} \) increases with increasing the intensity in the SP field.

### 2.4. Experimental Measurement

An optical setup was mounted for a fixed incident angle of 35° in order to record the reflection from the backside of the sample for s- and p-polarized incident light. This optical setup contained a broadband halogen fiber optic illuminator, a collimator, lens, Glan-Taylor calcite polarizer (GT10-A), an aperture, a sample chamber, a rotation stage, an Ocean spectrometer, a cell medium injector, and a smoke injector/withdrawer. The injection/suction was designed to pump cigarette smoke into/out of the chamber for the desired time duration. For measurement, we kept the gas knob open for 1 s and then closed it. Afterward, we recorded the reflection spectrum at 1, 60, and 120 s after the closure of the smoke flow. At the second step, the gas knob was opened and kept at open status for 3 s, and then, it was closed and the spectrum was recorded after 1, 60, and 120 s. The same procedure was repeated for the open status of 5 s, and 1, 3, and 5 s were called exposure time (\( T_e \)) and 1, 60, and 120 s were called maintenance time (\( T_m \)). Schematic of the optical setup mounted for recording the reflection of the sensing chip under various exposure and maintenance times of cigarette smoke is shown in Figure 1c. Besides recording the reflections for the cultured chips with and without fluorescence, the reflection spectrum of the non-cultured chips with the injection of smoke was recorded in various exposure and maintenance times. After recording the reflection spectrum in s and p-polarization of the incident light, the ellipsometry parameters were extracted using our written code in FORTRAN-written code, based on eq 1.
3. RESULTS AND DISCUSSION

In order to verify the structural geometry of the sensing chip and the fluorescence identity of the cells, SEM and fluorescent microscopic (Olympus fluorescent microscope) images are shown in Figure 2. The proliferation test was performed by flow cytometry with AnnexinV/PI markers. For demonstration of the live cells, the flow cytometry was carried out for three glass samples, as shown in Figure 2c–e. As seen in Figure 2F, more than 90% percentage of the cells were alive. In order to investigate similarly the 2D plasmonic chip, the flow cytometry test was also carried out for these chips with and without smoke. This test shows a significant difference between the cultured chip treated with and without smoke. The number of live cells significantly diminished under smoke from 81.65 to 47.5% and from 47.5 to 39.3%, and the majority of these cells went through the necrosis and apoptosis (Figure 3). The 2D plasmonic chip with a periodicity of 2.16 μm and a gold thickness of 30 nm was simulated using Lumerical FDTD Solutions. For increasing the accuracy, the mesh size was chosen to be 2 nm in the z-direction and 10 nm in the X–Y plane. The schematic of the unit cell and the reflection of the chip under s- and p-polarizations and their corresponding electric field distributions in the X–Z plane and at different vertical positions of the monitor (i.e., Z) are shown in Figure 4. As seen, the first plasmonic resonance in the spectral interval of 400–800 nm occurs at 454 and 476 nm for TE (s) and TM (p), respectively. After experimentally recording the reflection spectrum of the chips under s- and p-polarized incident light, the ellipsometry parameters of Δ and Ψ of nonfluorescent (Figures 5 and 6) cultured chips were recorded under exposure times of 1, 3, and 5 s and the maintenance time of 1, 6,
Figure 3. (a−c) Flow cytometry responses of the cultured GFP+ fibroblast cells on three 2D plasmonic substrates. Three times of repetition were carried out. (d) Comparative diagram of the flow cytometry responses of these samples showing the fatal effect of the smoke on the cells.

Figure 4. The simulations results of the 2D plasmonic structure. (a) Unit cell with a periodicity of 2.16 μm and a gold thickness of 30 nm. (b,c) Reflections under TM (p) and TE (s) incident light. (d) Corresponding electric field distributions in the X−Z plane and at different vertical positions of the monitor (i.e., Z).
and 120 s. As clear from Figures 4–6, there were multiple plasmonic resonances occurred due to the patterned geometry of the interface. As known, the plasmonic resonances appeared as the dips in $\Psi$ graphs and peaks in $\Delta$. There was a good match between simulation and experimental results. The main plasmonic resonances in the simulation occurred at 476, 628, and 775 nm; and in experimental results, they occurred at 495, 618, and 775 nm. Table 1 shows the spectral position and corresponding ellipsometry values of the resonances. In GFP+ cells (Figures 6 and 7), these multiple resonances vanished and there was one main plasmonic resonance dip appeared in $\Psi$ parameter, and correspondingly, one resonance peak value emerged in $\Delta$ parameter in the spectral interval of 700–800 nm. GFP can emit green fluorescence when being exposed to UV−vis light. Generally, the GFP has two excitation peaks at 395 and 470 nm and two fluorescence emission peaks at 509 nm and a shoulder at 540 nm. Therefore, the main plasmonic resonance at 476 nm in simulation (495 nm in experiment) had a good overlap with the excitation of this protein at 475 nm. In the case of fluorescent cells, the coupling of plasmonic resonance and fluorescence phenomenon occurred because of the overlap of the plasmonic resonance and the excitation wavelength of GFP.

The suppression of the multiple modes in case that we had GFP+ cells was due to this coupling and plasmophore entity not only could mutually enhance main plasmonic resonance and fluorescence emission but also could create new modes or suppress the modes previously seen for nonfluorescent cells. Table 2 shows the spectral position and corresponding ellipsometry values of the resonances for GFP+ cells cultured on the chips. Generally, it was observed that by increasing the exposure time, the phase shift ($\Delta$)
increased and the amplitude ratio decreased ($\Psi$). This means that by increasing the exposure time, much more difference in the phase of reflected light occurred between s- and p-polarized incident light. It was observed that the absolute values of $\Delta$ and $\Psi$ for plasmonic resonance increased considerably in GFP+ cells, which demonstrated the considerable effect of fluorescence and plasmonic coupling for enhancing the plasmonic resonance.

Table 1. Resonance Wavelengths and Their Corresponding $\Delta$ and $\Psi$ Values for Various Exposure (1, 3, 5 s) and Maintenance (1, 60, 120 s) Times in Normal (Non-fluorescent) Cells

| $T_e$ (normal cell) (s) | $T_m = 1$ s | $T_m = 60$ s | $T_m = 120$ s |
|------------------------|-------------|-------------|-------------|
| 1                      | $\Delta$: (518.88, 13.65), (627.02, 15.68), (820.86, 26.26) | $\Delta$: (528.01, 6.44), (635.46, 15.74), (780.53, 30.65) | $\Delta$: (517.58, 4.88), (627.44, 10.69), (785.76, 21.24) |
|                        | $\Psi$: (493.12, 36.67), (619.4, 39.91), (679.33, 42.86) | $\Psi$: (500.56, 30.84), (618.13, 30.96), (758.32, 33.49) | $\Psi$: (495.31, 33.60), (615.16, 34.28), (757.1, 35.83) |
| 3                      | $\Delta$: (528.01, 7.82), (628.71, 16.37), (778.92, 31.58) | $\Delta$: (531.49, 6.94), (626.59, 13.78), (772.07, 24.37) | $\Delta$: (530.62, 6.90), (627.86, 13.24), (751.83, 19.97) |
|                        | $\Psi$: (500.12, 30.62), (617.28, 32.07), (743.28, 33.78) | $\Psi$: (502.31, 32.55), (617.7, 33.87), (742.47, 34.44) | $\Psi$: (501.87, 32.55), (621.09, 33.49), (742.87, 35.16) |
| 5                      | $\Delta$: (529.32, 9.69), (626.17, 20.17), (778.12, 36.32) | $\Delta$: (525.41, 9.13), (627.44, 20.25), (774.9, 40.97) | $\Delta$: (534.96, 11.78), (629.55, 22.52), (776.51, 42.08) |
|                        | $\Psi$: (509.3, 27.75), (617.7, 29.07), (742.87, 31.59) | $\Psi$: (502.75, 29.38), (619.4, 29.40), (767.63, 29.95) | $\Psi$: (501.43, 25.36), (624.05, 26.92), (744.5, 29.49) |

Figure 7. Ellipsometric parameters of $\Delta$ and $\Psi$ for noncultured chips (a–c) and cultured chips with the fibroblast cells with GFP+ fluorescence (d–f). Maintenance time equals 1, 6, and 120 s under the exposure times of 1 (a,d), 3 (b,e), and 5 s (c,f).
Among the cells and smoke increased. As obtained from the graphs, the absolute value of $\Delta$ demonstrated this effect clearly. Figure 8 shows statistically the responses of the chips for air and cell interfaces. For $R_s$, the lowest standard deviation (SD) and standard error (SE) values were ($\pm 586, \pm 293$), ($\pm 379, \pm 189$), and ($\pm 579, \pm 410$) for Au/air, Au/cell, and PDMS/cell, respectively. For $R_p$, the lowest SD and SE values were ($\pm 339, \pm 169$), ($\pm 584, \pm 292$), and ($\pm 204, \pm 145$) for Au/air, Au/cell, and PDMS/cell, respectively. For $\Delta$, the lowest SD and SE values were ($\pm 2.34, \pm 1.17$), ($\pm 6.92, \pm 3.46$), and ($\pm 0.10, \pm 0.07$) for Au/air, Au/cell and PDMS/cell, respectively. For $\Psi$, the lowest SD and SE values were ($\pm 1.73, \pm 0.87$), ($\pm 0.95, \pm 0.47$) and ($\pm 2.59, \pm 1.83$) for Au/air, Au/cell, and PDMS/cell, respectively. The sample size for each plasmonic chip was four. As clear, at the interface of Au and dielectrics, the plasmonic resonance was clearly enhanced by GFP+ cells; however, it vanished when there was no plasmon excitation in the systems such as noncoated PDMS/dielectric interface. In comparison, as clear, when there were no cultured cells at the top surface of the sensing chip, there was no sharp resonances; however, in the case of GFP+ cultured chips, there were conspicuous resonances (Figure 9). These considerable resonances vanished in the case that we had no plasmons. The phase ($\Delta$) sensitivity graph in Figure 9 showed that for every second of maintenance, we encountered a considerable increase in the $\Delta$ values that were $0.15^\circ$ for $T_m = 1\ s$ and $0.24^\circ$ for $T_m = 3\ s$. This meant that there was a bigger split between the phases under s- and p-polarized incident light. We compared our technique with previously reported techniques on smoke/nicotine detection in Table 3.

Figure 10 shows the spectral position of plasmonphore resonances in $\Delta$ and $\Psi$ parameters for various $T_e$ and $T_m$. As seen, for normal cells without fluorescence, by increasing $T_m$, more smoke entered the chamber and an effective refractive index of the medium decreased because of bubbling. Therefore, there was a blue shift in the resonant wavelength. However, smoke could be more solved and homogenized in the medium with longer $T_e$ values. On the other hand, longer $T_m$ allowed better interaction of the smoky medium with the cells. For fluorescent cells, the biological interaction of the cells with the smoke showed varieties in the graphs for higher $T_m$ values. In this case, we observed blue shift because of the decreased effective refractive index of the medium by increasing $T_e$ from 1 s up to a threshold value of 3 s.

**Table 2. Resonance Wavelengths and Their Corresponding $\Delta$ and $\Psi$ Values for Various Exposure (1, 3, and 5 s) and Maintenance (1, 60, and 120 s) Times in Fluorescent Cells**

| $T_e$ (fluorescent cell) (s) | $T_m = 1\ s$ | $T_m = 60\ s$ | $T_m = 120\ s$ |
|-------------------------------|---------------|---------------|---------------|
| 1 | $\Delta$: (760.35, 38.01) $\Delta$: (759.94, 51.34) $\Delta$: (759.13, 56.50) | $\Psi$: (735.53, 37.27) $\Psi$: (733.48, 36.93) $\Psi$: (732.67, 36.58) | $\Delta$: (760.35, 38.01) $\Delta$: (759.94, 51.34) $\Delta$: (759.13, 56.50) |
| 3 | $\Delta$: (760.35, 37.02) $\Delta$: (762.78, 42.80) $\Delta$: (766.82, 65.86) | $\Psi$: (726.52, 37.40) $\Psi$: (722.68, 36.32) $\Psi$: (721.84, 35.24) | $\Delta$: (760.35, 38.01) $\Delta$: (759.94, 51.34) $\Delta$: (759.13, 56.50) |
| 5 | $\Delta$: (764.48, 48.63) $\Delta$: (768.03, 73.87) $\Delta$: (763.18, 50.80) | $\Psi$: (733.48, 37.44) $\Psi$: (731.44, 37.44) $\Psi$: (726.93, 37.40) | $\Delta$: (760.35, 38.01) $\Delta$: (759.94, 51.34) $\Delta$: (759.13, 56.50) |
Figure 9. (a) Phase sensitivity graph showing that for every second of maintenance, we encountered a considerable increase in the \( \Delta \) values that were 0.15° for \( T_m = 1 \) s and 0.24° for \( T_m = 3 \) s. (b) Comparative graph for GFP+ fibroblast cell in the presence and lack of plasmons. When there were no cultured cells at the top surface of the sensing chip, there were no sharp resonances; however, in the case of cultured chips, there were conspicuous resonances at around 550 and 640 nm. These considerable resonances vanished in the case that we had no plasmons.

| Table 3. Comparison between Detection Techniques of Nicotine/Smoke and Their Sensitivity |
|----------------------------------|----------------|------------|------------------|-------------------|
| detection method                | target material | sensitivity/concentration | Application                                      | ref   |
| chromatography/mass spectrometry | nicotine        | 0.84 μg/g       | analysis of free-base nicotine in tobacco leaf | 27    |
| electrochemical                  | nicotine        | 1.34 × 10^4 M   | aqueous and micellar media                      | 28    |
| gas chromatography               | smoke           | 23.3 μM         | environmental applications                      | 29    |
| nuclear magnetic resonance       | nicotine        | 20% synthetic in natural nicotine | food product authentication and adulteration detection | 30 |
| plasma mass spectrometry         | electronic cigarette | 0.396 μg/g for Cr | health                                         | 31    |
| optical (FP resonator)           | nicotine        | 1.24 μM         | biomedical detection                           | 32    |
| chemical                         | cigarette smoke | 0.010–1000 μM   | health, genotoxicity detection                  | 33    |
| electrical                       | nicotine        | 1000 M (DA), 0.7 mM (nicotine), 0.2 mM (sDNA) | detection of norepinephrine NEP, melatoninMEL and nicotine NIC | 34    |
| optochemistry                    | nicotine        | 10 nM (DA), 0.7 mM (nicotine), 0.2 mM sDNA (sDNA) | medical diagnosis                               | 20    |
| fluorescent imaging              | nicotine        | 0.98 μM         | detection of nicotine in urine solution and living cell | 21    |
| electrochemical                  | nicotine        | 10–200 mg/g     | natural planting environment                    | 35    |
| voltammetry                      | nicotine        | 0.01 mg/L       | liquids for e-cigarettes                       | 36    |
| omnidirectional image            | smoke           | 0.24 μM         | determination of the accurate location of a fire source | 37    |
| our method                       | smoke           | average phase sensitivity = 0.19°/s of maintenance | Health                                         | |

By increasing \( T_m \) more, the solved smoke particles increased the effective refractive index of the medium. This behavior was the same for normal cells without fluorescence. However, for longer \( T_m \) values, the behavior was different. For \( T_m = 120 \) s, the smoke and the cells had enough time for interaction, and considering the plasmophore phenomenon, this interaction could be more considerable. For this exposure time, the cells showed a red shift. The first red shift was due to the increased effective refractive index of the mixture of medium and smoke particles. The blue shift for longer exposure time was due to cell death and its corresponding volume decrease. For the values of \( \Psi \) and \( \Delta \), they behaved erratically because of the random entity of the gas and poor interaction for the normal, non-fluorescent cells. However, for the fluorescent case, \( \Psi \) and \( \Delta \) values decreased and increased for lower exposure times (i.e., 3 and 5 s), respectively. For longer exposure time (i.e., 5 s), the behavior was the same as previous up to maintenance time of 60 s; however, its behavior changed for longer maintenance time because of the cell death (Figure 11).

4. CONCLUSIONS

In this study, by benefiting from the coupling of the dipole of fluorescence emitters of GFP+ fibroblast cells and plasmons, we have investigated the nicotine-containing smoke reception by its related receptors in the cells. Using the highly-sensitive plasmonic-ellipsometry measurement technique, we have shown that the coupling of plasmon and fluorophore emission not only enhanced the plasmonic resonance response but also rationally enhanced the phase shift and the amplitude ratio between s- and p-polarized incident light which confirmed the coupling of the dipole of the fluorescence emitter and the plasmons of the metal nanostructure. For demonstrating the number of live cells before and after nicotine exposure, the flow cytometry test was performed. The effect of fluorescence in signal enhancement could be observed by comparing the optical responses of the cultured chips with and without GFP+ fibroblast cells. Similarly, the effect of plasmonics could be observed by cell culture on the metallic-coated PDMS substrate and the noncoated PDMS substrate. Both have shown the enhancement with several orders of magnitude which confirmed the effect of plasmophore in improvement of the detection signal. The phase (\( \Delta \)) sensitivity demonstrated that for 1 s increase of the maintenance time, we encountered a considerable increase in the \( \Delta \) values that were 0.15° for \( T_m = 1 \) s and 0.24° for \( T_m = 3 \) s. This increase meant that there was a bigger split between the phases under s- and p-polarized incident light. Benefiting from amplitude and phase sensitivity, we have shown...
the strong role of plasmophore resonances in investigating external stimulants such as smoke. This work could open new avenues toward studying various types of drugs in biological samples using a novel plasmophore platform.

**Figure 11.** $\Psi$ and $\Delta$ values for various exposure times (1, 3, 5 s) and maintenance times (1, 60, 120 s). Erratic behavior was seen for nonfluorescent cells. For fluorescent cells, $\Psi$ and $\Delta$ values decreased and increased for lower exposure times (i.e., 3, and 5 s), respectively. For 5 s, the behavior was the same as previous up to maintenance time of 60 s; however, its behavior changed for longer maintenance time because of the cell death.

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Notes
The authors declare no competing financial interest.

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