Electronic Supplementary Material (ESI) for Analyst.
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Supplementary Information

A smartphone biosensor based on analysing structural colour of porous silicon

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S1. Smartphone measurement procedures

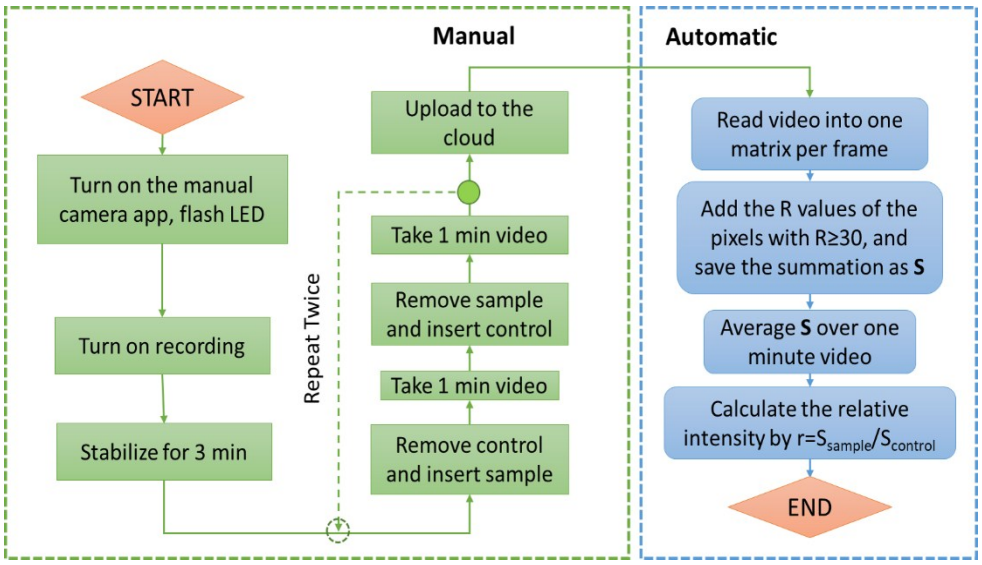

Figure S1. Test procedure of the smartphone. The left side is done manually and the right side could be fully automated.

Figure S1 provides an overview of the procedures used for the smartphone measurements. First, the control sample, a bare piece of silicon, is placed inside the 3D printed box in the designated position below the smartphone camera. The smartphone manual camera app is then turned on, the LED flash is set to 100%, and the settings for the camera focus, zoom, ISO, speed, exposure value, and white balance are adjusted to ensure that the image is not saturated. These settings are then fixed throughout the measurements. A video is then initiated with the control sample under the camera and recording continues for about 3 minutes until the light source is stabilized and the intensity fluctuations are significantly reduced. Next, the control sample is removed and the PSiM sample is inserted into the platform, and a short video of approximately 1 min duration is taken. Then the PSiM sample is removed, the control sample is placed back into the 3D printed box, and a short video of approximately 1 min duration is taken to be used for normalizing the intensity data from the PSiM sample. The PSiM and control samples are iteratively measured a total of three times to evaluate the stability and repeatability of the system.

In order to obtain and process the data from the smartphone, the videos are uploaded to a computer and read by a MATLAB code that turns each video into a series of RGB images (24 frames per second). Each frame comprises three matrices containing R, G, and B intensity values, respectively, for all the pixel locations. The R values of pixels with R≥30 are summed together as ***S*** for each frame of the video with the exception of a few frames at the beginning and end of the video. Then, an average *S* value for each video (S_{sample} and $S_{control}$ for the PSiM and control sample, respectively) is determined by calculating the average *S* value for approximately 500 frames whose *S* value falls within the 30^{th}-70^{th} percentile of all values within the 1-minute video. The relative intensity measured by the smartphone is calculated by taking the ratio of S_{sample} and $S_{control}$, as given in Eq. S1. The standard deviation (σ) is calculated based on consideration of the three independent measurements of S_{sample} and $S_{control}$ taken for each PSiM preparation condition (e.g., each APTES exposure or each streptavidin concentration), as given in Eq. S2.

Relative intensity:
$$r = \frac{S_{sample}}{S_{control}} \tag{S1}$$

Standard deviation:
$$\sigma = \sqrt{\frac{1}{3}\sum_{i=1}^{3}(r_i - \bar{r})^2} \qquad \text{where} \quad \bar{r} = \frac{1}{3}(r_1 + r_2 + r_3) \tag{S2}$$

S2. Bulk refractive index spectral sensitivity of PSiM

Figure S2 shows a linear relationship between the spectral shifts of the PSiM measured by the spectrometer after adding different concentrations of glucose solution (0 – 50 g/L). The refractive indices of the glucose solutions with different concentrations are determined based on data reported in Ref. 1. A linear fit to the data suggests that the RIU sensitivity of the PSiM is approximately 350 nm/RIU.

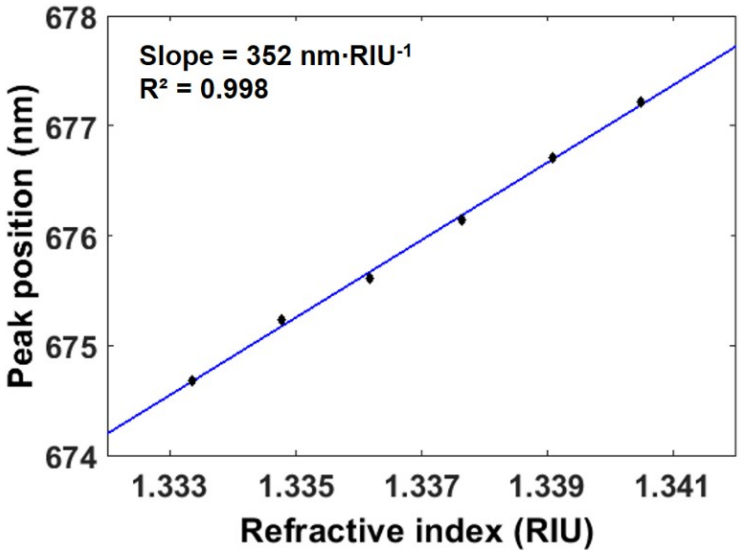

Figure S2. Shift of PSiM reflectance (measured at peak wavelength on long wavelength side of resonance) after adding different concentrations of glucose solution, as measured by a spectrometer. A linear fit is shown (blue line).

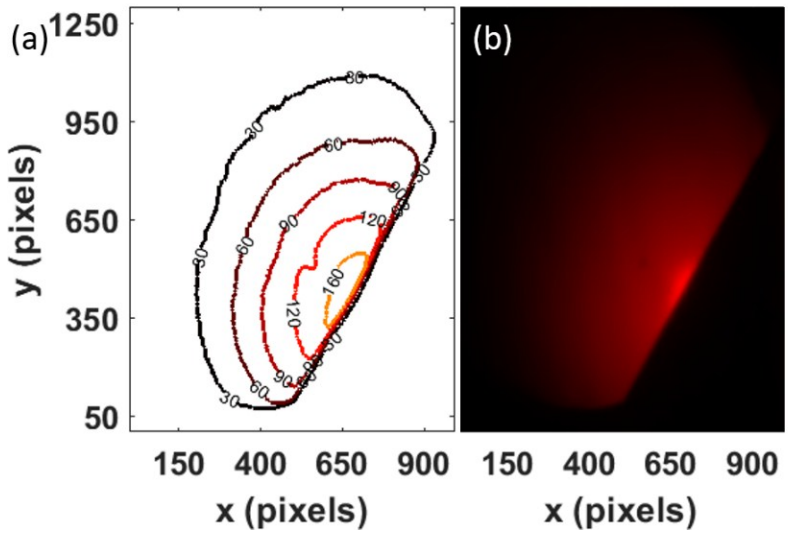

Figure S3. (a) R value contour map of a PSiM sample extracted from one frame of a video recorded by the smartphone sensing platform. (b) Image of PSiM sample (i.e., video frame) corresponding to the R value contour map shown in (a). The sharp interface between the red and black regions of the image that leads to a strong gradient in the contour map is due to the presence of the black tape that blocks a portion of the emitted light from the smartphone LED.

S3. Smartphone image and data processing

S4. Stability of the PSiM-smartphone biosensor system

In order to assess the stability and reproducibility of the PSiM-smartphone biosensor system, three repeat measurements were taken for each experimental condition of the PSiM. Each measurement comprised a 1 min video of the PSiM followed by a 1 min video of the control sample. The PSiM and control samples were removed and then reinserted into the 3D printed box holding the smartphone between each set of measurements. Figure S4 shows the *S* value as a function of time during each of the three videos for the PSiM ("measure") and control bare silicon ("control") samples. We note that the time duration for removing and reinserting the samples is very short compared to the time duration for which the sample is measured. Good stability and reproducibility is demonstrated as the samples are taken in and out of the box. The percent intensity changes between measure 1 and measure 2, measure 2 and measure 3, and measure 3 and measure 1 are all less than 0.1%

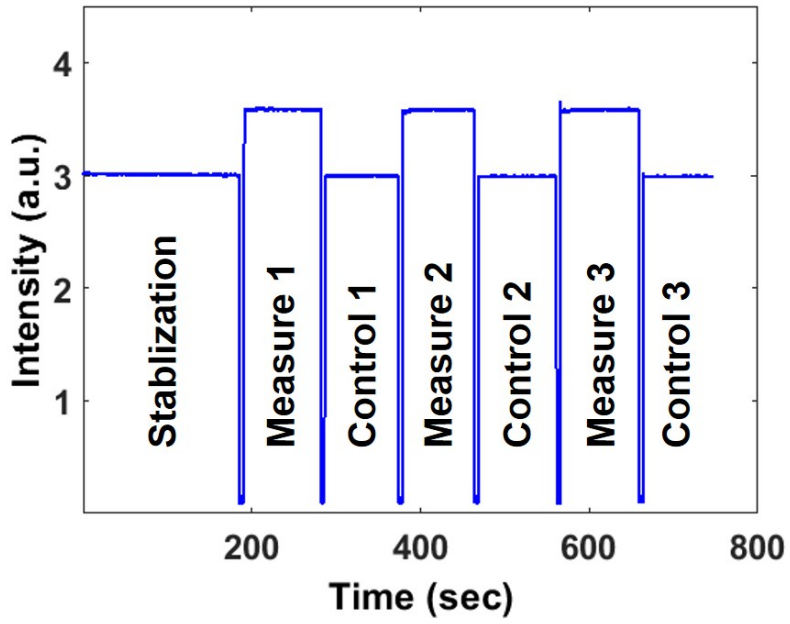

Figure S4. Smartphone measured intensity as a function of time for three independent measurements of a PSiM and control bare silicon sample. Good stability of the system is demonstrated when samples are removed and subsequently reinserted into the 3D printed box holding the smartphone.

S5. Root mean square deviation and Bland-Altman analysis

Root-mean-square deviation:
$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\lambda_i - \hat{\lambda})^2} \quad \text{(S3)}$$

In Eq. S3, $\hat{\lambda}$ is the peak wavelength of the PSiM on the long wavelength side of the resonance as calculated from the linear fit in Fig. 3b, and λ_i is the peak wavelength measured by the spectrometer. Three independent measurements are taken after each APTES exposure to the PSiM, and 21 total values (i.e., n =21) are considered in the calculation.A Bland-Altman plot was generated by transforming the relative intensities measured by the smartphone to peak wavelengths using the linear fit in Fig. 3b, and then these values are compared to the peak wavelengths measured by the spectrometer.

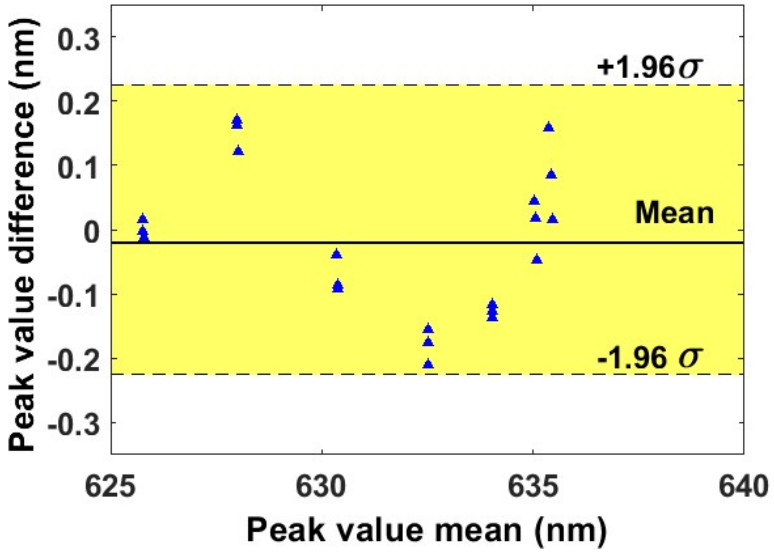

Figure S5. Bland-Altman plot of smartphone and spectrometer measurements of PSiM exposed to APTES (data shown in Fig. 3). The dotted lines enclosing the yellow region represent the 95% confidence interval for the peak value difference.

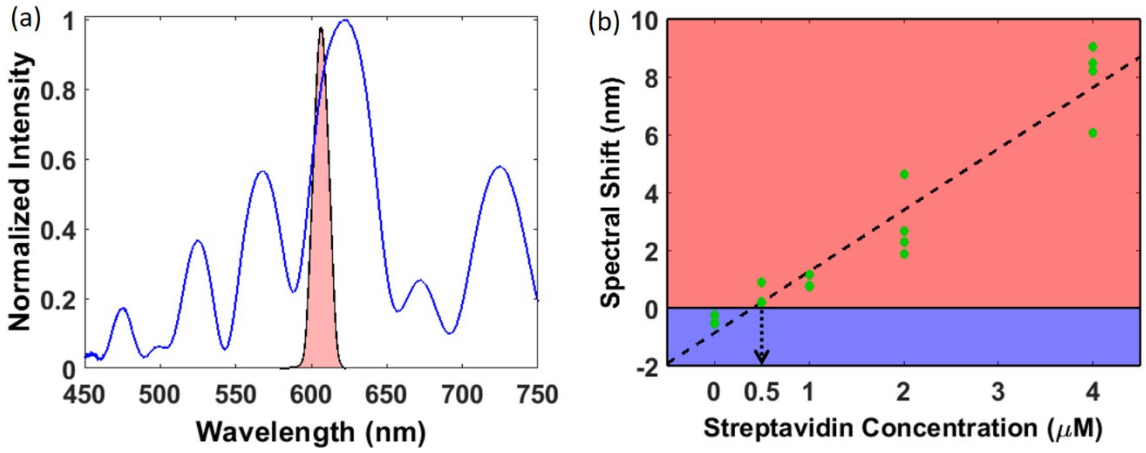

Figure S6. (a) Spectrometer measurement of the reflectance spectrum of the PSiM before adding streptavidin (blue curve) and the smartphone light transmitted through filter (red shaded area). Note that the relative position of the reflectance spectrum and bandpass of the filter may be slightly different for the smartphone measurement. (b) Spectral shift of PSiM after adding different concentrations of streptavidin molecules, as measured by a spectrometer with reference to the reflectance peak on the long wavelength side of the microcavity resonance. With a concentration of 0 μM (i.e., no streptavidin molecules and only solvent exposed to the sample), there is a slight blueshift of the spectrum, suggesting minor instability of the PSiM surface functionalization during the experiment. The data suggest a linear relationship between the PSiM response and streptavidin concentration exposed to the PSiM in the reported concentration range.

S6. Spectrometer measurements of streptavidin attachment

S7. Streptavidin sensing with separate PSiM samples

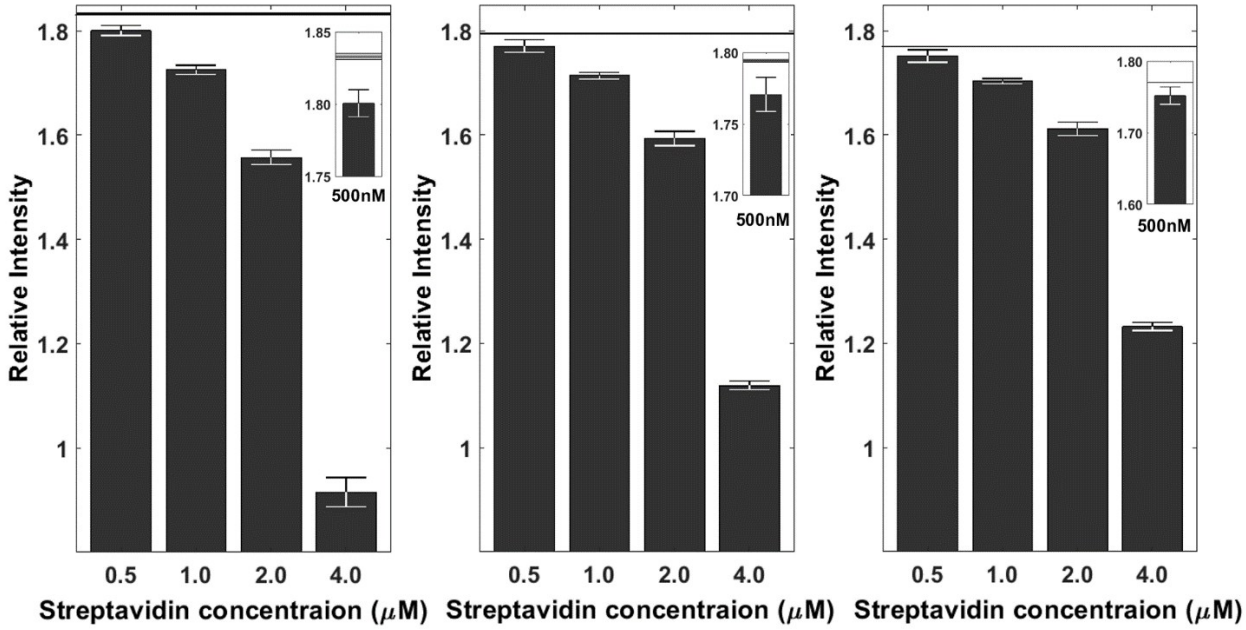

Figure S7. Streptavidin sensing experiment carried out on three separate PSiM samples. The measurements show the same trend in the relative intensity changes for different concentrations of streptavidin solutions exposed to the different samples. Error bars represent ±3σ for the three smartphone relative intensity measurements taken after each PSiM is exposed to a given concentration of streptavidin molecules. Differences in the measured relative intensity values between the different PSiM samples is explained by the different initial microcavity resonance positions with respect to the bandpass of the filter used in the smartphone sensing system. The thickness of the solid line at the top of each graph represents the 3σ value of the relative intensity of the PSiM measured before streptavidin infiltration. For all three PSiM samples, a streptavidin concentration of 500 nM can be clearly distinguished (insets).

Reference

1. H. Sobral and M. Pena-Gomar, *Applied Optics*, 2015, **54**, 8453-8458.