SUPPORTING INFORMATION

Single-cell photonic nanocavity probes

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Supplementary Note I – Device assembly

Figure S1 – Illustration of device assembly. Once semiconductor templates have been fabricated they are aligned underneath a microscope for positioning. A droplet of epoxy is coated onto the edge of a fiber using a sharp electrical probe tip (I). The fiber is then aligned parallel to the membrane using a micropositioner and then lowered into contact with the suspended membrane (II). After a brief pause to allow for adhesion, the fiber is retracted away, carrying the template with the beam at the tip (III).

Templates were fabricated as described in the Methods, and contained small tabs or bridges which connected the templates to the surrounding substrate. The non-nanobeam portion of the template was perforated with large holes to allow for the wet etch to release the membrane. Template sizes, shapes, and tabs were optimized to prevent membrane collapse upon undercutting. Methods to tear away the membrane without breaking the structure were also optimized. It should also be noted that such large undercut structures will exhibit strong bowing effects due to built-in strain¹. This causes templates to warp significantly; however once the templates are torn away the strain is removed and the membranes relax. We used ITW Devcon 5 minute epoxy for the bonding process. This whole process works for silicon membranes and is likely to also work for any semiconductor or dielectric material with expected differences in material flexibility and brittleness.

Figure S2 – SEM pictures of constructed probe devices. **a**, View of the backside of the membrane bound to the fiber edge. The membrane lies flat along the outside of the fiber with minimal bending or undulation. **b**, Zoom-in image of the epoxy bond joint where the membrane is attached to the fiber. Also seen is a leftover template tab. **c**, Close-up of the edge of the membrane showing MBE-grown oval defects as well as the three embedded QD layers. **d**, Intermediate zoom image of the device from Figure 1b. **e**, A second probe device imaged from a different angle, also displaying a clean construction. **f**, Zoom-in of **e**, showing the nanobeam portion with cleanly defined holes with an evident taper defect cavity. Scale bars: 50 μm (**a**), 2 μm (**b**), 1 μm (**c,f**), 10 μm (**d**), 20 μm (**e**).

Template structures ranged anywhere from 200-300 microns long, with a little more than half overlapping the fiber for structural support (Fig. S2a). Once the epoxy cured, the structures were very stable and would never detach in solution, a critical necessity for any kind of chemical functionalization or sensing. Even if the epoxy swelled in aqueous solution, it was never noticed since the joints are far from the nanobeam. As seen in Figure S2c, our GaAs membrane has numerous pit-shaped defects which we believe are due to oval defects incorporated during MBE growth of the material². These pits are located everywhere and in conjunction with dry etch surface roughness limit our cavity quality factors to a few thousand. Nonetheless, for protein sensing with wavelength shifts of 2-4 nm, a quality factor of 1,000 or greater is more than sufficient for detection.

Supplementary Note II – Probe insertion

Figure S3 – Details of cell probing and beam flexibility. **a**, Image of a nanoprobe hovering above the dish surface. The template is defocused and it is hard to see details of the membrane (e.g. the through holes). **b**, Image of a nanoprobe with the membrane flush against the petri dish. The template is in focus and it is possible to see the large one micron-sized holes. **c**, Image of a probe in a cell with the focus placed on the top surface of the membrane, which is known to be on the substrate. **d**, Here the focus is

placed on the top surface of the cell, which is several microns above the substrate surface. The beam is obscured, indicating the beam is inside the cell. **e, f**, Flexibility testing of the nanobeam by deflecting it with a 200 nm wide tip Tungsten electrical probe. Scale bars, 20 μm .

Video S1 – Live insertion of a photonic crystal cavity inside a single PC3 cell. The video clip shows a typical experiment of probing a single cell with the device by sliding it in sideways and retracting out sideways. The cell deforms both upon entry and exit of the beam.

The process of probing a cell begins by bringing a mounted probe into the field of view of the microscope. Since the probe is mounted at a 30-40 degree angle from the substrate, the beam is pointing down when the device is hovering over the substrate. Therefore the membrane is defocused and it is hard to see any features (Fig. S3a). When the device is lowered, the membrane flexes and smoothly lies on the substrate surface as shown in (Fig. S3b), where now the features of the template are in focus. The probe can then be slid sideways until it punctures a cell from the side as seen in Figure 1e.

We know that our beams go inside rather than on top of the cells by varying the focus of the image. If we focus on or near to the substrate we see that the beam and membrane are defined well (Fig. S3c); however, when we focus on the top of the cell we find that the beam is no longer clearly seen (Fig. S3d). Experimentally, there are additional cues for when the beam has successfully penetrated a cell. Upon entry, the cells typically indent until the beam is inside at which point they elastically relax back to their original shape. Upon exit, the cell edge protrudes and sticks slightly to the beam until it lets go and elastically relaxes to its original position. These effects can be seen in Supplementary Video S1.

The GaAs material is extremely flexible as seen in Figures S3e-f. For such thin membranes and for such narrow beams, the GaAs membrane can bend significantly before any breakage. Nonetheless, the material is stiff enough to penetrate the much softer cells.

Supplementary Note III – Material properties

Figure S4 – Laser induced photo-oxidation of GaAs in water. SEM image of a large GaAs membrane with a central two-dimensional PC cavity. The sample was submerged in water and irradiated with a continuous wave 830 nm laser with pump power less than 1 mW for ten minutes. The GaAs is clearly damaged and is flaking off in large chunks in the region where the laser pump was. Scale bar, 2 μm .

Figure S5 – Laser oxidation of cavity inside a cell. a-c, Sequence of spectra for a nanoprobe that was not coated with a nanolaminate inside a cell for various time points. **a**, Initial spectrum of cavity inside cell. **b**, Spectrum of cavity one minute after insertion. **c**, Spectrum of cavity two minutes after insertion. The fringes on the background emission are from Fabry-Perot reflections off of the fiber face.

Gallium arsenide has been shown to be susceptible to laser-induced oxidation in dry air environments³. This has been used in the past to fine tune PC cavity resonances by controlled amounts. Typically for this to occur the laser pump power must be in the 10s of mW to sufficiently heat the material and catalyze the oxidation reaction. We found that when our GaAs was submerged in an aqueous environment, the threshold for observing oxidation was drastically reduced, and even pump powers as low as 100 μ W could significantly oxidize cavities in minutes (Fig. S4). This causes rapid blueshifting of a cavity wavelength (as seen in Fig. S5) since the GaAs is converted into oxide which has a lower refractive index.

Since heating is unlikely in such a thermally conductive medium and with such low pump powers, it is likely that some photochemical mechanism promotes the oxidation reaction. Laser-driven oxidation in water was also observed in Ref. 4. We find that in other solutions such as isopropanol or acetone, which are devoid of oxidizing water molecules, NO material degradation or wavelength shifting is observed, reinforcing the suggestion that oxidation is the primary factor.

In order to combat this detrimental effect, we first tried using an alumina only barrier; however we still observed rapid oxidation, in agreement with former findings of alumina not serving as a barrier to dry oxidation⁵. We turned to the recently developed encapsulation strategy of an alumina/zirconia blend coating. This alumina/zirconia nanolaminate is superior to neat alumina because it suppresses the formation of microscopic voids and nanocrystals much more, thereby limiting permeation pathways and serving as a diffusion blocking layer. The nanolaminate coats all surfaces, including the interior of PC holes, because the plasma ALD deposition is conformal⁶. In our application, this diffusion blocking layer prevents the reaction of water molecules with the GaAs surface and prevents laser-assisted oxidation. Coated cavities can be pumped in water solution for long periods of time with no degradation (we have tested for several hours and have seen no changes). We also note that cavities redshift 10-20 nm after the nanolaminate coating is applied depending on the layer thickness. The subsequent 26 nm redshift from Fig. 2d is due to the higher refractive index water surroundings and is in addition to the first 10-20 nm imposed by the nanolaminate.

Supplementary Note IV – Optical properties

Comparison of relative collection efficiencies of light from our fiber probe versus a standard microphotoluminescence setup shows that we actually have better coupling of light through the fiber. The probe spectrum in air from Figure 2b was pumped with 150 μW through a 100x objective with a spot size of 2 μm . Meanwhile the probe spectra in the cell and cell medium were pumped with 450 μW of laser power through a 20x objective with a 10 μm spot size. Therefore pumping is approximately 25 times more efficient with the microphotoluminescence setup for the micron-scale cavity. Meanwhile Figure 2 shows the collection intensity is about three times greater for the cavity in air for a pump power that is three times lower (the same integration time was used). Therefore, after normalizing by the pumping efficiency, the fiber-based collection is actually 2.8 times greater. This value can likely be easily increased by using larger multimode fibers, further optimizing the fiber-cavity angle and distance, or by engineering the radiation emission pattern from the cavity.

The quality factors of our cavities do not seem to be affected by the reduced index contrast of solution environments, nor by the plastic cell substrate. Ordinarily one would expect the quality factors to drop in both of these circumstances due to the smaller photonic bandgap of the cavity in water as well as the leakiness of radiation into the substrate. In our case, our cavities already have a Q factor of only 2,000 due to fabrication roughness and QD absorption. Therefore this Q loss dominates the overall Q factor and the solution/substrate environment does not make a substantial difference. In fact, as noted, the Q factor slightly increases in the water environment, likely due to the fact that longer wavelength emission is absorbed less by the wetting layer and by shorter wavelength QDs. In the future, it may be possible to achieve ultra-high Q values in this low index environment by using alternative optimized cavity designs⁷.

Also, we only observe a small, 0.2 nm wavelength difference between the nanocavity placed in cell medium versus inside of a cell. The refractive index of cell medium is 1.35, while the refractive index of cytoplasm is between 1.35 and 1.38, depending on the protein content⁸. Therefore because the refractive index contrast is so low between the two states we only observe a minor wavelength redshift.

Supplementary Note V – Cell viability assay

Figure S6 – Control samples for cell viability. **a**, Phase contrast image of healthy cells that were not modified. **b**, Phase contrast image of dead cells that were treated in methanol for 1 hour. **c-d**, Greyscale images of green fluorescence from the respective above panels. **e-f**, Greyscale images of red fluorescence from the respective above panels.

Images for control dead and alive samples are shown in Supplementary Figure S6. Healthy cells exhibit a bright green fluorescence and minimal red fluorescence while dead cells exhibit low green fluorescence and bright red fluorescence, particularly from the nuclei. For cells probed with a nanobeam cavity, we typically observe that after washing with reagents, those cells that are dead or dying will detach from the substrate and wash away. All cells probed or loaded with a nanobeam that remain on the petri dish are viable, as measured from fluorescence.

Supplementary Note VI – Beam loading

Figure S7 – Process of loading cells. **a**, A beam is inserted into a cell as normal. Then a second micromanipulator brings a sharp tungsten electrical probe tip into the field of view. **b**, Pressure is applied from the metal tip onto the joint between the nanobeam handle and the nanobeam, causing the nanobeam to snap off. The triangular mark in the picture is a scratch mark in the petri dish from the metal tip. **c**, The fiber is retracted revealing the untethered beam. **d**, Both probes removed with the end result of a loaded cell. Scale bars, 20 μm .

The cell loading process is as depicted in Supplementary Figure S7. A tungsten metal probe tip is used to snap off the beam by applying pressure to the joint where it is connected to the rest of the template. Since the beam is about half a micron wide, this break is easy to accomplish. Nanobeams of any size can be inserted or loaded into cells. The length of the cavity on the beam is about six microns, which is determined by the number of holes we patterned (20). Therefore much smaller beams can be inserted in cells. Also, by eliminating a few hole periods or by scaling the wavelength down the cavity could be made as small as a few microns long.

Figure S8 – Tracking loaded cells. **a-c**, Orientation tracking of a single cell with a nanobeam inside it. This cell is different from the one in Figure 4. The nanobeam is seen to rotate at various time points. Times are at 30, 51, and 54 hours post beam insertion for **a**, **b**, and **c**, respectively. **d**, Cell from Figure 4 immediately after beam loading on day 1. The beam is only partially penetrating the cell. **e**, Same cell three hours later. The beam has been completely internalized by the cell. **f**, Same cell 4.5 days after initial beam loading. The cell is undergoing a second mitotic division. The cell has also migrated considerably from the initial loading location and the reference scratch mark is visible to the right. Scale bars, 20 μm .

Unique to our study is the observation that cells can survive and proliferate even with large nanoprobe inside them. As seen in Figure 4 and Supplementary Figure S8f, cells can divide and produce normal new cells with one of the cells containing the beam. We find that 44% ($n = 9$) of cells that are loaded last three days or more and some as long as eight days. The question of how normal these loaded cells are requires further investigation with gene expression profiling. It is likely that some cellular activity is modified by the presence of the large probe; however, as mentioned above the probe can be shrunk in size by a factor of 3-5 in the future. In any case, by remaining in the cell the probe can be a useful reporter of optical information for various sensing applications over a long period of time.

Figure S9 – PL in loaded cells. **a**, PL of a cavity probe before loading a single cell when pumped in air. **b**, PL of the same cavity probe after being broken off and loaded into a cell.

We also examine the cavity properties after being loaded into a cell, no longer tethered to the original template and fiber. Figure S9a shows a spectrum of a cavity probe prior to cellular insertion. We then proceed to break off the nanobeam cavity from the full GaAs template according to the procedure outlined above. We measure the spectrum of the internalized nanobeam four hours later using the original multimode fiber as a collection optic. The only change in the nanobeam during this period is a slight rotation due to cell movement. Figure S9b shows this spectrum revealing once again that our cavity properties remain robust and the only major change is a redshift due to the higher refractive index water.

Figure S10 – SEM pictures of loaded and normal cells. **a**, 45-degree perspective of the cell from Figure 4e. **b**, Close-up of **a**, showing nanobeam holes disappearing into the cell. **c**, 45-degree perspective of the cell from Figure 4d. **d**, Close-up of **c**. **e**, Picture of a normal cell that was dehydrated by critical point drying. **f**, Picture of a normal cell that was dehydrated by HMDS. Scale bars: 5 μm (**a**, **c**, **e**), 2 μm (**b**, **d**), 20 μm (**f**).

Our loaded cells exhibit very similar morphology to non-probed cells. Supplementary Figures S10e-f show two cells that were not probed for samples that underwent critical point drying and HMDS drying, respectively. In both cases the cells look very much like those that were loaded. As expected, the cells dried by HMDS flattened out considerably compared to those dried by critical point drying. There is significant organic debris scattered around the GaAs material and this is likely due to the fixing/dehydration process. For accurate sensing, one would want to minimize the non-specific sticking of material and this will be possible in future experiments through successive washing steps.

Supplementary Note VII – Protein detection

Figure S11 – SEM pictures of a stuck bayonet-type nanoprobe device. The membrane is seen here bent completely by 90 degrees from fiber edge to fiber facet. The bumps visible on the membrane are chunks of AlGaAs from the sacrificial layer which occasionally get stuck to the GaAs during processing. Scale bar, 50 μm .

Nanoprobes consisting of the original spear or bayonet-shaped GaAs template do not work for chemical or protein sensing because they are mechanically compromised after one retraction from aqueous solution. When the probe is pulled out of water, capillary forces cause the membrane to stick to the fiber facet as the water evaporates as shown in Supplementary Figure S11. Interestingly enough, the membranes do not break because they are so flexible, but are otherwise rendered useless for subsequent activity. The alternative design shown in Figure 5a, however, is robust against mechanical buckling and works just fine for repeat solution immersions.

Figure S12 – Organic layer film thicknesses. Plot of organic layer thicknesses as measured by ellipsometry for the chemical reaction steps.

We measure film thicknesses of each organic coating step by performing ellipsometry on a control chip of silicon that has undergone the same chemical reaction steps as the GaAs chips and is also terminated in a silica-ALD layer. We model the entire SiO_2 /organic layer stack as SiO_2 due to the similar refractive indices, and are consistent throughout to get the plot shown in Supplementary Figure S12. Measured thicknesses have reasonable values ranging from 10-25 Å for the organic monolayers, similar to those reported in prior literature. For the photonic devices, we can measure the cavity wavelength after each chemical step and find that the redshift follows proportionally with the amount of organic deposited (e.g. the redshift after 1 nm of biotin deposition is about half the value of redshift after 2 nm of SA deposition).

One difference that we did observe was that the redshift after aminosilanization for the nanoprobe device was significantly greater than from regular chips. In fact we measure a redshift of about 20 nm for nanoprobe devices versus a few nm for chip devices indicating a much thicker APTES layer. This is not surprising because APTES tends to polymerize surfaces and grow thicker layers with either increasing reaction time or faster kinetics^{9,10}. Here we react for the same time but speculate that the chemical reaction kinetics for the nanoprobe are much greater than for solid substrates. Likely, there is increased diffusion of silane molecules to the very exposed probe in solution compared to a flat, two-dimensional chip surface. Meanwhile, the biotin and SA addition steps did not show an increased reaction speed for the nanoprobe because these steps are mostly self-limiting to begin with.

Figure S13 – Label-free sensing for many devices. Scatter plot of 12 nanobeams from two GaAs chips that were biotinylated and not biotinylated to test for magnitude of specific and non-specific binding redshifts.

Supplementary Figure S13 shows the statistics of many devices on two chips for the Streptavidin detection. Results are very consistent and show similar peak shift values with an average of 2.57 nm for specific binding and 0.45 nm for non-specific binding. Values were calculated by fitting the peaks with Lorentzian profiles.

It is very important to have reproducible results in order to accurately determine wavelength shift information. The majority of label-free sensing from photonic cavities is in passive transmission measurements, typically in silicon material¹¹. There are no ambiguities about heating or charge-based wavelength shifting since there is no optical absorption in transparent cavities. In our case, we use an active photoluminescence measurement which does involve optical absorption. With regard to heating, we find that if we keep our laser pump powers at 50 μW or below (measured before the objective), then heating is a quite negligible. Considering that we have 30% lens losses, 30% reflection loss, 25% spot size/beam area overlap, and 23% pump absorption for 220 nm thick GaAs, the total amount of absorbed pump power is only 1.4 μW , which is not expected to cause any device heating¹. Meanwhile, the steady-state electron and hole carrier densities is expected to be less than 10^{16} cm^{-3} due to the high non-radiative surface recombination, corresponding to a free-carrier dispersion blueshift of less than 0.1 nm (Ref. 12). Therefore, neither heating nor charge-carrier effects influence our wavelength measurements and our active PL measurement gives an accurate reading of the cavity refractive index environment for label-free sensing.

The amount of biomolecules captured can be approximately quantified using the following formula¹³:

$$\sigma_P = \delta\lambda \frac{\varepsilon_0 \lambda}{2\pi\alpha_{ex}\sqrt{n_m^2 - n_s^2}} \frac{n_s^2}{n_m} \frac{1}{S} \quad (S1)$$

Where σ_P is the surface density of biomolecules, λ is the wavelength of the cavity, $\delta\lambda$ is the cavity wavelength shift, ε_0 is the permittivity of free space, α_{ex} is the excess polarizability of the molecule, n_m is the sensor refractive index, n_s is the analyte medium refractive index, and S is the bulk refractive index sensitivity of the sensor. An additional factor accounting for the geometry of the PC resonator could be included here but the above equation gives an accurate estimate of bound molecules. For the nanoprobe device measured in Figure 5f, we have $\lambda = 1,254$ nm, $\delta\lambda = 3.7$ nm, $n_m = 3.5$, $n_s = 1$ (air), $\alpha_{ex} = 4\pi\varepsilon_0(3.2 \cdot 10^{-21})$ cm³ (from Ref. 11), and $S = 78$ nm/RI (from the 26 nm wavelength shift of the cavity in water in Figure 2). Equating, we find the surface density of molecules to be $2 \cdot 10^{10}$ mm⁻², or one protein molecule for every 50 nm². Considering the size of streptavidin¹⁴ to be approximately 5.5 nm, this indicates a fairly good coverage of 60%. Of course, a more thorough surface study is necessary to confirm the details of protein binding.

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