Supplementary Information

Pixeled metasurface for multiwavelength detection of vitamin D

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Supplementary Note 1: Details on geometrical parameters of the pixeled metasurfaces

The geometrical parameters pertaining to the various metasurface pixels for the CS- and SS-type designs are detailed in Figures S1 and S2, respectively. For each pixel, also shown are the numerically predicted and experimentally measured values of the resonance wavenumber (ν_{num} and ν_{exp}, respectively); a generally good agreement is observed, with differences ranging between 0.4% and 11%. The significantly larger departure (21%) observed for pixel $SS_{2,1}$ is an outlier attributable to a local substrate contamination that occurred during the final cleaning process.

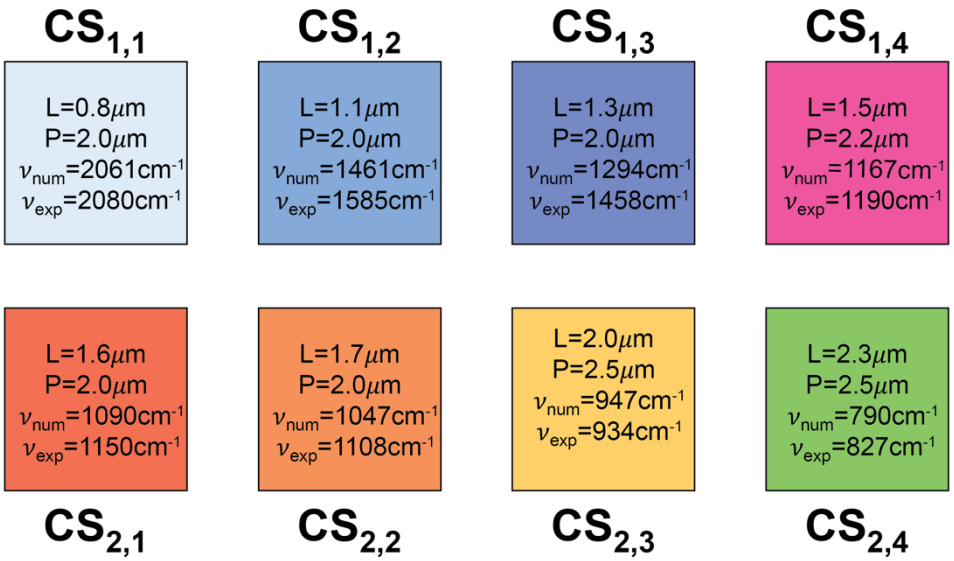

Figure S1: Geometrical parameters pertaining to the CS-type metasurface pixels. The arm width is fixed at W= 200 nm. Also shown are the numerical and experimental values of the resonance wavenumber (ν_{num} and ν_{exp}, respectively).

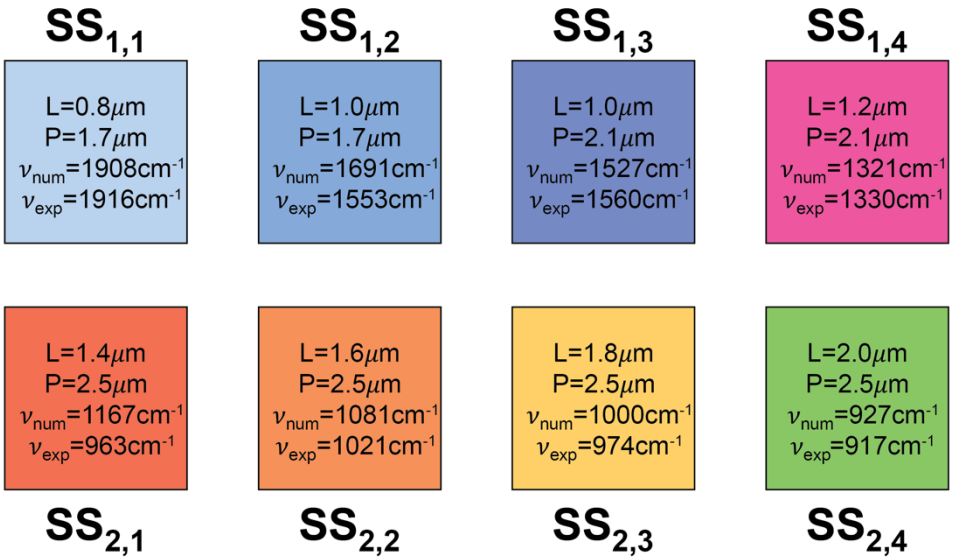

Figure S2: Geometrical parameters pertaining to the SS-type metasurface pixels. The arm width is fixed at W= 110 nm. Also shown are the numerical and experimental values of the resonance wavenumber (ν_{num} and ν_{exp}, respectively).

Supplementary Note 2: FTIR characterization of vitamin D3 and metasurface functionalization

As mentioned in the main text, the 25-hydroxyvitamin D3 (25(OH)D3) was analyzed by FTIR spectroscopy so as to assign the main vibrational bands generated by its chemical groups [S1]. To this aim, 25(OH)D3 solid samples were ground into a fine powder and analyzed by using a Jasco FTIR 4100 spectrometer, via the attenuated total reflection (ATR) technique. A film of the dry compound, from which most of the free water was removed, was placed on the ATR crystal. Figure S3 shows the SEIRA reflectance spectra pertaining to four representative pixels ($CS_{1,3}$, $CS_{2,4}$, $SS_{1,2}$, $SS_{2,4}$). The 25(OH)D3 used in our experiments was vitamin D3 25-hydroxy monohydrate (CAS Number: 63283-36-3, MW: 418.65 g/mol), obtained from LCG group (Germany). A sheep monoclonal antibody against 25-hydroxy vitamin D3 (clone 2F4) from Bioventix (UK) was used for capturing the hormone.

The amount of antibody IgG adsorbed on the whole area of the chip was estimated as follows: assuming the space occupied by a single antibody as sphere with a diameter corresponding to the maximum extension of the IgG (20 nm in our case), and considering the area exposed to the droplet as the whole area of the chip (i.e., 1 cm^2), we need 25×10^{10} IgG, which in moles amounts to 4.16×10^{-13}, i.e., ~0.4 pmol. Since the mass value of an IgG is 147,000 g/mol, this is equivalent to 61.15 ng of IgG. We prepared a solution of 130 ng of IgG in 1mL, infused the chip with 0.5 mL of solution, and assumed that 0.442×10^{-12} molecules are adsorbed on the whole chip. The minimum amount of 25(OH)D3 monohydrate which can bind to the antibody monolayer on the entire area of the device (1 cm^2), corresponding to the lowest concentration we detect (i.e., 18 pg in one droplet of 0.5 mL, or equivalently 36 pmol) is given by 0.0431 pmol (being the MW of the molecule 418.65 g/mol). Considering that the illuminated area was 100×100 μm^2, and therefore a factor of 10^{-4} with respect to the whole area needs to be considered, we conclude that we detect 4.31 amol of 25(OH)D3.

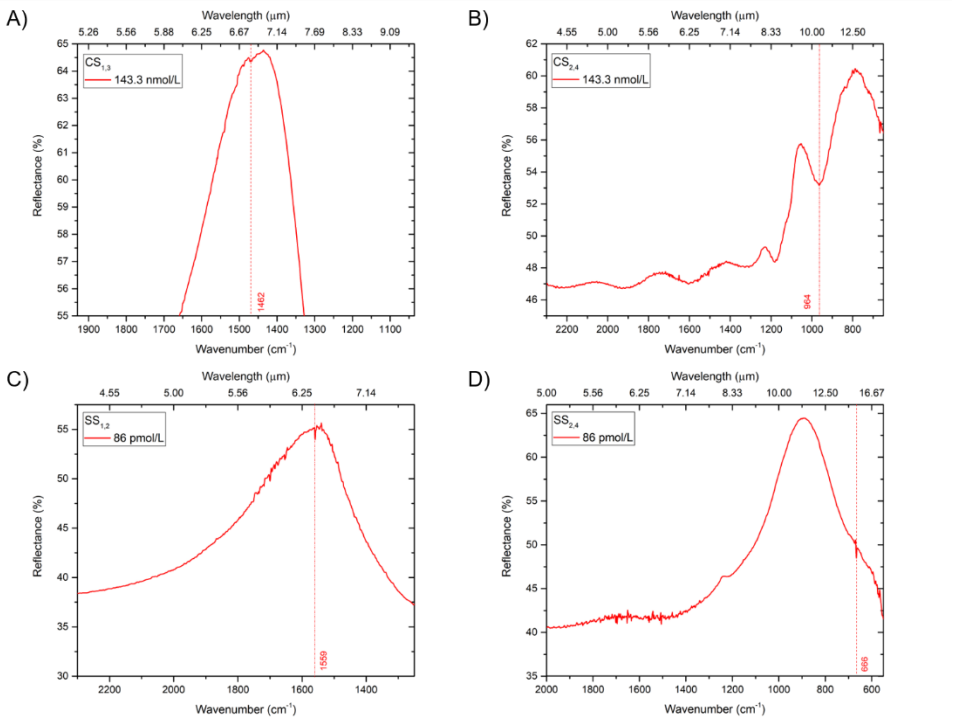

Figure S3: SEIRA reflectance spectra pertaining to representative pixels with difference concentrations of 25(OH)D3. (A) Pixel $CS_{1,3}$ (L = 1.3 μm, P = 2.0 μm, W = 200 nm) with 143.33 nmol/L concentration. (B) Pixel $CS_{2,4}$ (L = 2.3 μm, P = 2.0 μm, W = 200 nm) with 143.33 nmol/L concentration. (C) Pixel $SS_{1,2}$ (L = 1.0 μm, P = 0.7 μm, W = 110 nm) with 86 pmol/L. (D) Pixel $SS_{2,4}$ (L = 2.0 μm, P = 2.5 μm, W = 110 nm) with 86 pmol/L concentration.

Supplementary Note 3: Details on the EF Estimation

In Eq. (1) of the main text, A_{SEIRA} denotes the effective NA area on which the field is localized. As reported in the literature [9,17], and as can be observed in Figures 1E and 2E, the field is mainly localized on the tips of the NAs arms. For the CS-type geometry, we estimate the active area for each NA as the sum of four semi-circles with 200 nm diameter (see Figure S4A for illustration), i.e., $A_{NA} \approx 6.28\times10^4$ nm^2. Depending on the value of the period, this yields different values of the active area; for instance, we obtain $A_{SEIRA} = 1.6\times10^8$ nm^2 for P = 2 μm, and $A_{SEIRA} = 10^8$ nm^2 for P=2.5 μm. Recalling that the area of the NAs exposed to the light is $A_0=10^{10}$ nm^2, the ratio A_0/A_{SEIRA} (also called geometrical enhancement factor) for these two cases is 64 and 100, respectively.
For the SS-type geometry, we estimate the active area for each NA as the sum of 8 semi-circles with 120 nm diameter (see Figure S4B for illustration), i.e., $A_{NA} \approx 4.5\times10^4$ nm^2. Accordingly, we obtain $A_{SEIRA} = 7.3\times10^7$ nm^2 for P=2.5 μm, and $A_{SEIRA} = 1.6\times10^8$ nm^2 for P=1.7.μm, corresponding to geometrical factors of 68 and 142, respectively.

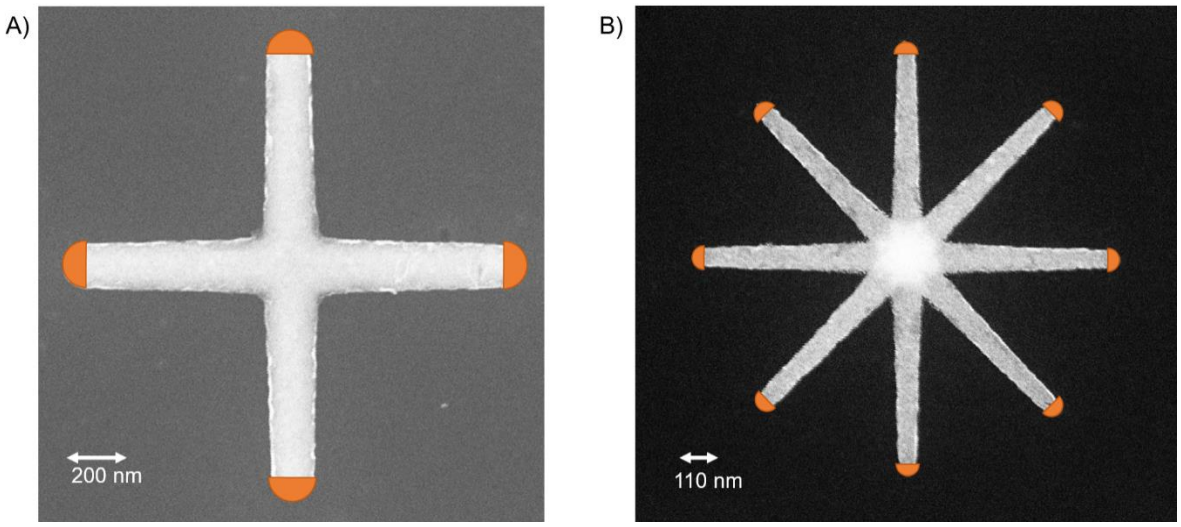

Figure S4: (A), (B) Scanning electron micrographs of $CS_{2,4}$ and $SS_{2,4}$ NAs, respectively.

Supplementary References

[S1] Coates J, Interpretation of infrared spectra, A practical approach. In: Meyers, ed. Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation. New York, USA, John Wiley & Sons Ltd, 2006.