Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Application of Metasurface-Enhanced Infra-Red Spectroscopy to Distinguishing Between Normal and Cancerous Cell Types

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Design details for the periodic unit cells of the plasmonic metasurfaces

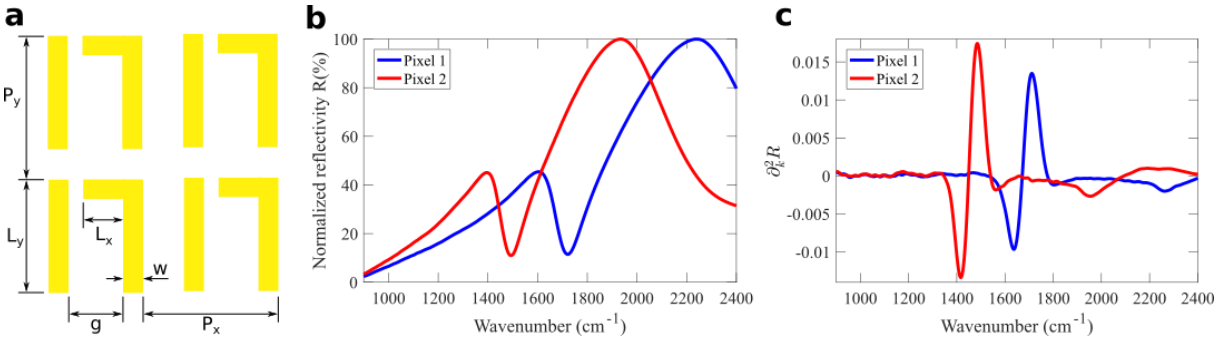

Fig. S1 (a) 2x2 array of plasmonic metasurface unit cells. Each unit cell consists of two Au antennas fabricated atop of a CaF_2 substrate. Two different scalings of the unit cells were used in this study to create two spectrally distinct arrays (pixels 1 and 2). The corresponding scalings and wavenumbers of resonances are given in Table S1. For pixel 2 (scaling factor 1), the dimensions are as follows: P_x = 3.15 µm, P_y = 2.7 µm, L_x = 0.72 µm, L_y = 1.8 µm, g = 0.99 µm, w = 0.36 µm. **(b)** Experimentally measured normalized reflection spectra $R(k)$ of both pixel types used in this work. Each pixel has the lateral dimension of $120 \times 120\ \mu m^2$, i.e. it contains more than $1{,}600$ periodic unit cells. For all practical purposes, such array can be considered infinitely large. The broad reflectivity peak corresponds to the dipole resonance (k_D in Table S1). The sharp reflectivity feature at the lower wavenumber (k_F in Table S1) corresponds to the Fano interference between the broad dipole and sharp quadrupole resonances [1], [2]. This feature is referred to as „Fano resonance“ in the main text. **(c)** Second derivative spectra $\partial_k^2 R$ of the reflectances in (b). The Fano resonance produces the largest feature in the second derivative spectra, the dipole resonance produces a much smaller feature. For both metasurfaces (Pixel 1 and Pixel 2) the Au thickness is $t = 70$ nm.

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Pixel numbers, corresponding scaling factors and resonance frequencies

Pixel no.	Scaling factor	k_D (cm^{-1}) ± 5%	k_F (cm^{-1}) ± 5%
1	0.86	2240	1670
2	1.00	1935	1450

Table S1 Pixels used in the cell sensing experiments. The columns refer to: (I) the numeric label of pixels, (II) the in-plane dimension scaling factor, (III) wavenumber of the dipole resonance (k_D) (IV) wavenumber of the Fano resonance (k_F).

Dependence of the magnitude of the Fano feature on cell coverage

A metasurface resonance is known to be a collective effect [3], [4], [5]. This means that a single pair of plasmonic antennas does not produce the sharp Fano feature that is observed for very large 2D arrays of periodic unit cells. The details of how many unit cells are necessary for a collective resonance to emerge strongly depends on the unit cell structure [4]. It is an area of active research. For antenna arrays, there is more in-plane interaction in the direction perpendicular to the antennas. For the dielectric Pi-structure [4] a 5 x 5 array of unit cells gives rise to collective effects. For the plasmonic metasurface we have used for this study, an array of 3 x 3 unit cells is thought to give rise to a collective Fano feature.

Such collective effects are likely to be strongly affected by the placement of biological cells atop of the metasurface. That is because the typical lateral size of an adhering epithelial cell is around $10 - 15\mu m$, which is of the same magnitude as the collective interaction length between the array-forming antenna pairs. For example, a given periodic unit cell of a metasurface can be covered by a cell, whereas the adjacent one may not be. That has important implications for collective interactions between the adjacent unit cells because they can no longer be considered to be identical. Specifically, those periodic units of a metasurface that have a biological cell attached to them experience a significantly higher refractive index (and as a result experience a redshift of the Fano resonance wavenumber k_F) compared to those not covered by the cells.

In order to investigate the nature of collective interactions, we have conducted experiments with a range of different cell coverages: from very sparse to moderate and large. The leading hypothesis is that the collective effects will persist for very sparse coverage, and will greatly diminish for moderate cell coverage. These considerations were fully borne out by the results of our experiments that are presented in Figure S2. As the cell coverage increases, the prominence of the Fano feature, as seen in reflectivity, diminishes. The Fano feature, in general, became weaker as cell coverage went from 1-2 cells to 20-30 cells in the analyzed region (100 μm × 100 μm area from which spectra were collected). This is clearly reflected in both reflectivity spectra (Fig.S2a) and the first-derivative spectra (Fig.S2b). For low coverage the peak corresponding to the Fano resonance is redshifted. For moderate coverage, the prominence of Fano feature is not clearly predictable solely from coverage. For large coverages, the Fano feature essentially disappears and is difficult to discern in the spectra. On the other hand, the prominence of the weaker vibrational lines (such as Amide II) steadily grows with coverage.

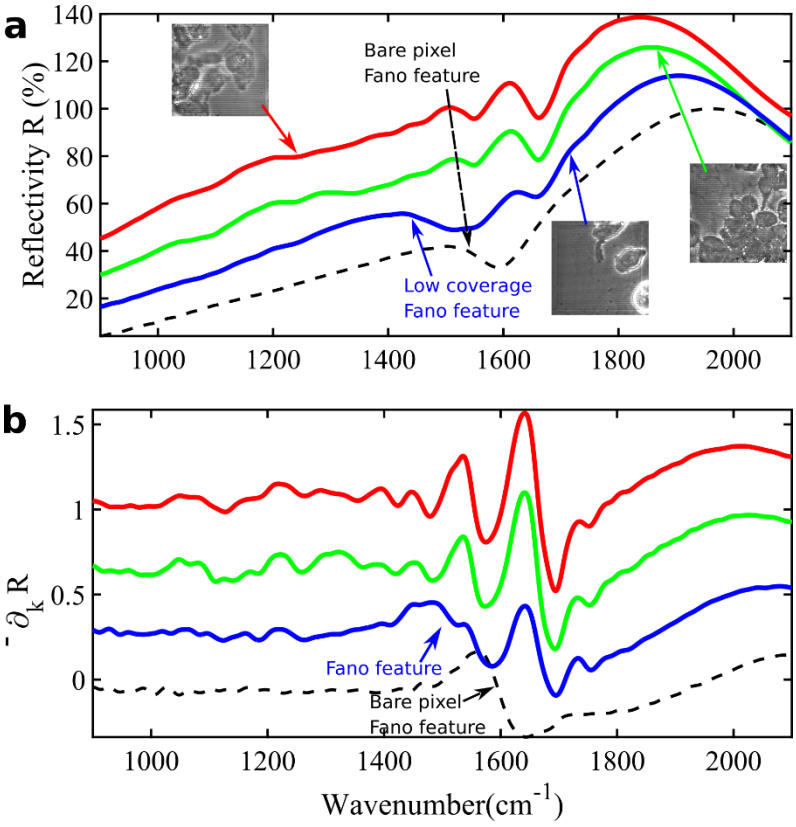

Fig. S2 Decrease of Fano feature with increasing cell coverage of pixels for four experimental runs with fixed CCD 841 cells. (a) The Fano feature in reflection spectra weakens as the cell coverage increases, apart from an expected redshift. The dashed curve shows bare spectra with no cells. All spectra are normalized so the dipole peak is 100%. The spectra are vertically shifted for clarity. (b) In derivative spectra, the Fano feature is seen to be redshifted by about $100\ \mathrm{cm}^{-1}$ for low coverage (blue line) compared to bare metasurface (dashed line). For higher cell coverages the Fano feature disappears completely. The spectra are vertically shifted for clarity. The metasurface pixels used to collect these illustrative spectra had in-plane dimension scaling of 0.92 (compared to 0.86 and 1.00 used in the rest of the study).

The reason for such behaviour is that for the metasurface unit cells covered with cells the dipole and Fano resonance frequencies (k_D and k_F) are both redshifted (by δk_i, where $i = D,F$). The dipole resonance is broad ($\delta k_D \ll k_D/Q_D$, where $Q_D \sim 3$ is the quality factor of the dipole resonance), and as a result the spectra remain similar, albeit with an overall redshift that is proportional to cell coverage. For the Fano resonance, however, the shift is comparable to the width of the Fano resonance ($k_\mathrm{F} \sim k_\mathrm{F}/Q_\mathrm{F}$, where $Q_\mathrm{F} \sim 10$ is the quality factor of the quadrupole resonance). Since the metasurface is only partially covered with cells, the Fano resonance feature is "washed out" starting from moderate cell coverage.

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