Supporting Information for:

A Smartphone-Enabled Plasmonic Nanosensor

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Synthesis of Gold Nanoparticles

Gold nanoparticles in sizes ranging from roughly 10 nm to 130 nm were synthesized according to Bastus et al. All glassware involved in the synthesis procedure was cleaned with aqua regia. A 150-mL solution of 2.2 mM trisodium citrate was heated to boiling in a 250-mL three-neck round-bottom flask with a condenser attached to the center neck. Once the solution reached boiling, 1 mL of 25 mM $HAuCl_4$ was added to the flask via injection through a septum on the side neck. The solution was boiled for 10 minutes to allow growth of gold seeds. After 10 minutes, the solution was left to cool to 90˚C.

Once at 90˚C, 1 mL of 25 mM $HAuCl_4$ was added to the gold seed solution. The solution was left to stir for 30 minutes while maintaining the temperature at 90˚C. After 30 minutes, another 1 mL of 25 mM $HAuCl_4$ was added and allowed to react for 30 minutes. A 55-mL aliquot of the resulting nanoparticle solution was then extracted and stored at room temperature for further use and analysis. This first extraction was labeled Generation 1 (G1). To obtain larger gold nanoparticles, the reaction in the round bottom flask was continued by adding 53 mL of boiling MQ water, 2 mL of 60 mM sodium citrate, and two sequential additions of 25 mM $HAuCl_4$ as described above. This process was performed a total of nine times, with each extraction labeled according to its growth generation (G1 through G9). The nanoparticle solutions were used to produce sensor chips as described below.

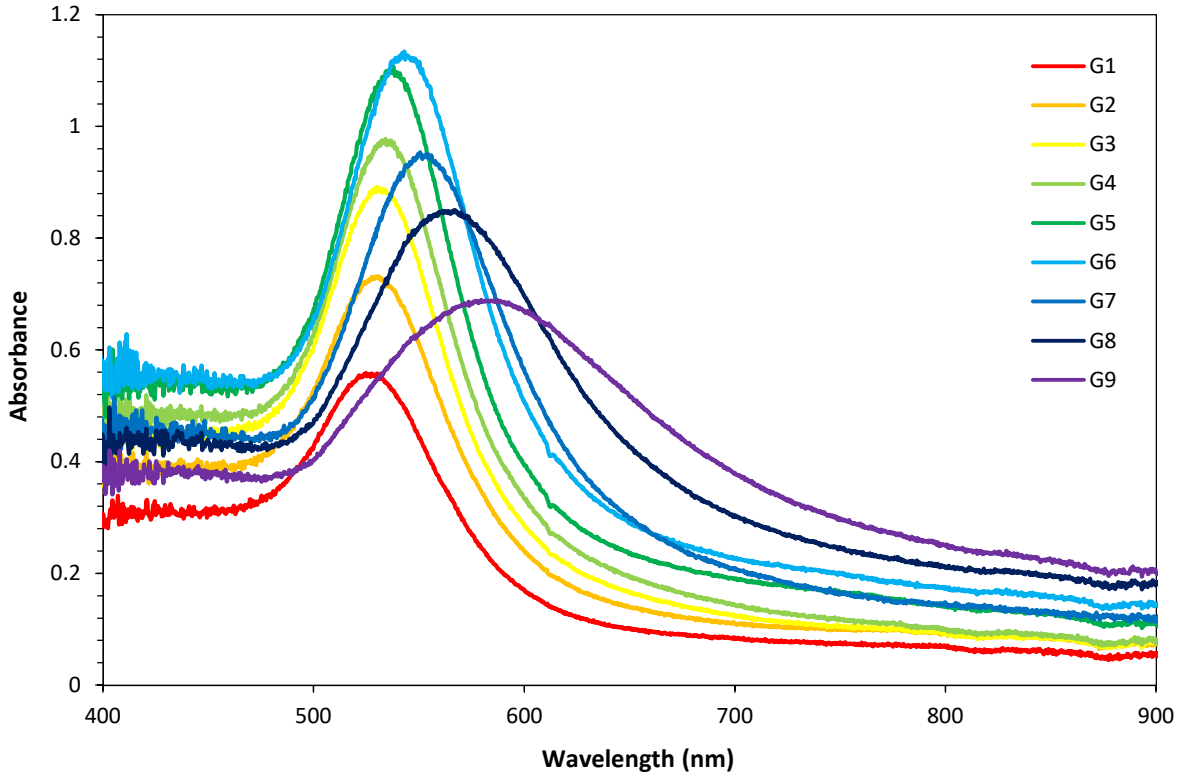

Figure S1. Solution Spectra of Gold Nanoparticles. The absorbance spectra of Au nanoparticles synthesized according to the Bastus et al. procedure were measured with an Ocean Optics Flame VIS-NIR Fluorescence Spectrometer. The nanoparticle solution was diluted 1:2 with Milli-Q water. The solutions are labeled according to their growth generation, ranging from G1 (generation 1) to G9 (generation 9).

Sensor Chip Fabrication

Prior to drop-casting on silane-functionalized glass coverslips, aliquots of the nanoparticle solution were centrifuged according to the parameters given below on an Eppendorf 5402 centrifuge with rotor F-45-18-11. Following centrifugation, the supernatant was removed and the particles were resuspended to their original volume with MQ water.

Nanoparticle Diameter (nm)	Speed (rpm)	Time (min)
34.1	10,000	10
59.8	8,000	10
81.5	8,000	6
114.5	6,000	6

Scanning Electron Microscopy

Sensor chips were coated with 5-10 nm of graphitic carbon and imaged using an FEI Sirion XL30 FEG Scanning Electron Microscope operating at a 10kV acceleration voltage. SEM images were processed using the particle analysis feature in ImageJ in order to quantify the nanoparticle sizes. The table below displays the average particle diameter, standard deviation in particle diameter, and total number of particles analyzed (N) for each nanoparticle generation used in this study.

Nanoparticle Generation	Nanoparticle Diameter (nm)	Standard Deviation (nm)	N
G3	34.1	6.7	1881
G5	59.8	7.7	365
G7	81.5	7.6	244
G9	115.6	16.6	140

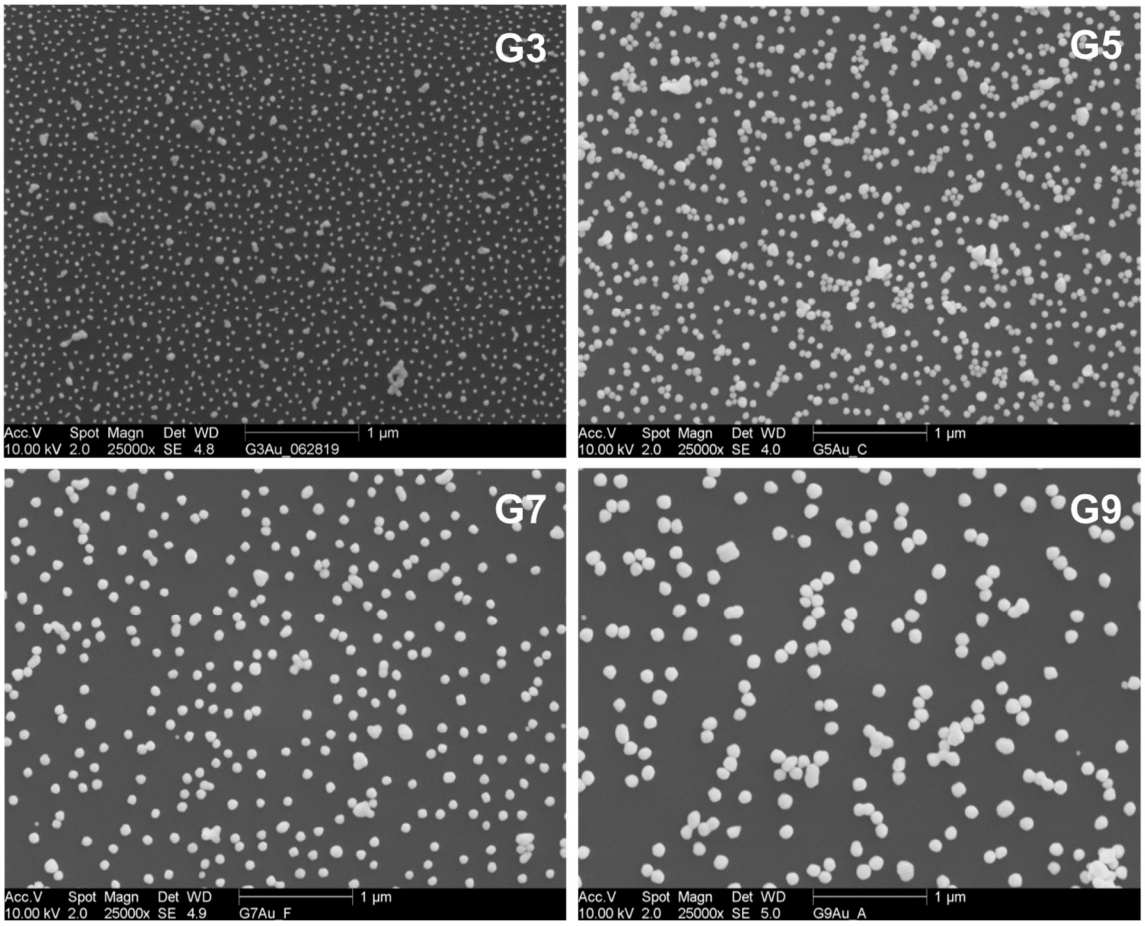

Figure S2. Scanning electron microscope images of sensor chip surfaces. All images were collected at 25,000x magnification.

Biosensing Experiment

A G7 sensor chip was incubated in a 1 mM ethanolic solution of 75% $CT(PEG)_{12}$ and 25% $MT(PEG)_4$ for 48 hours in order to achieve a mixed self-assembled monolayer comprising PEG-thiol groups with either a methyl or a carboxyl terminal group. The sensor chip was then washed with 100 mM MES and submerged in a 1 mM biotin, 100 mM MES, and 100 mM EDC solution for one hour in order to functionalize the carboxyl-terminated self-assembled monolayer groups with biotin. Ten photos of the sensor chip were taken before and after the one-hour incubation. Next, the sensor chip was subsequently washed with 100 mM MES followed by a wash with 100 mM PBS, and ten photos were captured of the sensor chip in PBS. Finally, the sensor chip was submerged into a solution of 400 nM anti-biotin in 100 mM PBS for one hour, with photos taken every 10 minutes to record anti-body binding. After the one-hour incubation, the sensor chip was washed and placed in a solution of 100 mM PBS, and ten photos were taken.

Conversion of spectra to tristimulus values

Reflectance or absorbance measurements, such as those shown in Figure S3, were converted to the CIE XYZ color space created by the International Commission on Illumination (CIE) in 1931. The 2-degree (standard observer) color matching functions for XYZ (based on experimental data collected by Wright and Guild) are shown in Figure S4. The D65 standard daylight illuminant, also specified by CIE, is shown in Figure S5. These data were downloaded as an Excel file with data points every 1 nm from the Munsell Color Science Lab at the Rochester Institute of Technology.

https://www.rit.edu/science/munsell-color-science-lab-educational-resources?ref=rit-search#useful-color-data

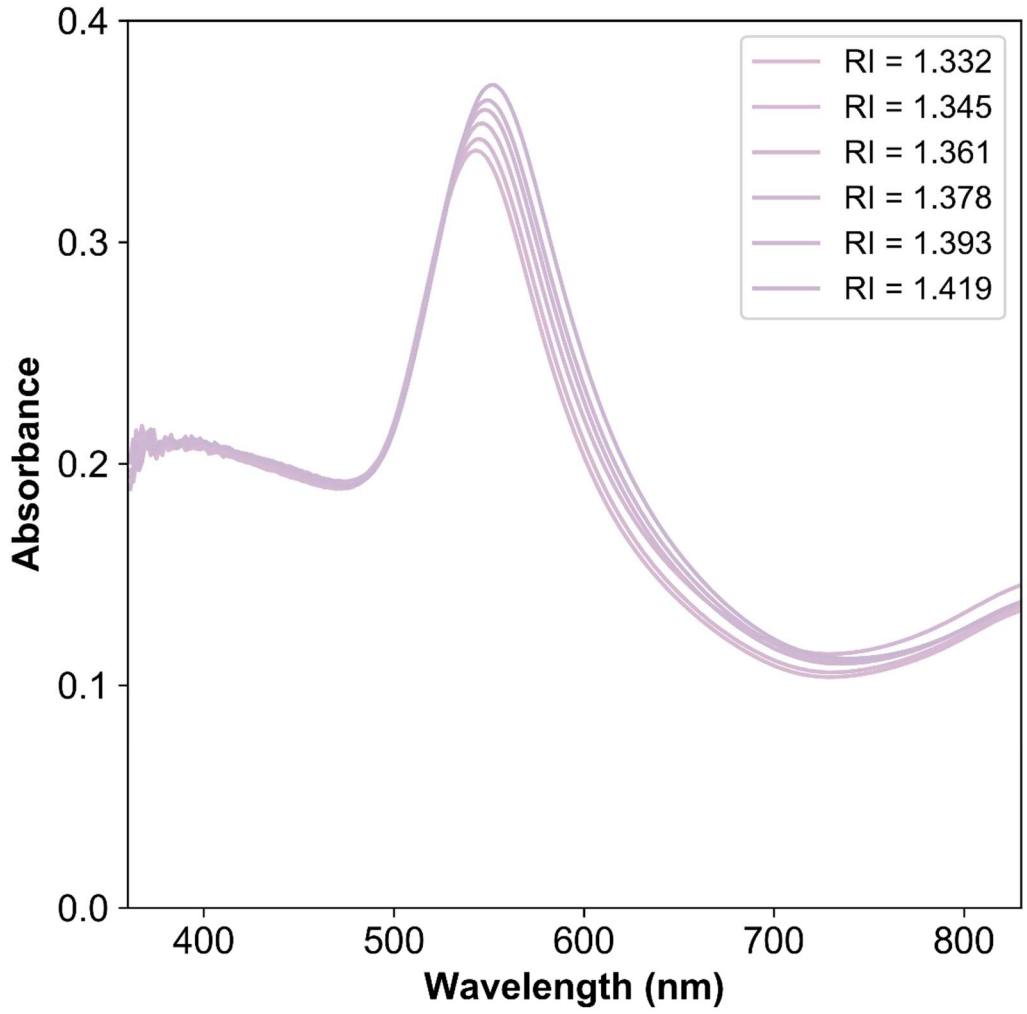

Figure S3. Absorbance spectra of G7 gold nanoparticles in sucrose solutions. Each of these spectra represent the average of spectra from seven different sensor chips in the same sucrose solution.

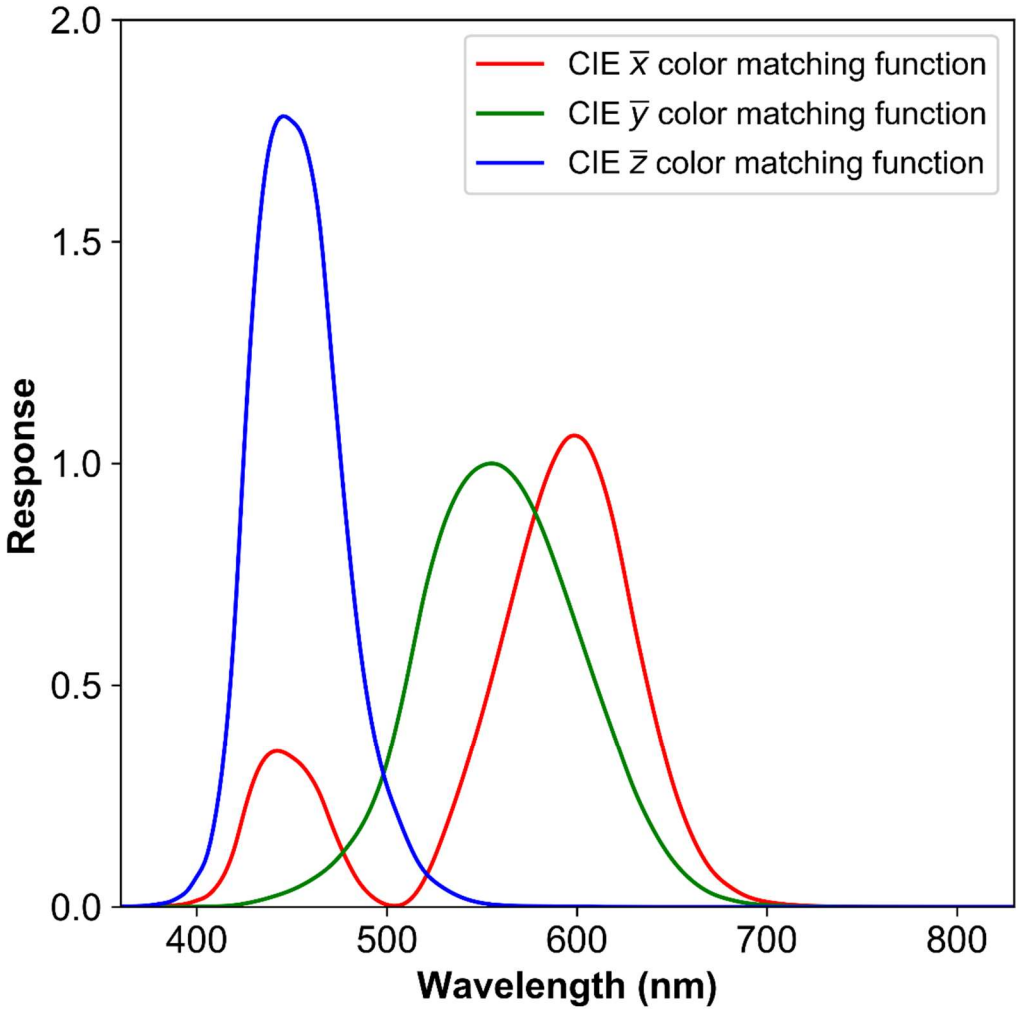

Figure S4. CIE 1931 XYZ color matching functions. All values were interpolated to wavelengths to match the measured absorbance spectra using the function <`scipy.interpolate.interp1d`> with a "cubic" fit.

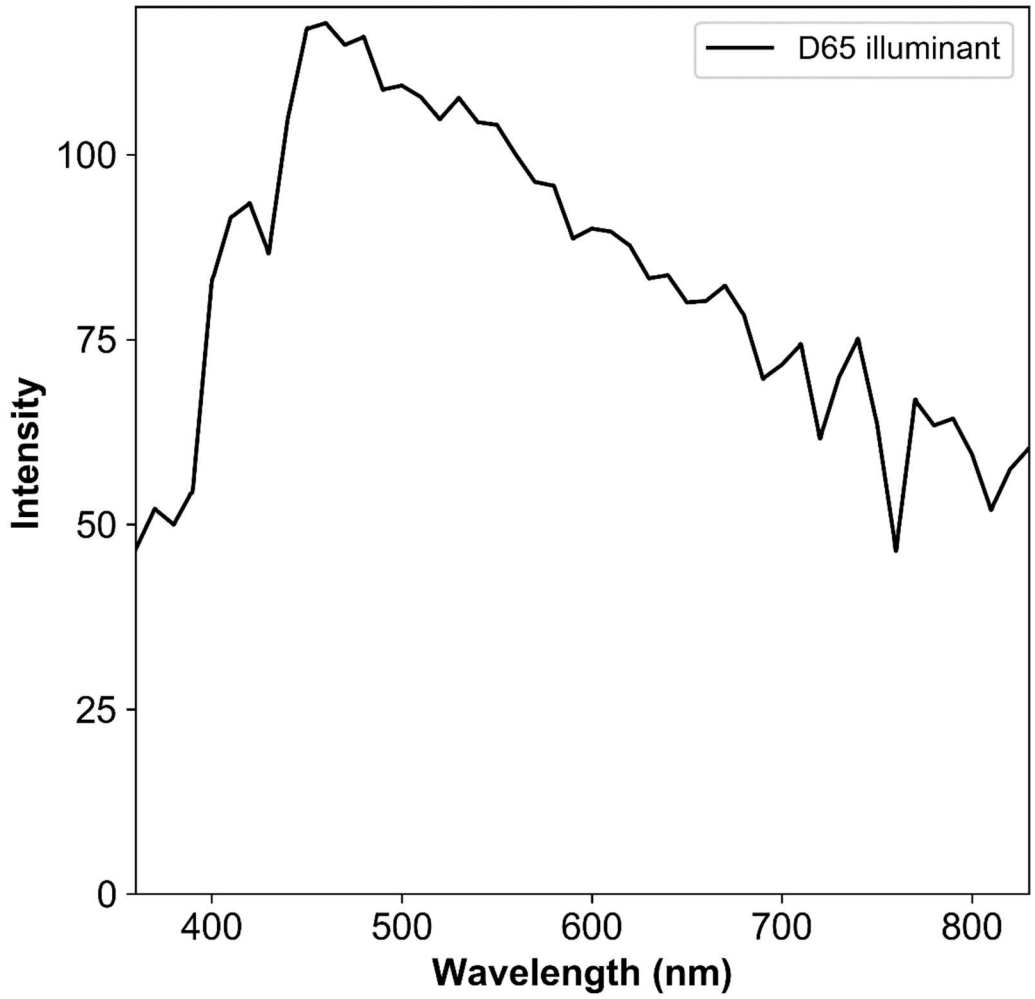

Figure S5. D65 illuminant intensity. This is the standard illuminant specified by CIE and used to represent daylight in the northern hemisphere. The color temperature is approximately 6500K. All values were interpolated to wavelengths to match the measured absorbance spectra using the function `<scipy.interpolate.interp1d>` with a "cubic" fit.

Absorbance spectra were converted to tristimulus values using the following equations:

$$N = \int_{360}^{830} CIE\ \bar{y} * D65\ illuminant$$

$$X = \frac{\int_{360}^{830} CIE\ \bar{x} * D65\ illuminant * \ 10^{-Absorbanc}}{N}$$

$$Y = \frac{\int_{360}^{830} CIE\ \bar{y} * D65\ illuminant * \ 10^{-Absorbance}}{N}$$

$$Z = \frac{\int_{360}^{830} CIE\ \bar{z} * D65\ illuminant * \ 10^{-Absorbance}}{N}$$

The equation for N represents the unattenuated intensity of the Y color channel and is used to normalize the calculated values of XYZ. The integrals were calculated using the trapezoid rule in a Python script (`<scipy.integrate.trapz>`). The numerators in these equations represent the intensity for each color channel after attenuation by nanoparticle absorbance. As an example, results of this calculation are shown for a G7 sensor chip in water in Figure S6.

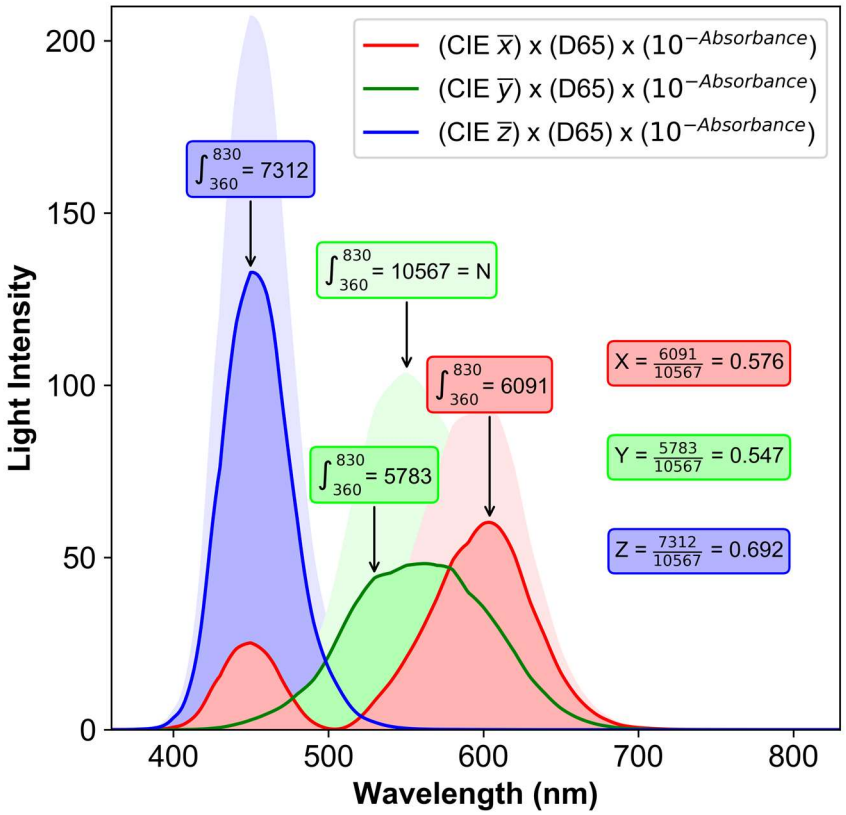

Figure S6. CIE XYZ of G7 gold nanoparticles in water.

Conversion of XYZ tristimulus values to RGB and HSV values

Once the XYZ values were determined, the tristimulus values in other color spaces were then calculated based on them. Linear (not gamma compressed) values for sRGB were calculated by matrix multiplication as shown in the equation below. The matrix below is specified by IEC publication 61966-2-1:1999 and was downloaded from the International Color Consortium (ICC) website specifications of the SRGB color space.

http://www.color.org/srgb04.xalter

XYZ to linear sRGB:

$$\begin{bmatrix} \mathrm{R}_{linear} \\ \mathrm{G}_{linear} \\ \mathrm{B}_{linear} \end{bmatrix} = \begin{bmatrix} 3.2406255 & -1.537208 & -0.4986286 \\ -0.9689307 & 1.8757561 & 0.0415175 \\ 0.0557101 & -0.2040211 & 1.0569959 \end{bmatrix} \begin{bmatrix} X \\ Y \\ Z \end{bmatrix}$$

These values were then gamma corrected as shown in the piecewise function immediately below:

$$RGB_{gamma} = \begin{cases} 12.92 * \mathrm{RGB}_{linear} & if\ \mathrm{RGB}_{linear} < 0.0031308 \\ 1.055 * \mathrm{RGB}_{linear}^{\left(\frac{1}{2.4}\right)} - 0.055 & otherwise \end{cases}$$

$$RGB_{gamma} = \begin{cases} 1 & if RGB_{gamma} > 1 \\ 0 & if RGB_{gamma} < 0 \end{cases}$$

These gamma corrected sRGB values were then used to calculate HSV values:

$$V = \max(R, G, B)$$

$$C = V - \min(R, G, B)$$

$$S = \begin{cases} 0 & if\ V = 0 \\ \frac{C}{V} & otherwise \end{cases}$$

$$H_S = \begin{cases} 0 & if\ C = 0 \\ \left(\frac{G - B}{C} + 0\right)/6 & if\ V = R \\ \left(\frac{B - R}{C} + 2\right)/6 & if\ V = G \\ \left(\frac{R - G}{C} + 4\right)/6 & if\ V = B \end{cases}$$

$$H = \begin{cases} H_S + 1 & if\ H_S < 0 \\ H_S & otherwise \end{cases}$$

Adjustment of Hue Origin and Direction of Rotation

The origin of hue was shifted from red (nominally 0 or 1) to green (1/3) to avoid crossing of the origin during sensing measurements. A counter clockwise (CCW) hue angle of rotation (G < R < B) was defined as positive so that increasing refractive index would produce an increasing hue angle.

$$H_{org} = \begin{cases} H - Origin + 1 & if\ H < Origin \\ H - Origin & if\ H \geq Origin \end{cases}$$

$$H_{dir} = \begin{cases} H_{org} & if\ direction = CW \\ 1 - H_{org} & if\ direction = CCW \end{cases}$$

The color wheel below illustrates the range of calculated hue angles for nanoparticle absorbance spectra, where the start of the range represents the reddest particles (G3 in air), the end of the range represents the bluest particles (G9 in 50% sucrose), and the hashmarks represent the change for a G7 sensor chip going from water to 50% sucrose.

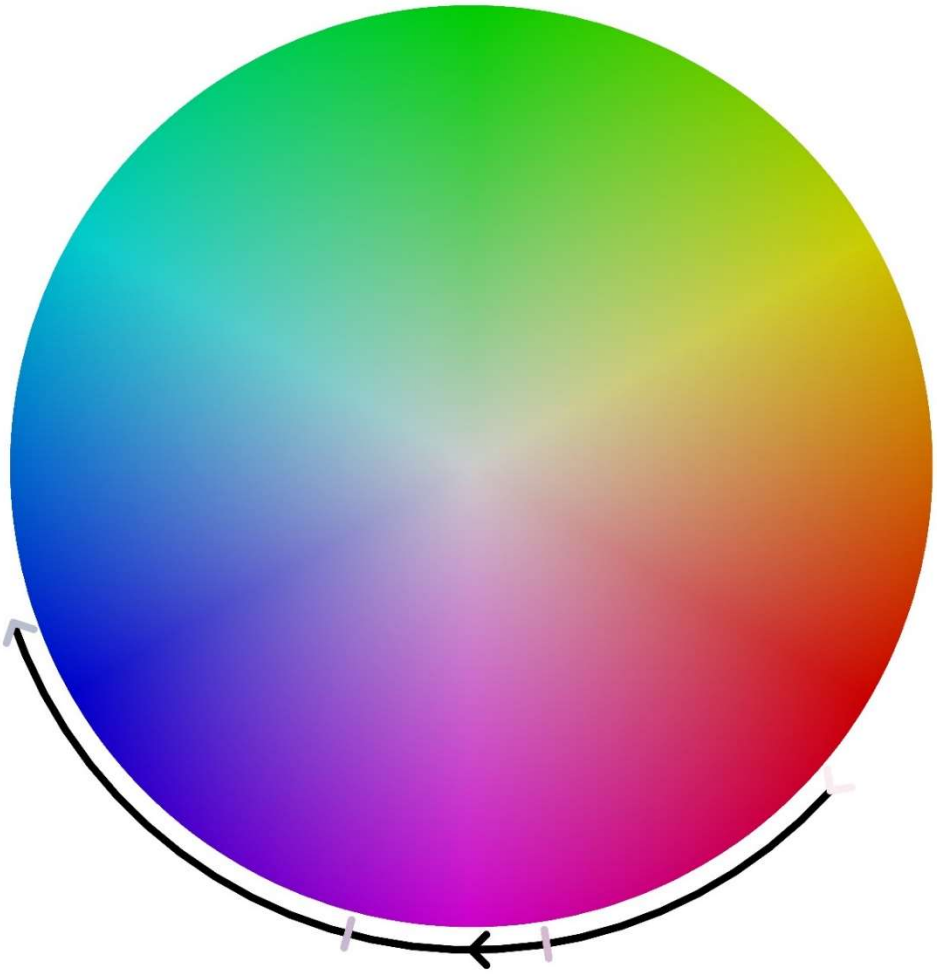

Figure S7. Hue wheel displaying the range of angles measured in this study.

Conversion of XYZ tristimulus values to $L^*a^*b^*$ color space

The XYZ tristimulus values were used to calculate CIE $L^*a^*b^*$ using the equations below as specified by CIE 015-2004, where X_r, Y_r, and Z_r represent the X, Y, and Z values for the D65 illuminant that is unattenuated by an absorbance spectrum.

$$X_r = \frac{\int_{360}^{830} CIE\ \bar{x} * D65\ illuminant}{N}$$

$$x_r = \frac{X}{X_r}$$

$$y_r = \frac{Y}{Y_r}$$

$$z_r = \frac{Z}{Z_r}$$

$$f_x = \begin{cases} \sqrt[3]{x_r} & \text{if } x_r > (24/116)^3 \\ \frac{841}{108} x_r + \frac{16}{116} & \text{otherwise} \end{cases}$$

$$f_y = \begin{cases} \sqrt[3]{y_r} & \text{if } y_r > (24/116)^3 \\ \frac{841}{108} y_r + \frac{16}{116} & \text{otherwise} \end{cases}$$

$$f_z = \begin{cases} \sqrt[3]{z_r} & \text{if } z_r > (24/116)^3 \\ \frac{841}{108} z_r + \frac{16}{116} & \text{otherwise} \end{cases}$$

$$L^* = 116 f_y - 16$$

$$a^* = 500(f_x - f_y)$$

$$b^* = 200(f_y - f_z)$$

Conversion of RGB to rgb chromaticity color space

The rgb chromaticity values represent the RGB tristimulus values normalized to the sum of the RGB pixel intensities.

$$T = R + B + G$$

$$r = \frac{R}{T}$$

$$g = \frac{G}{T}$$

$$b = \frac{B}{T}$$

Modeling Nanoparticle Extinction Spectra using Mie Theory

The Johnson and Christy values for complex refractive index (n and k) for gold were used in the Python Mie Scattering package (https://pymiescatt.readthedocs.io/en/latest/index.html) to calculate theoretical extinction spectra for gold nanoparticles of various sizes and in various refractive index environments (using the function `<MieQ_withWavelengthRange>`). Examples of the calculated extinction coefficients are shown in Figure S9 (effect of size) and S10 (effect of refractive index). To convert the extinction coefficients to tristimulus values, an optical density coefficient of 0.063 was used to produce the best match to experimental spectra.

https://refractiveindex.info/?shelf=main&book=Au&page=Johnson

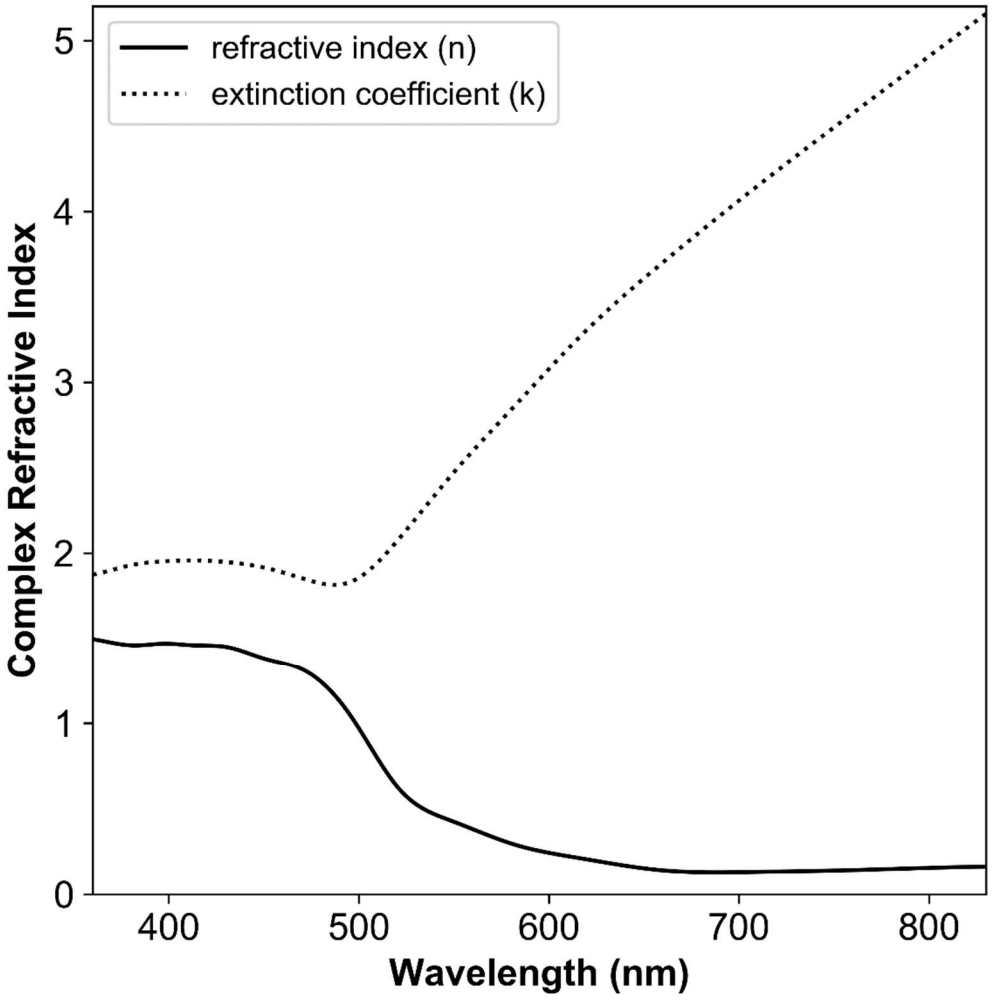

Figure S8. P. B. Johnson and R. W. Christy. Optical constants of the noble metals, *Phys. Rev. B* **6**, 4370-4379 (1972). All values were interpolated to a wavelength scale 360 to 830 nm in 0.1 nm increments using the function `<scipy.interpolate.interp1d>` with a "cubic" fit.

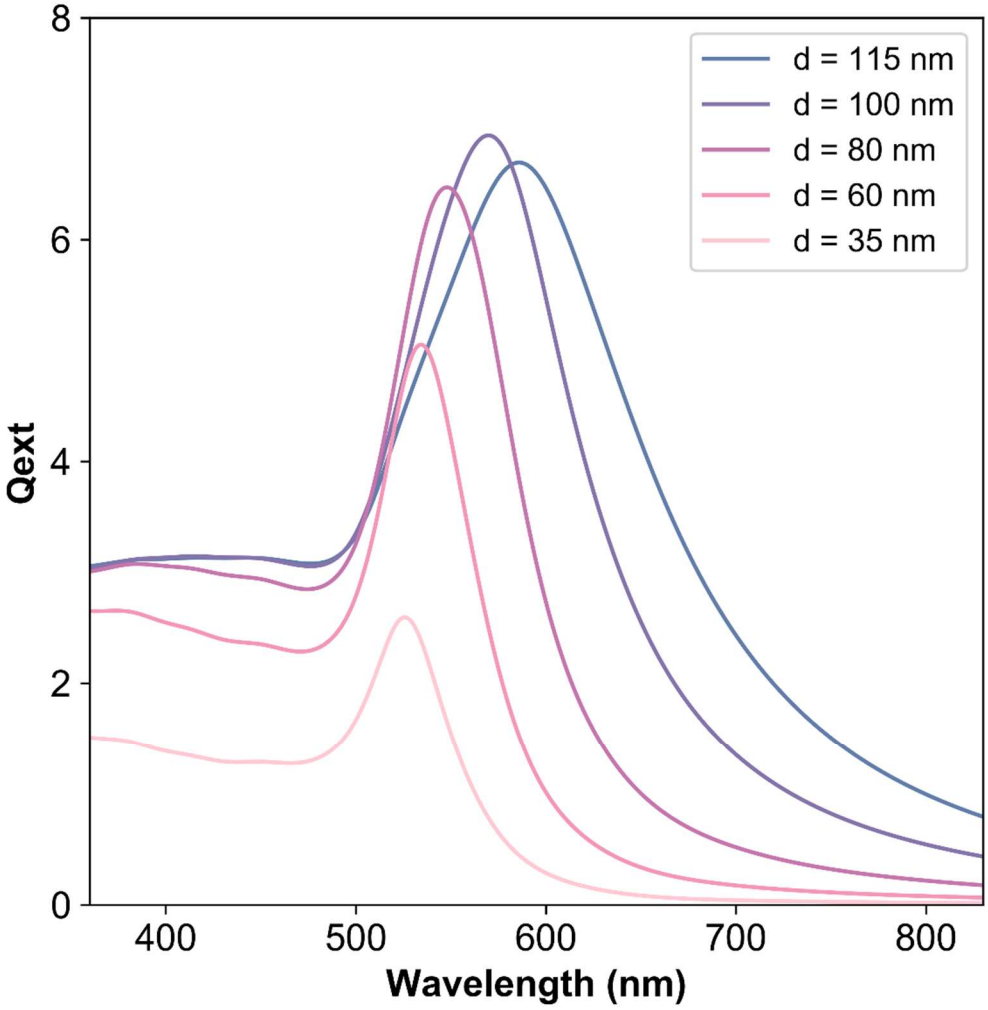

Figure S9. Extinction coefficients of Au spheres of varying diameter in water (RI=1.333).

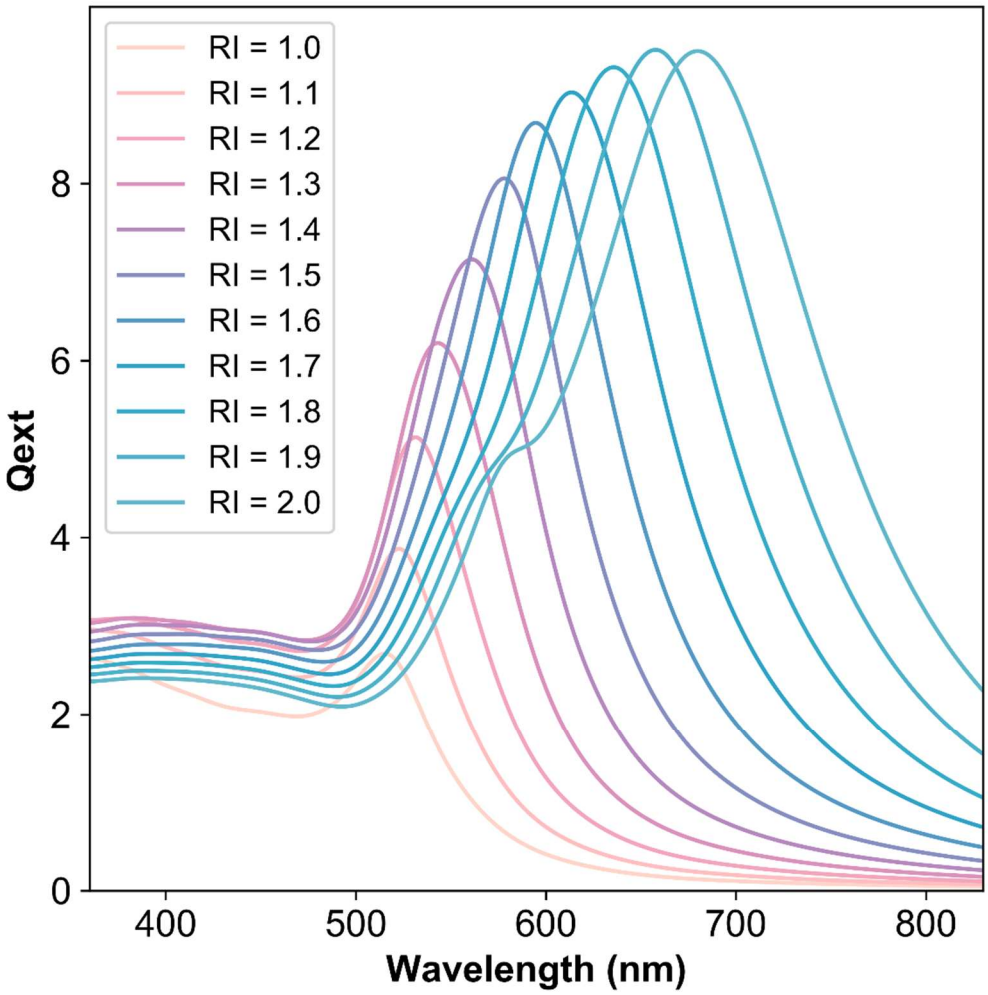

Figure S10. Extinction coefficients of 80 nm monodisperse Au spheres in RI 1 to 2.

Calculated Qext values such as those shown in figures S9 and S10 were scaled by a coefficient of 0.063 to produce a close match to the intensity of experimental absorbance spectra. The scaled spectra were then converted to CIE XYZ tristimulus values following the same procedure used for experimental spectra. The CIE XYZ values allowed us to calculate 12 other color parameters: RGB, HSV, L*a*b*, and rgb chromaticity. The color behavior of these parameters for 80 nm Au particles is shown below, where the x-axis has a range from RI = 1 to RI = 2, and the vertical droplines represent the refractive indices of water and 50% sucrose. All values have been scaled 0 - 1 for ease of comparison, and the y-axis height is 1.2.

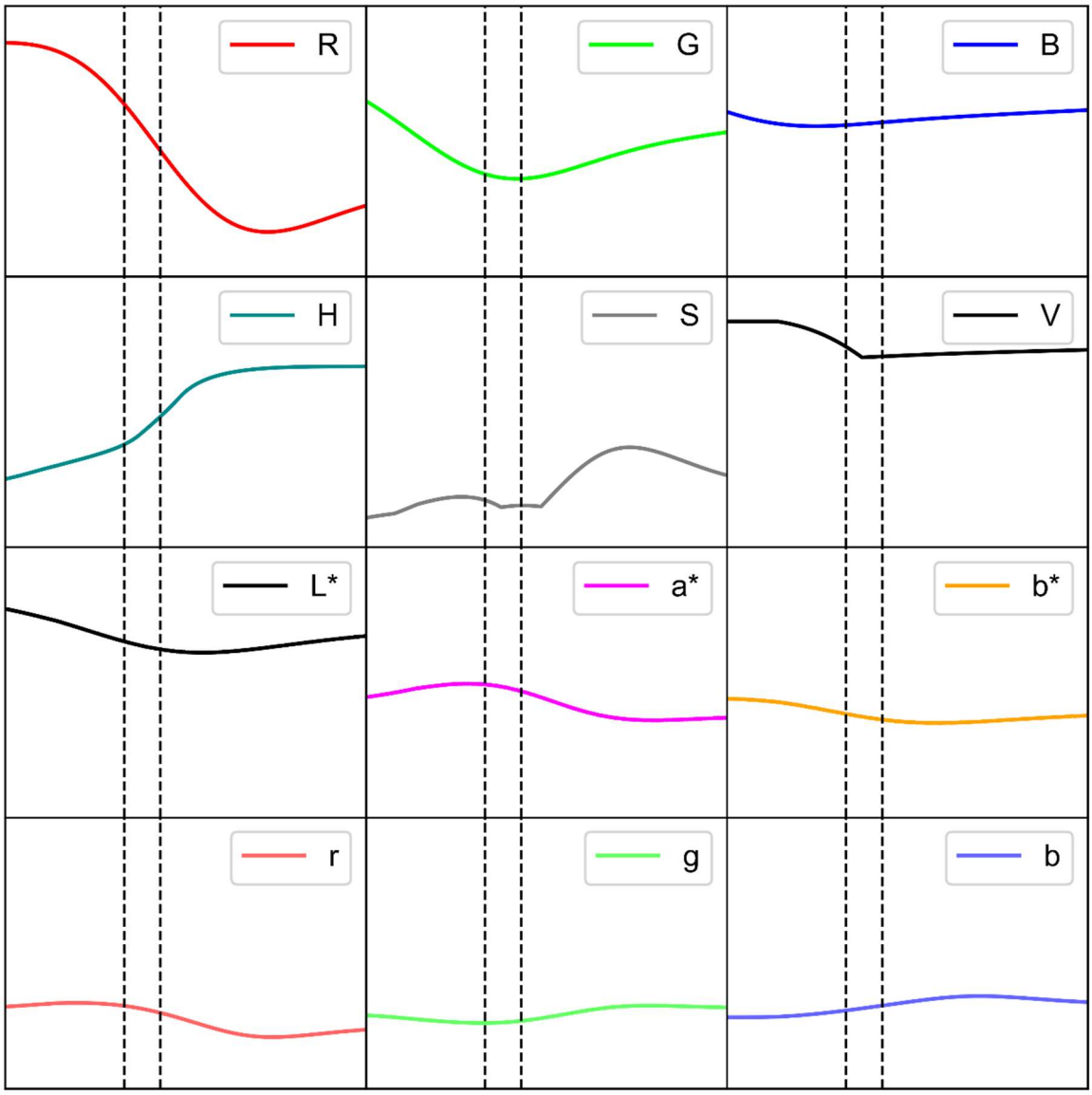

Figure S11. Color behavior of 80 nm monodisperse Au spheres in RI 1 to 2.

Image Analysis

All image analysis was performed using an automated script developed in Python. After an image file is opened, unsigned integers (0 to 255) are converted to floating point values to prevent rounding error. A fixed rectangular portion of the image (shown in red below) is selected for white balancing, where the length of the rectangle encompasses 10% to 90% of the image width, and the height of the rectangle encompasses 50% to 67% of the image height.

Figure S12. Raw (left) and white balanced (right) images of a nanoparticle sensor chip in water.

To white balance the image, the mean RGB intensities are quantified in the white balance region. Here, for example, RGB_{mean}= (0.6548, 0.7215, 0.6768). A scale factor is calculated for each channel such that all pixel intensities are scaled to a value of 65% of the maximum pixel intensity (0.65*255). The unique scaling factors are applied to each color channel to rebalance the color across the entire image. For example, here R_{factor}= 0.9926, G_{factor}= 0.9009, B_{factor}=0.9604.

Conversion of the image to HSV and CIE L*a*b* color spaces

First, a mask is developed that excludes pixels in the image that have a saturation < 6%. We then find the center of the largest contour of connected pixels left unmasked. The estimated center is 50% of width, 25% of height, both ±20% (inside green rectangle, Figure S13). We calculate the minimum enclosing rectangle of the largest contour to define the region of interest (ROI) (Figure S14).

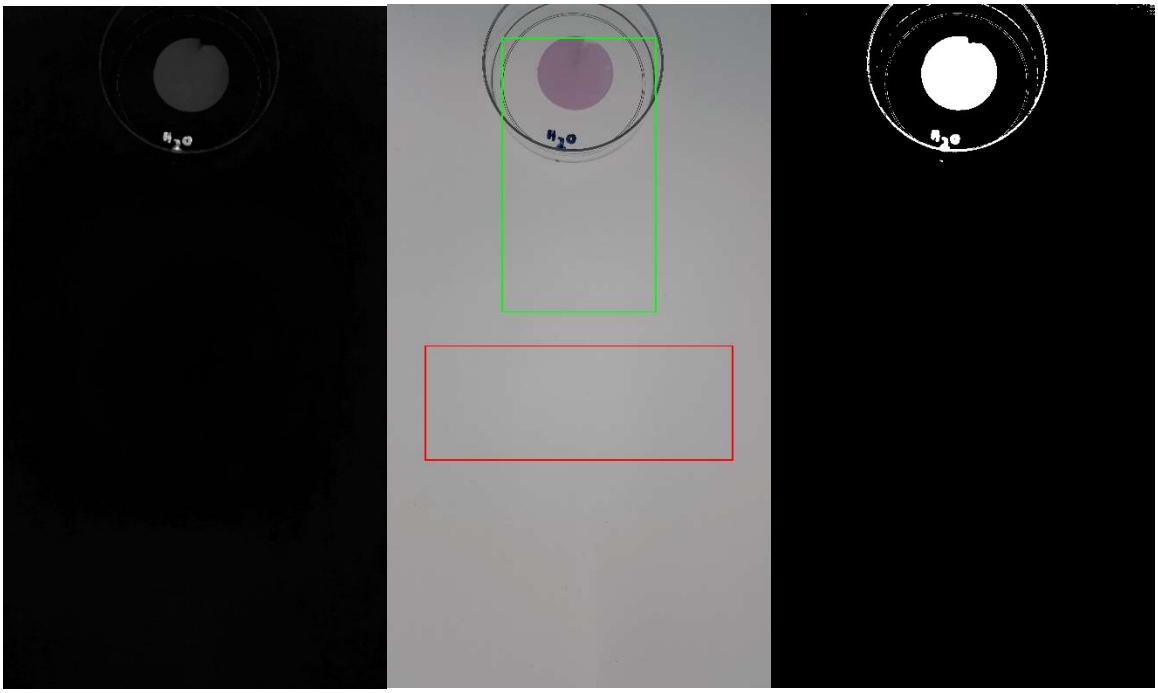

Figure S13. Greyscale Saturation (S=1 is white, S=0 is black) from the HSV color space (left), white balance and sensor ROI regions (center), and saturation mask (right).

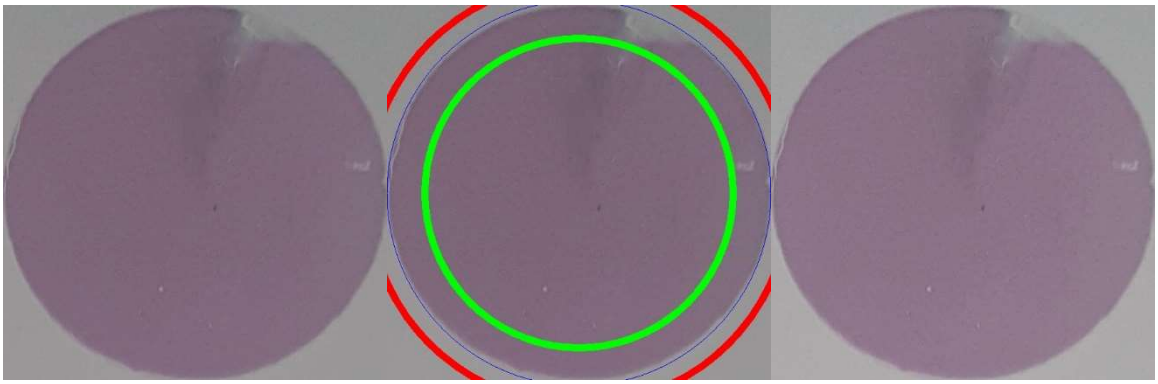

Figure S14. Identified ROI based on minimum bounding rectangle (left), white balance (outside red lines) and sensor material (inside green line) regions (center), and ROI after local white balance (right).

We calculate the center and radius of the minimum enclosing circle of the largest contour (blue circle, center panel in Figure S14), and divide the ROI into two regions:

- Local White balance region = rectangular ROI – sensor circle with the radius increased by 10% (red outline, center panel in Figure S14). A local white balance is performed using the pixels from the red line to the outer edges of the rectangular ROI using the same procedure outlined for the initial global white balance.
- Sensor ROI = circle with the same center as that of the largest contour but with the radius reduced by 20% (green circle, center panel Figure S14) and with saturation ≥ 6%.

These calculations produce the final sensor ROI (Figure S15) that is used for color analysis (Figure S16).

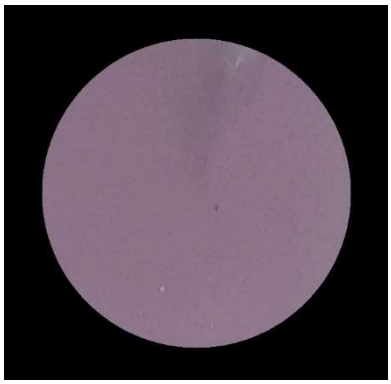

Figure S15. Sensor ROI after masking and white balancing (both global and local).

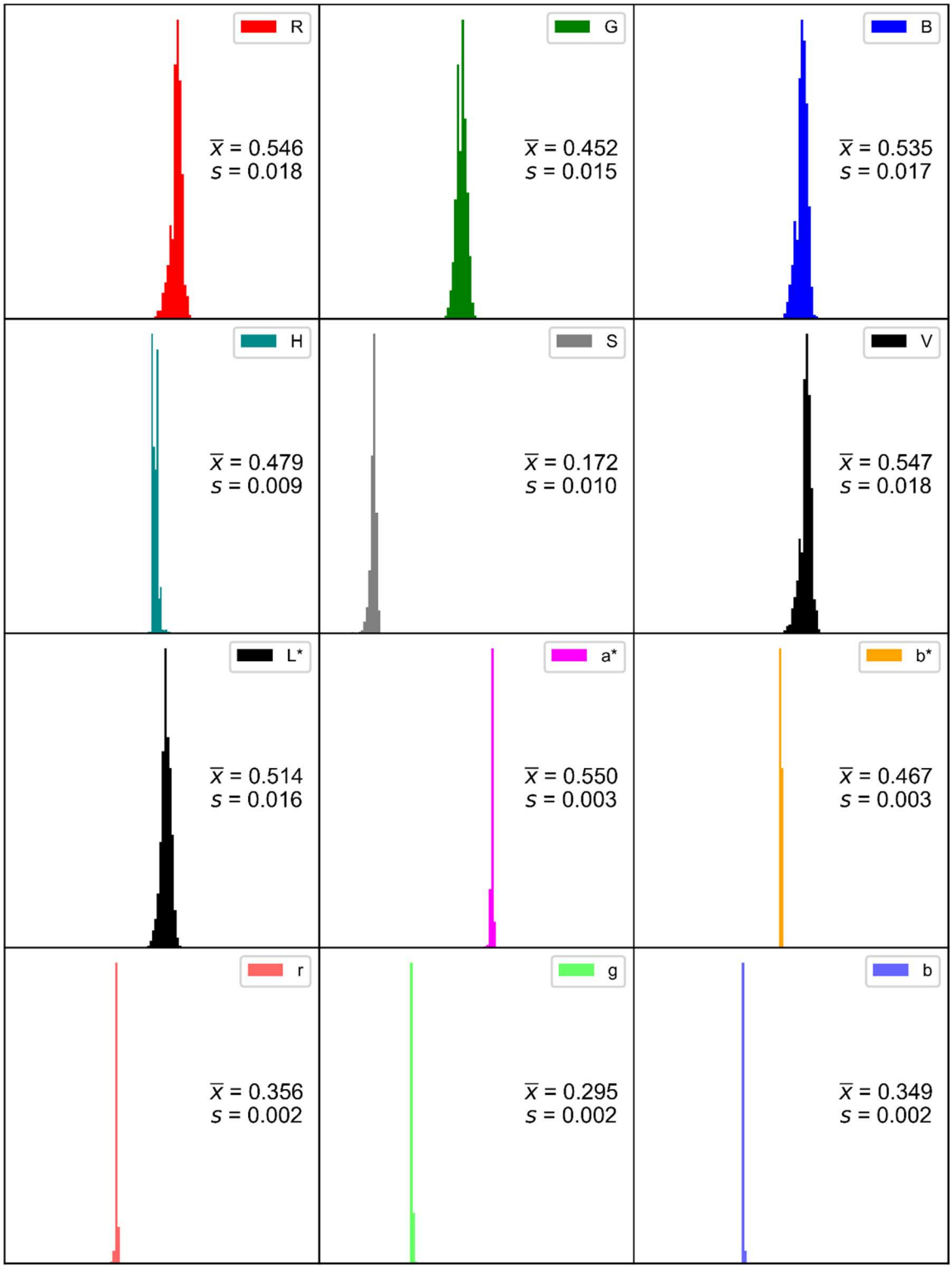

Figure S16. Normalized histograms along with the calculated mean and standard deviation of each color channel from the sensor ROI in Figure S15.