Supporting Information

Broadly Available Imaging Devices Enable High Quality Low-cost Photometry

Dionysios C. Christodouleas[1], Alex Nemiroski[1], Ashok A. Kumar[1] and George M. Whitesides[1,2,3*]

[1] Department of Chemistry & Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA

[2] Wyss Institute for Biologically Inspired Engineering, Harvard University, 60 Oxford Street, Cambridge, MA 02138, USA

[3] Kavli Institute for Bionano Science & Technology, Harvard University, 29 Oxford Street, Cambridge, MA 02138, USA

*Corresponding author E-mail: gwhitesides@gmwgroup.harvard.edu

Experimental procedures

Construction of *camera-based photometer*. We first constructed a five-sided box from cardboard and lined the inside with black fabric. A base formed by adhering a sheet of foam (1" thick) underneath a perfboard provided a surface on which to solder components and fix them in place. On to this perfboard, we soldered a series circuit consisting of two 9-V batteries (connected electrically in parallel and in separate battery holders), a toggle switch, a potentiometer (to adjust brightness), and a planar light source. We finally placed the box on the base and used a biopsy punch to create a 6-mm hole in the lid of the box, centered on the planar light source, for the camera. The imaging distance was 15.5 cm. A schematic of the *camera-based photometer* is shown in Figure S-2. Although a larger backlight (e.g. from a tablet) could illuminate the whole plate at once, the camera would have to be moved much farther away to reduce the angular spread in optical path lengths through the samples.

Preparation of solutions of dyes. We prepared solutions of the dyes with the following concentrations. Disperse orange 3 (2.81, 8.43, 14.05, 28.10, 42.15, 56.20 μM), methyl orange (0.06, 0.24, 0.50, 1.00, 2.00, 4.00 μM), fluorescein (2.00, 4.00, 6.00, 8.00, 10.00, 16.00 μM), DPPH (1.40, 2.81, 5.62, 8.43, 11.24, 16.86, 22.48, 28.10 μM), eosin Y (0.54, 1.00, 2.00, 2.69, 6.00, 8.00, 10.00, 13.45 μM), rhodamine B (0.16, 0.78, 1.17, 2.91, 3.92, 5.83, 8.74, 11.66 μM), trypan blue (1.04, 2.08, 4.16, 6.24, 8.33, 10.41, 16.65, 20.82 μM), prussian blue (1.00, 2.00, 4.00, 6.00, 10.00, 14.04, 19.20 μM), malachite green (0.06, 0.24, 0.50, 1.00, 2.00, 4.00 μM), methylene blue (1.00, 2.00, 4.00, 6.00, 8.00, 10.00, 15.70, 21.98 μM), chlorophyll b (1.1, 2.2, 4.4, 6.6, 8.8 μM).

Preparation of calibration lines of dyes. We added 400 μL of different solutions into each well of a 96-well microtiter plate (we prepared one plate per dye) and read the absorbance on a microplate spectrophotometer at the peak absorbance of these solutions (Table S-2). Then, we captured the images of the microtiter plates contained these solutions using the *flatbed scanner* and the *camera-based photometer*. We read the RGB values of three pixels (chosen at random from within the image of each of the wells that contained these solutions) with Image J or Microsoft Paint and recorded the mean values C_k of each group of RGB values. We calculated the RGB-resolved absorbance A_k of each sample using Equation 3 of the main text ($A_k \equiv -\log_{10}\left[\frac{C_k^{(S)}}{C_k^{(B)}}\right]$ and the guidelines for the selection of appropriate color value.

Concentration of solutions of dyes shown in Figure 2. Each row of the 96-well microtiter plate contained 8 solutions of one of 11 different compounds, the four left columns at the same, higher concentration and the four right columns at the same, lower concentration (see Supporting Information for the concentration of the solutions : i) disperse orange 3 (Left: 70.2 μM / Right: 28.1 μM), ii) methyl orange (Left: 29.3 μM / Right: 14.6 μM), iii) fluorescein (Left: 20.0 μM / Right: 10.0 μM), iv) 2,2-diphenyl-1-picrylhydrazyl (DPPH; Left: 16.8 μM / Right: 8.4 μM), v) eosin Y (Left: 8.0 μM / Right: 4.0 μM), vi) rhodamine B (Left: 5.8 μM / Right: 1.4 μM), vii) trypan blue (Left: 10.4 μM / Right: 6.2 μM), viii) prussian blue (Left: 19.2 μM / Right: 10.0 μM), ix) malachite green (Left: 6.0 μM / Right: 4.0 μM), x) methylene blue (Left: 10.0 μM / Right: 4.0 μM), xi) chlorophyll b (Left: 8.8 μM / Right: 4.4 μM).

Analytical protocols of the six photometric assays. We summarize the type of assay (*e.g.*, ELISA, enzymatic assay) used for each test, and special characteristics of the absorbance

peaks that are used as an analytical signal in Table S-4. The absorbance spectra of the chromogenic products of the assays are shown in Figure S-13. The analytical characteristics of the calibration lines of the assays are shown in Figure S-6. The protocols we followed are the following:

Lactate assay: We followed the "Instructions for Use" supplied with the Lactate Assay Kit (BioVision). The kit contained all the necessary reagents (Lactate Assay Buffer, Lactate Probe, Lactate Enzyme Mix and a 100 nmol/μL Lactic Acid Standard solution). We first prepared solutions of lactate at different concentrations (40, 80, 120, 160, 200 μg/mL) to construct a calibration curve. We then diluted the human serum samples (human serum Innovative Research Inc) 1:10 with Lactate Assay Buffer. We added 50 μL of standard or sample per well and then added 50 μL of the Reaction Mix (consisting of 46:2:2 Lactate Assay Buffer, Lactate Probe, Lactate Enzyme Mix). We covered the wells with a sealing tape and incubated for 30 minutes in room temperature protected from light. Then, we read the absorbance on a microplate spectrophotometer at a wavelength of 570 nm and captured an image of the plate using the *flatbed scanner* in transmittance mode or the *camera-based photometer*.

Low-density lipoprotein (LDL) ELISA: We followed the "Instructions for Use" supplied with the LDL Human ELISA Kit (Abcam). The kit included a 96-well plate, with LDL specific antibodies immobilized onto each well, and all the necessary stock reagents (LDL standard, biotinylated LDL, Streptavidin-Peroxidase Complex, Chromogen Substrate, Wash Buffer). We first prepared working solutions of the necessary reagents. We then prepared solutions of LDL at different concentrations (6.25, 25, 50, 100, 200 μg/mL) to construct a calibration curve. We also dilute 1:50 the human serum samples (human serum Innovative Research Inc). We added 25 μL of standard or sample per well and immediately added 25 μL of biotinylated LDL. We covered

the wells with a sealing tape and incubated for two hours at 37 ˚C. We then washed each well five times with 200 µL of wash buffer manually. Then we added 50 µL of the Streptavidin-Peroxidase Complex to each well and we incubated them for 30 minutes. We then washed each well five times with 200 µL of wash buffer manually. We then added 50 µL of the Chromogen Substrate (containing TMB) in each well and we incubated them for 12 minutes. Then we added 50 µL of Stop Solution to each well and immediately read the absorbance on a microplate spectrophotometer at a wavelength of 450 nm and captured an image of the plate using the *flatbed scanner* in transmittance mode or the *camera-based photometer*.

Anti-*Treponema pallidum IgG Human ELISA:* We followed the "Instructions for Use" supplied with the Anti-Treponema pallidum IgG Human ELISA Kit (Abcam). The kit included a 96-well plate, with recombinant Treponema pallidum antigens immobilized onto each well, and all the necessary stock reagents (IgG Sample Diluent, Stop Solution, Washing Solution, Treponema pallidum anti-IgG HRP Conjugate, TMB Substrate Solution, Treponema pallidum Positive Control, Treponema pallidum Cut-off Control, Treponema pallidum Negative Control). We first prepared working solutions of the necessary reagents. We then dilute 1:10 the human serum samples (VIROTROL® Syphilis Total Controls, human serum Innovative Research Inc). We added 100 µL of controls or sample per well and covered the wells with a sealing tape and incubated for one hours at 37 ˚C. We then washed each well three times with 300 µL of wash buffer manually. Then we added 100 µL of the Treponema pallidum anti-IgG HRP Conjugate to each well and we incubated them for 30 minutes. We then washed each well three times with 300 µL of wash buffer manually. We then added 100 µL of the TMB Substrate Solution in each well and we incubated them for 15 minutes. Then we added 100 µL of Stop Solution to each well and immediately read the absorbance on a microplate spectrophotometer at a wavelength of 450 nm

and captured an image of the plate using the *flatbed scanner* in transmittance mode or the *camera-based photometer*.

Cyanomethemoglobin Assay: We followed the "Instructions for Use" supplied with the Drabkin's Reagent (Sigma Aldrich). We first prepared the Drabkin's Solution by reconstituting one vial of the Drabkin's Reagent with 1000 mL of water, and adding 0.5 mL of a 30% Brij 35 solution. We then prepared a Cyanmethemoglobin Standard Solution containing 18 mg/mL of human hemoglobin in Drabkin's Solution. We prepared solutions of cyanmethemoglobin at different concentrations (60, 80, 100, 120, 160, 180 mg/mL) to construct a calibration curve. We also diluted 1:250 the blood samples (Meter Trax Control Level 1, Meter Trax Control Level 2 and Meter Trax Control Level 3) in Drabkin's Solution and incubated for 15 minutes in room temperature. We added 350 μL of the solutions per well and then we read the absorbance on a microplate spectrophotometer at a wavelength of 540 nm and captured an image of the plate using the *flatbed scanner* in transmittance mode or the *camera-based photometer*.

Bicinchoninic acid (BCA) assay: We followed the "Instructions" supplied with the Micro BCA Protein Assay Kit (Thermo Scientific). We first prepared the Micro BCA Working Reagent by mixing 25 parts of Micro BCA Reagent MA, 24 parts of Micro BCA Reagent MB and 1 part of Micro BCA Reagent MC. We diluted the samples (antibodies raised in rabbit, mouse or goat) 1:50 in Phosphate Buffer Saline, pH 7.4. We then prepared solutions of albumin at different concentrations (1.0, 5.0, 10.0, 20.0, 40.0 μg/mL) to construct a calibration curve. We added 150 μL of controls or sample and 150 μL of the Micro BCA Working Reagent in each well and incubated for 2 hours at 37 ˚C. We then let the solutions to cool to room temperature and we read the absorbance on a microplate spectrophotometer at a wavelength of 560 nm and

captured an image of the plate using the *flatbed scanner* in transmittance mode or the *camera-based photometer*.

Griess Assay: We followed the "Instructions" supplied with the Griess Reagent (Sigma Aldrich). We first prepared the Griess Solution by reconstituting Griess Reagent with 250 mL of water. We then prepared solutions of nitrite ions at different concentrations (1.0, 7.0, 15.0, 30.0, 60.0 μM) to construct a calibration curve. We added 200 μL of controls or samples (tap or bottled water) and 200 μL of the Griess Reagent in each well and incubated for 15 minutes in room temperatures. We read the absorbance on a microplate spectrophotometer at a wavelength of 540 nm and captured an image of the plate using the *flatbed scanner* in transmittance mode or the *camera-based photometer*.

Calculation of RGB-resolved Absorbance using the *flatbed scanner*. We captured the images of the microtiter plates contained the samples using the *flatbed scanner* in transmittance mode. Using the software bundled with the scanner (Epson), we disabled all automatic correction functions (Figure S1) and saved all images as Joint Photographic Experts Group (JPEG) files. We next read the RGB values of three pixels (chosen at random from within the image of each of the wells that contained solutions of the test compounds) with Image J or Microsoft Paint and recorded the mean values C_k of each group of RGB values. We finally calculated the RGB-resolved absorbance A_k of each sample using Equation 3 of the main text $A_k \equiv -\log_{10}\left[\frac{C_k^{(S)}}{C_k^{(B)}}\right]$ and the guidelines for the selection of appropriate color value.

Calculation of RGB-resolved Absorbance using the *camera-based photometer*. The planar light source used in the *camera-based photometer* was large enough to illuminate 32 wells

of a standard 96-microtiter plate, and therefore, we required three separate images (Figure S2A-C) to capture an entire plate. We adjusted the settings on the camera of the cell-phone (LG OPTIMUS F3 4G LTE) during image capture to the following: i) Focus: Auto, ii) ISO: 400, iii) White balance: Auto, iv) Brightness: 0.0, v) Image size: 5M (2560×1920), vi) Color effect: None. The cell-phone automatically saved images in the Joint Photographic Experts Group (JPEG) format. After importing the images to a personal computer, we read the RGB values of three pixels (chosen at random from within the image of each of the wells that contained solutions of the test compounds) with Image J or Microsoft Paint and recorded the mean values C_k of each group of RGB values.

To eliminate the influence of the slight spatial gradients in the intensity of light emitted by the planar light source, we used the following procedure. For each assay, we first captured a baseline image of 32 wells of a standard 96-microtiter plate filled with blank solutions and then calculated $C_k^{(B)}$ for each well. The relative standard deviation of the $C_k^{(B)}$ values of different wells in this baseline measurement was ~2 % (N=32 wells). We next captured the sample image of 32 wells filled with 28 samples and 4 blank solutions. To account for any changes in the white balance from image to image (cell-phone cameras typically adjust white balance automatically), we estimated a white balance correction factor $W = \text{mean}\left\{C_k^{(B,\,\text{sample})}\right\} / \text{mean}\left\{C_k^{(B,\,\text{baseline})}\right\}$, where $C_k^{(B,\,\text{sample})}$ are the RGB values of the 4 blank solutions in the sample image and $C_k^{(B,\,\text{baseline})}$ are the RGB values of the corresponding wells in the baseline image. We next corrected the color values of the sample image by $C_k^{(S)} = WC_k^{(S,\,\text{raw})}$. We finally calculated the RGB-resolved absorbance A_k of each sample using these corrected values, Equation 3 of the main text ($A_k \equiv -\log_{10}\left[\frac{C_k^{(S)}}{C_k^{(B)}}\right]$), and the guidelines for the selection of appropriate color value.

Estimation of the spectral sensitivity S(λ) and the gamma correction factors γ_k for RGB-based images sensors. Each individual pixel sensor of a RGB-based imaging device employs a CCD or CMOS photodetector with a spectral responsivity $R(\lambda)$, and red, green and blue color filters, each with transmittance $F_k(\lambda)$, where k = {R, G, B}. The total spectral sensitivity of each pixel sensor is $S_k(\lambda) = R(\lambda)F_k(\lambda)$. Although we cannot measure these individual contributions to $S_k(\lambda)$ independently, we estimated this function by using an external light source, a monochromator, and a fiber optic cable to deliver a pseudo delta-function inputs to the pixel sensors such that $I_n(\lambda) = i_n\delta(\lambda - \lambda_n)$, where λ_n is the central wavelength, $i_n = \int_0^\infty I_n(\lambda)d\lambda$ is the total intensity of the narrowband input with index (defined by the monochromator). We determined the relationship between the measured color values and sensitivity of each channel to be $C_{k,n} = \beta_k\left(\int_{\lambda_1}^{\lambda_2} i_n\ \delta(\lambda - \lambda_n)\cdot T\cdot S_k(\lambda)d\lambda\right)^{\gamma_k} = \beta_k\left[i_n T S_k(\lambda_n)\right]^{\gamma_k}$, where T accounts for all the losses between the monochromator and the RGB detector. Through an independent measurement of i_n and with a fiber-coupled spectrophotometer, we estimated S_k (up to unknown factors of γ_k, β_k, and T) by Equation S-1.

$$S_k(\lambda_n) = \left(\frac{1}{\beta_k}C_{k,n}\right)^{1/\gamma_k} / i_n T \qquad \text{(Eq. S-1)}$$

Because S_k is approximately continuous between the different λ_n that we measured, we used Mathematica to interpolate the collected values $C_k(\lambda_n) \to C_k(\lambda)$ and $i_n \to i(\lambda)$ to form the continuous function $S_k(\lambda_n) \to S_k(\lambda)$. Inspection of Equation 6 from the main text shows that the unknown linear factors β_k and T will drop out and therefore not contribute to the measurement of absorbance.

To calculate the values of γ_k for the RGB sensor, we first measured the RGB-resolved absorbance values $A_k^{(measured)}$ for 11 different dyes at different concentrations. We then calculated the expected RGB-resolved absorbance $A_k^{(expected)}$ by substituting the interpolated function $S_k(\lambda)$, given in Equation S-1, into Equation 6, and used the absorption spectra $A(\lambda)$ of all the dyes (measured on by a spectrophotometer) to calculate A_k for each color channel by Equation S-2.

$$A_k = -\gamma_k \log \frac{\left[\int_{\lambda_1}^{\lambda_2} L(\lambda) \times 10^{-A_S(\lambda)} \times \left(\frac{C_k(\lambda)}{i(\lambda)}\right)^{1/\gamma_k} d\lambda\right]}{\left[\int_{\lambda_1}^{\lambda_2} L(\lambda) \times 10^{-A_B(\lambda)} \times \left(\frac{C_k(\lambda)}{i(\lambda)}\right)^{1/\gamma_k} d\lambda\right]} \qquad \text{(Eq. S-2)}$$

We plotted all the values of $A_k^{(measured)}$ *vs* $A_k^{(expected)}$, and used γ_k as a single free parameter fit the data to unity slope (within $\pm\ 0.001$), shown in Figure S-12. Using this approach, we determined γ_k for the scanner to be $\gamma_R = 0.617$, $\gamma_G = 0.472$, $\gamma_B = 0.498$, and γ_k for the camera to be $\gamma_R = 0.724$, $\gamma_G = 0.525$, $\gamma_B = 0.778$.

Definition of Standard and Peak Absorbance. The spectral intensity $I(\lambda)$ of light that is transmitted by an arbitrary sample is $I(\lambda) = I_0(\lambda)10^{-A(\lambda)}$, where $I_0(\lambda)$ is the spectral intensity of the light source and $A(\lambda)$ is the absorbance of the sample at any given wavelength λ. The transmittance of the sample, relative to free space, therefore, is $T(\lambda) = I(\lambda)/I_0(\lambda) = 10^{-A(\lambda)}$ and $A(\lambda) = -\log T(\lambda)$. In general, $A(\lambda) = \varepsilon(\lambda) \times d \times c + A_b(\lambda)$ (known as the Beer-Lambert law), where $\varepsilon(\lambda)$ is the molar extinction coefficient of the sample, d is the optical path length through the sample, and c is the concentration of the sample, and $A_b(\lambda)$ is the absorption of the

background (or blank). To eliminate the contribution of the background absorption of the solution (not related to the concentration of the sample), the absorbance is typically defined relative to a blank solution, as shown in Equation S-3.

$$A = A_s(\lambda) - A_b(\lambda) = -\log\left[\frac{I_s(\lambda)}{I_b(\lambda)}\right] = \varepsilon(\lambda) \times d \times c \qquad \text{(Eq. S-3)}$$

The peak absorbance A_{peak} occurs at the wavelength λ_{peak} where A is a maximum, and is also linearly related to c. After calibrating a system with standard concentrations of a known dye, measuring A_{peak} enables a highly sensitive determination of an unknown concentration c of the known dye. This measurement is the standard method of determining the absorbance of a sample and requires sophisticated equipment (a spectrophotometer) that incorporates a monochromator or a narrowband filter to isolate the bandwidth of the measurement narrowly around λ_{peak}, and to tune the value of λ_{peak} to match spectral characteristics the compound being tested.

The narrowband absorbance defined by Eq. S-3 is a special case of the more generalized broadband absorbance defined by Equation 4 in the main text. The spectral distribution of a spectrophotometer is controlled by a diffraction grating that disperses the light so that each element of the photodetector array only receives a very narrow band of wavelengths. We can approximate this behavior by replacing the broadband spectral sensitivity by a delta-like function such that $S_k(\lambda) \rightarrow S_k(\lambda)\delta(\lambda - \lambda_0)$, where λ_0 is the central wavelength of the light diffracted to each pixel. Assuming that $\gamma_k = 1$ for the photodetector array, Equation 4 of the main text reduces to Eq. S-4.

$$A = -\log\left[\frac{\int_{\lambda_1}^{\lambda_2} L(\lambda)\cdot 10^{-A_S(\lambda)}\cdot S(\lambda)\cdot\delta(\lambda-\lambda_0)\,d\lambda}{\int_{\lambda_1}^{\lambda_2} L(\lambda)\cdot 10^{-A_B(\lambda)}\cdot S(\lambda)\cdot\delta(\lambda-\lambda_0)\,d\lambda}\right] = -\log_{10}\left[\frac{L(\lambda_0)\cdot 10^{-A_S(\lambda_0)}\cdot S(\lambda_0)}{L(\lambda_0)\cdot 10^{-A_B(\lambda_0)}\cdot S(\lambda_0)}\right] = A_S(\lambda_0) - A_B(\lambda_0) \qquad \text{(Eq. S-4)}$$

Choice of RGB color system rather than grayscale or HSV. Although we are free to explore any of the available methods for representing color intensity, we chose to use the R, G, or B values because: i) most digital imaging devices use the RGB color system to digitize the image; ii) RGB values of the pixels of an image are easily read using commonly available software (*e.g.* Microsoft Paint, Adobe Illustrator, Image J); iii) RGB values can be considered as metrics of the total light intensity within certain bandwidths corresponding to the light that passes through the red, green and blue filters that are present on the surface of the CCD/CMOS photodetector. The intensity of the gray scale value (x) and the hue (H) component of hue-saturation-value (HSV) color system are other parameters that have been used in the past to correlate the color of the sample with the concentration of an analyte.[13] These values are simply linear (gray scale) or nonlinear (hue) combinations of the recorded RGB values, and are defined by Equation S-5 and Equation S-6.

$$x = 0.299R + 0.587G + 0.114B, \qquad \text{(Eq. S-5)}$$

$$H = \begin{cases} \cos^{-1}\left[\dfrac{\left(R - \sqrt{G} - \sqrt{B}\right)}{\sqrt{\left(R^2 + G^2 + B^2 - RG - RB - GB\right)}}\right], & \text{if } G \geq B \\ 360 - \cos^{-1}\left[\dfrac{\left(R - \sqrt{G} - \sqrt{B}\right)}{\sqrt{\left(R^2 + G^2 + B^2 - RG - RB - GB\right)}}\right], & \text{if } B \geq G \end{cases} \qquad \text{(Eq. S-6)}$$

Spectral peaks, however, typically overlap most strongly with only one RGB color channel. Combining these RGB values into grayscale or HSV values, therefore, adds information about light intensity that is not related to the concentration of the sample, and consequently, makes these color values less sensitive to changes in concentration than the raw RGB values.

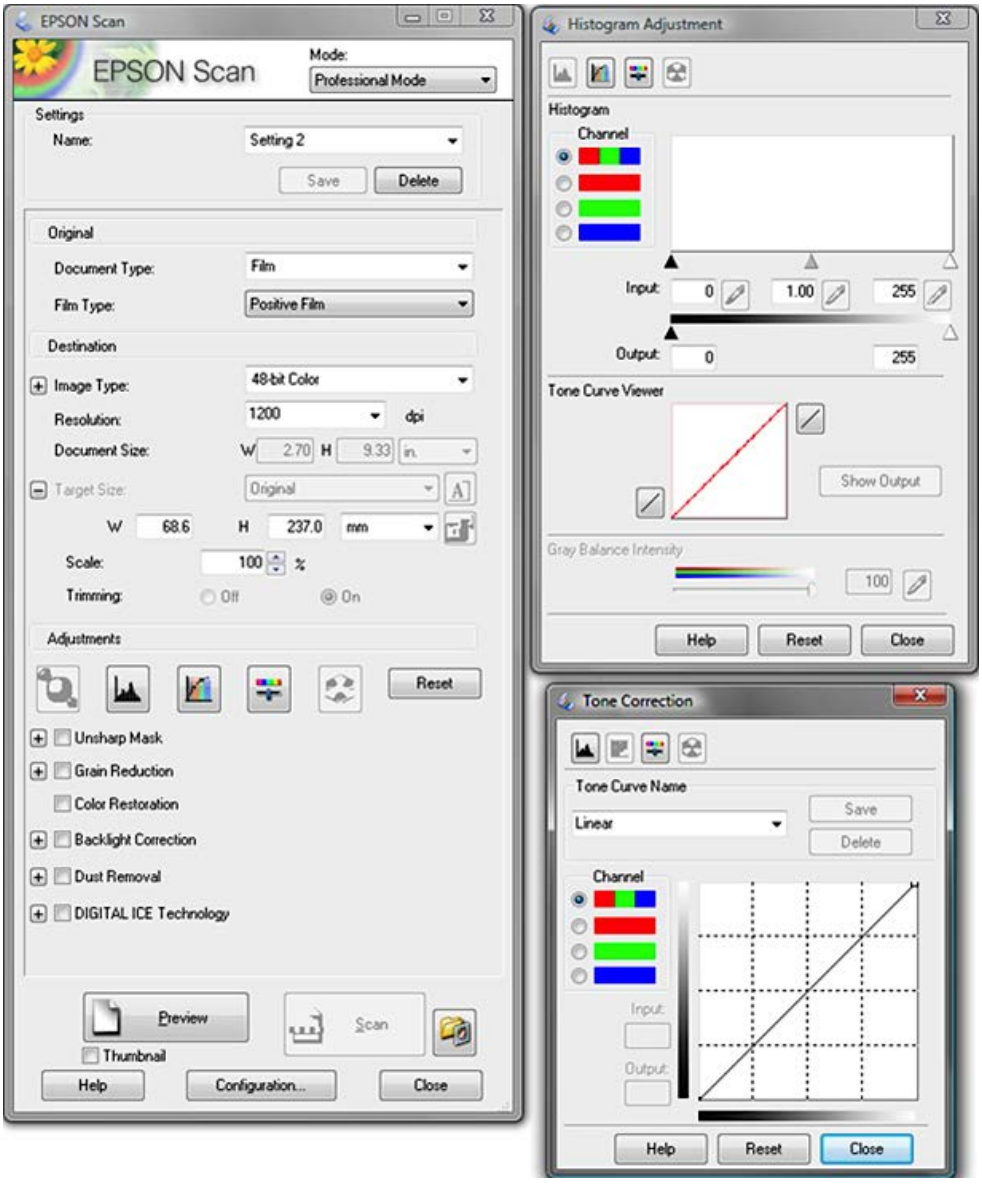


Figure S-1. Screenshots of the control window of the software of the scanner

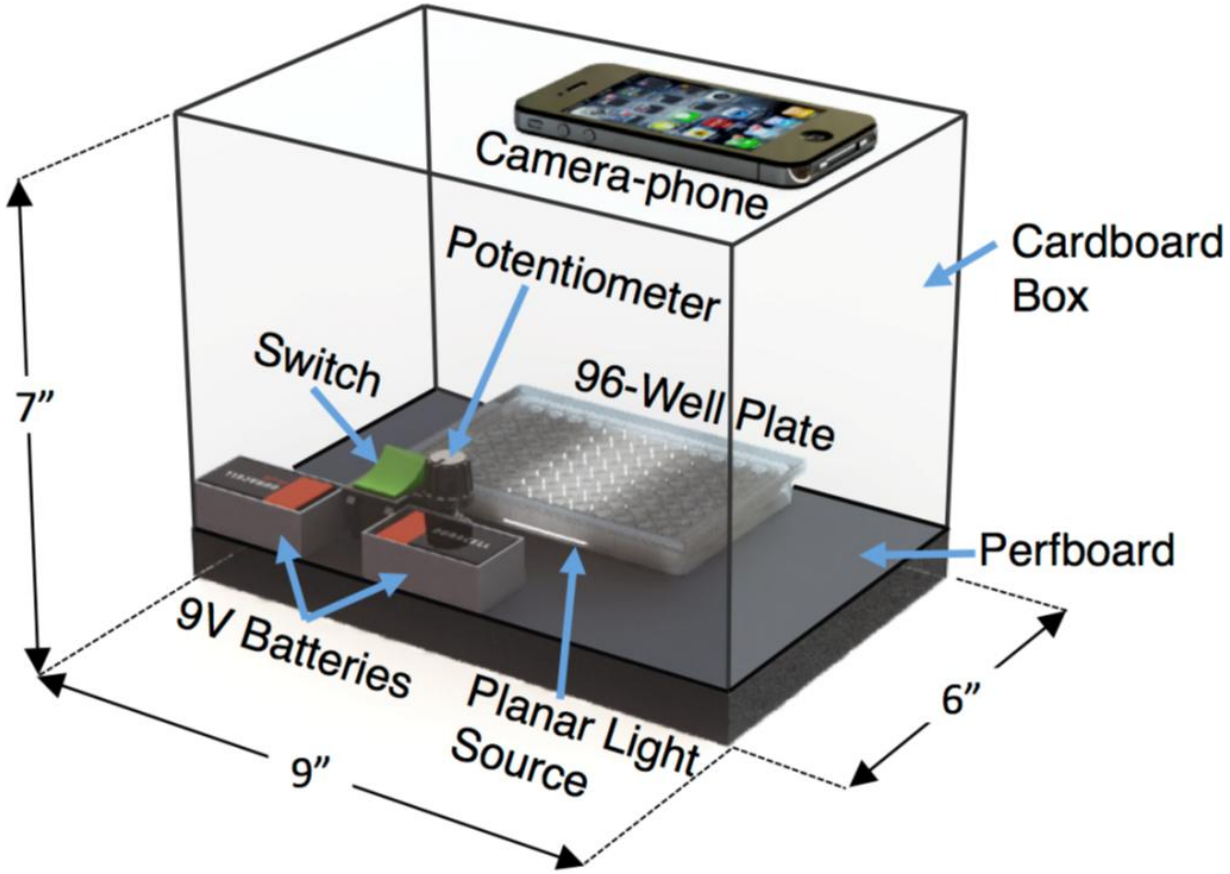


Figure S-2. Schematic of the *camera-based photometer*

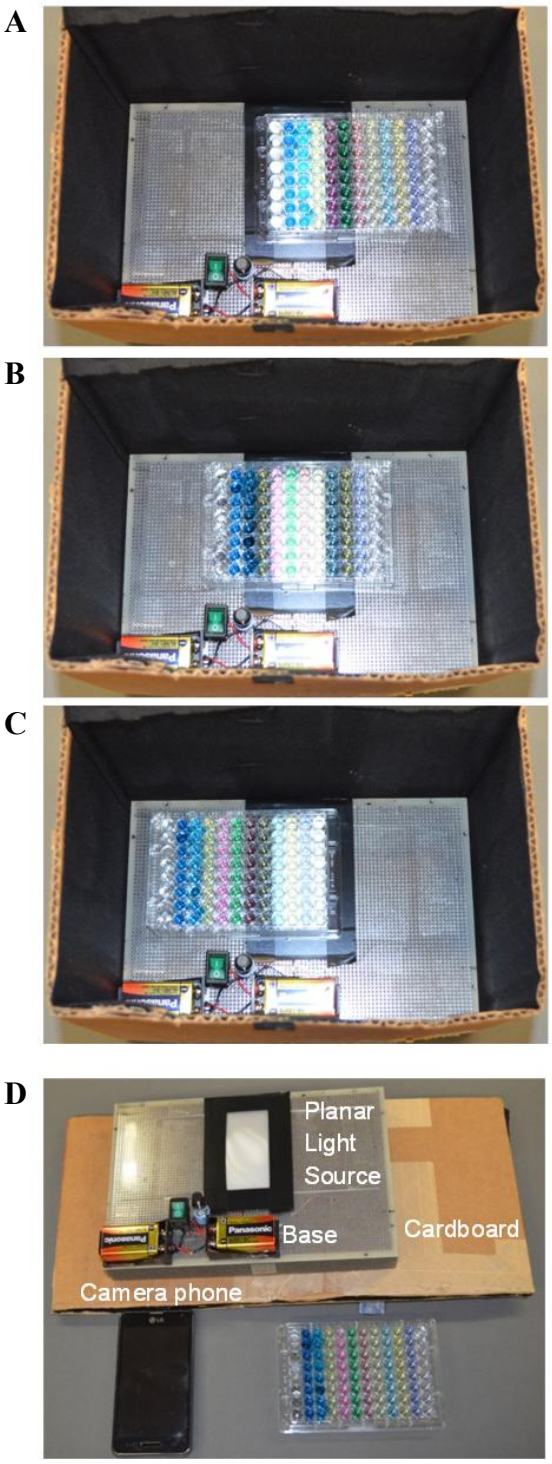


Figure S-3. A-C) Images of a standard microtiter plate containing solutions of 11 chromogenic dyes inside the *camera-based photometer*. D) Image of the *camera-based photometer* disassembled, including a cell-phone, a microtiter plate containing colored dyes, a folded cardboard box, and the base.

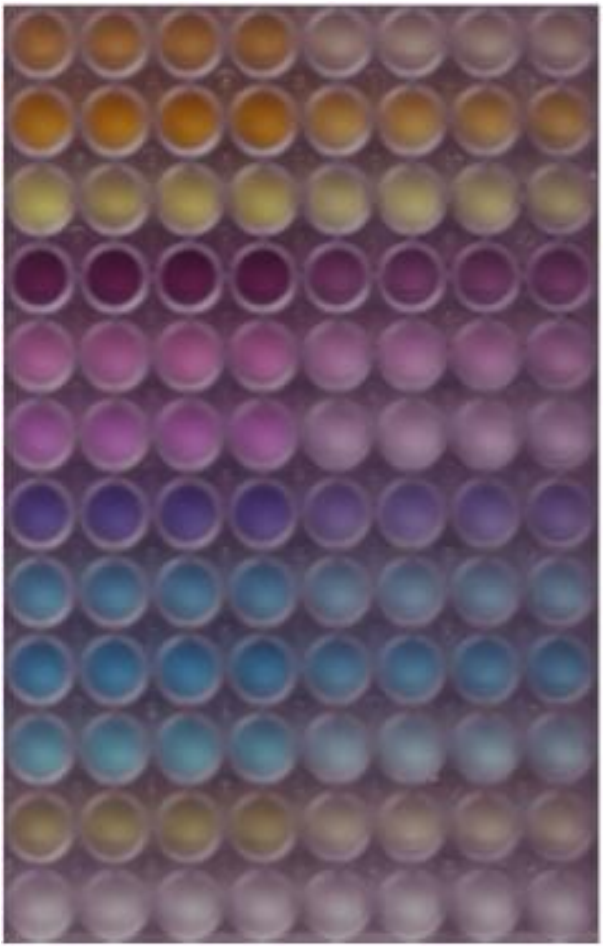


Figure S-4. An image of a microtiter plate, containing solutions of 11 dyes (one per row; lowest/twelfth row contains an aqueous blank) in two concentrations (four left columns *vs.* four right columns) captured by the CIS scanner in reflectance mode

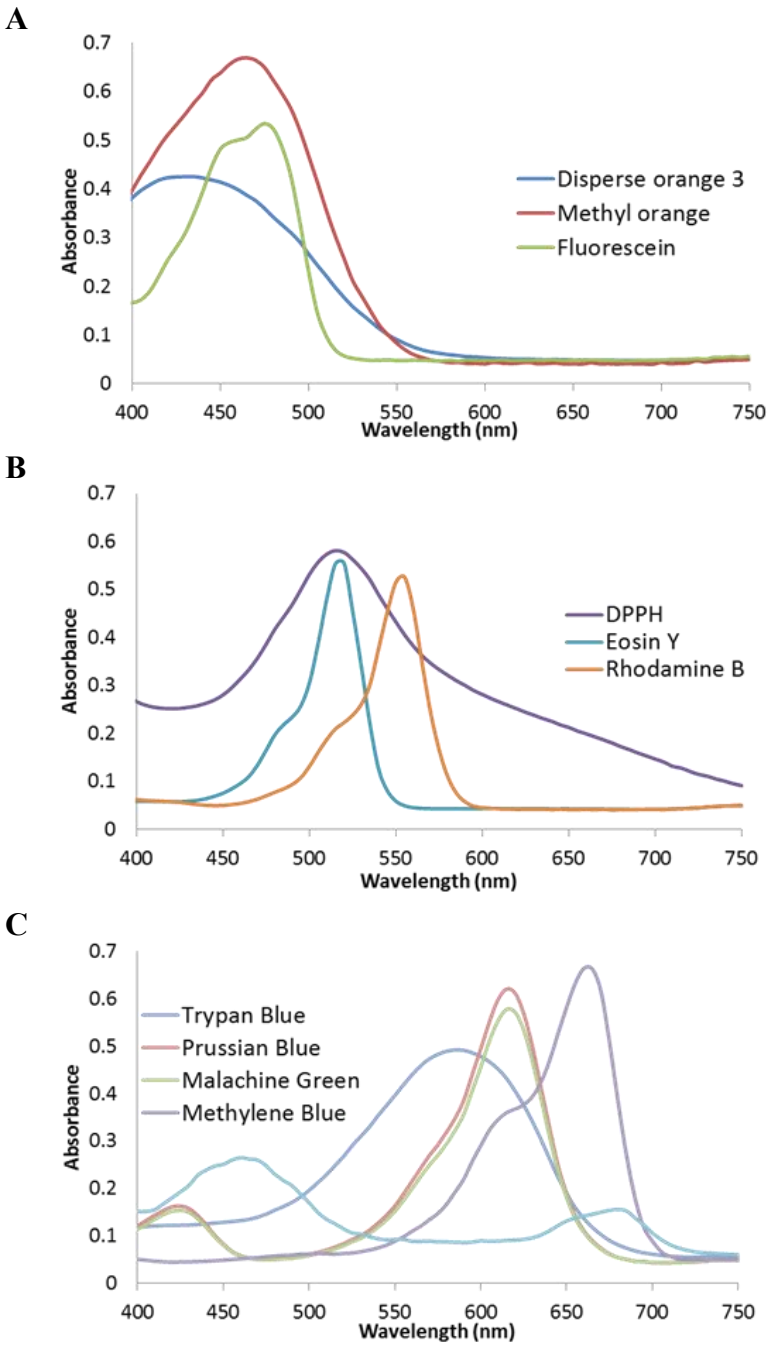


Figure S-5. A) Absorbance spectra of disperse orange 3 in ethanolic solution (7.0 μM), methyl orange in aqueous solution (29.3 μM), and fluorescein in aqueous solution (20.0 μM). B) Absorbance spectra of DPPH in methanolic solution (16.8 μM), eosin Y in aqueous solution (8.0 μM), and rhodamine B in aqueous solution (5.8 μM). C) Absorbance spectra of trypan blue in aqueous solution (10.0 μM), prussian blue in aqueous solution (19.2 μM), malachine green in aqueous solution (6.0 μM), methylene blue in aqueous solution (10.0 μM), and chlorophyll b in aqueous solution (8.8 μM).

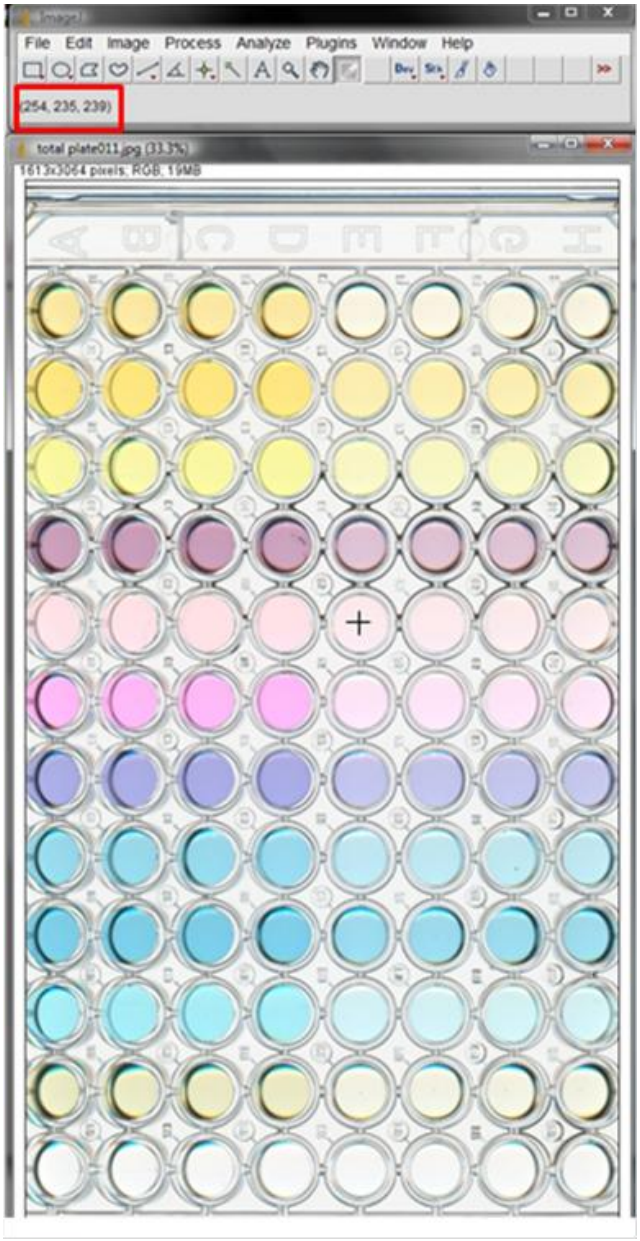


Figure S-6. A screenshot that shows how to use Image J to read the RGB color value of a well containing a solution. The RGB values are outlined by the red box.

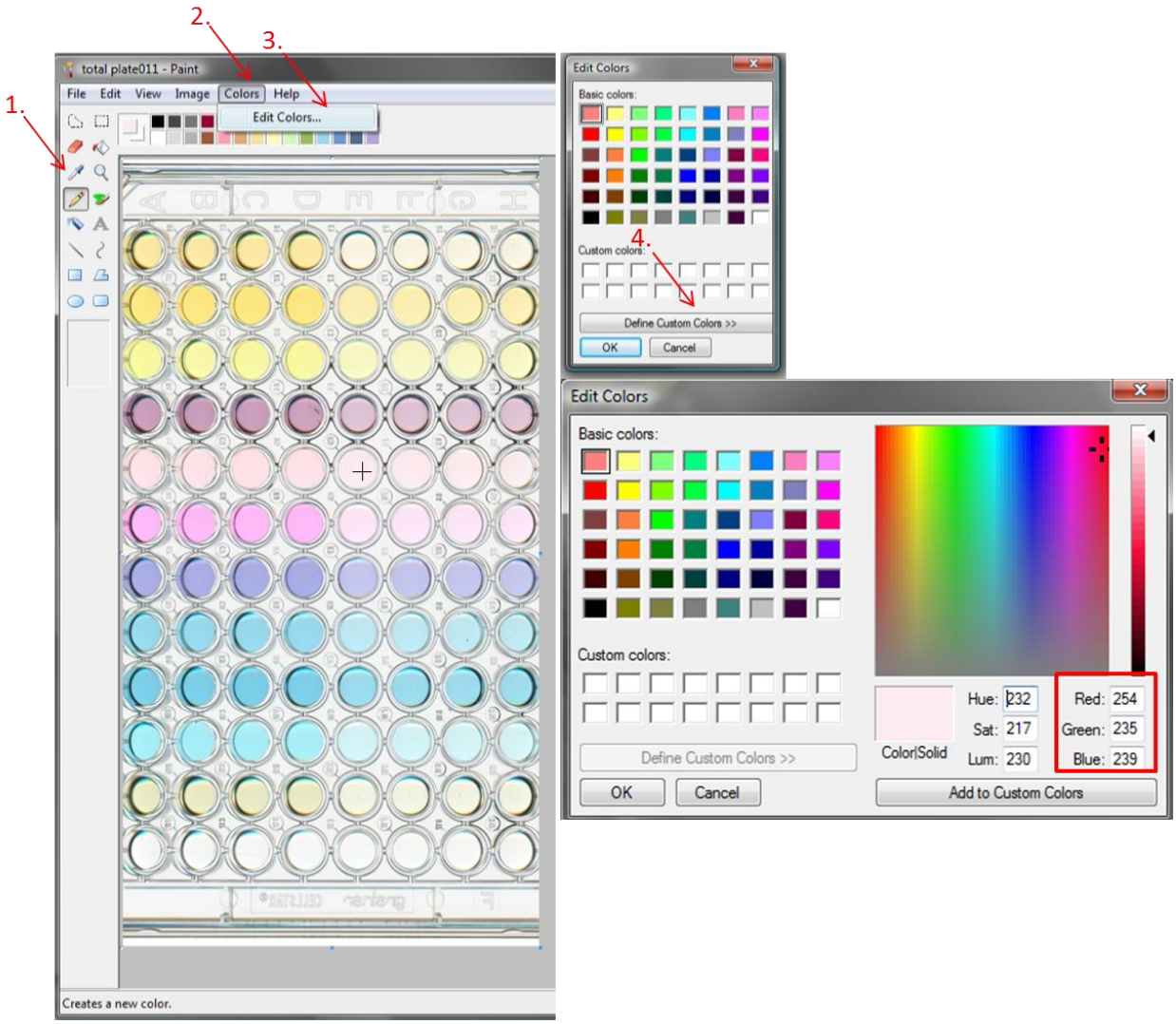


Figure S-7. A screenshot that shows how to use Microsoft Paint to read the RGB color value of a well containing a solution. The necessary steps prior to the reading are indicated: 1) choose eye-dropper tool and select a pixel, 2) select Colors, 3) select Edit Colors, 4) select Define Custom Colors. Finally, read out the RGB values (outlined by the red box).

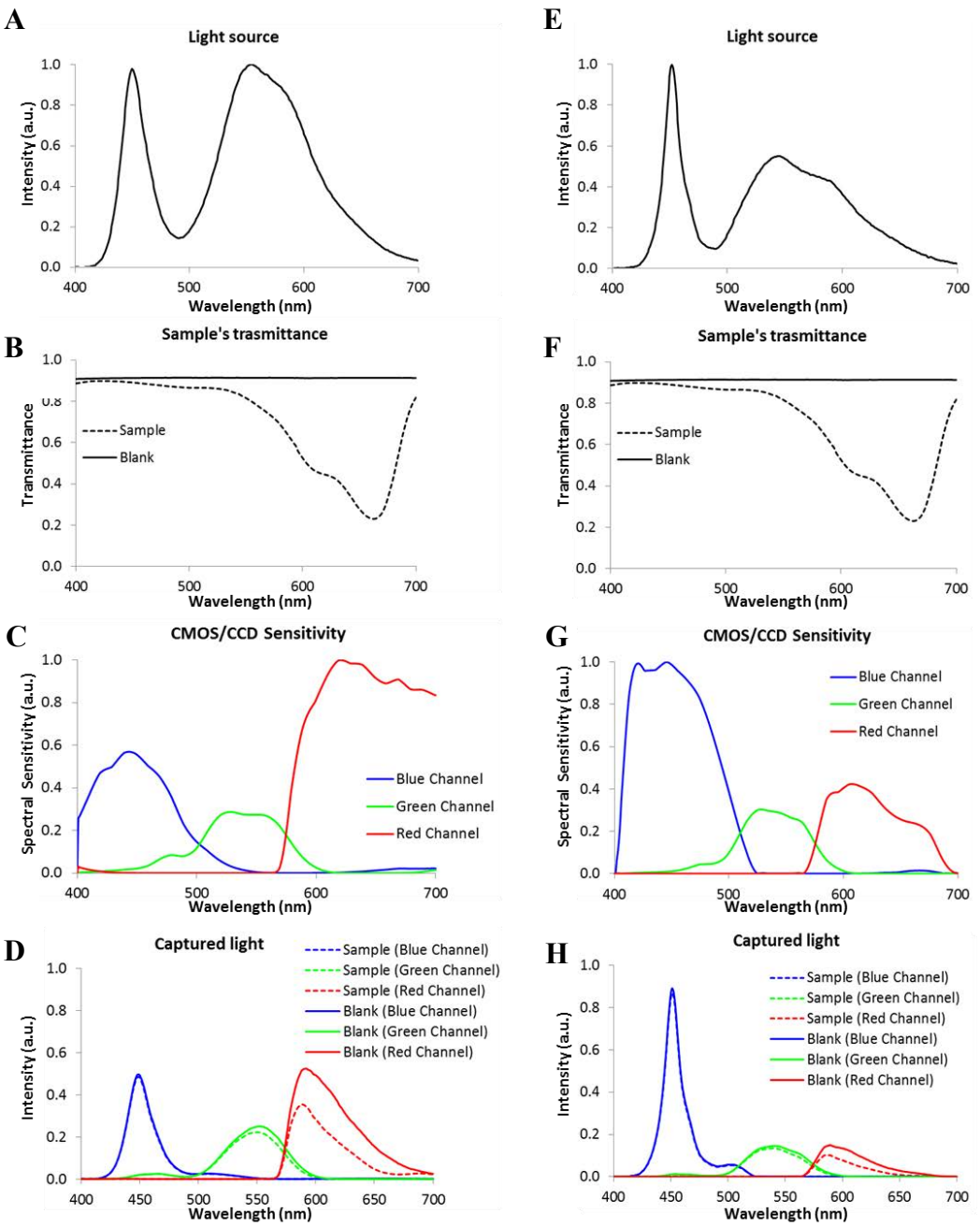


Figure S-8. i) Characterization of the spectrum of the light emitted from the light source of the *flatbed scanner* (A) or the planar light source (E). ii) Transmittance spectra of a 10-μM methylene blue solution (sample) and DI water (blank) measured in the *microplate spectrophotometer* (B, F). iii) Spectral sensitivity, $S_k(\lambda)$, of the image sensor of the scanner (C) and the cell-phone camera (G). iv) The light intensity that reach the CCD/CMOS detector of the *flatbed scanner* (D) and the cell-phone camera (H) during the imaging of a 10 μM methylene blue solution (sample) and DI water (blank).

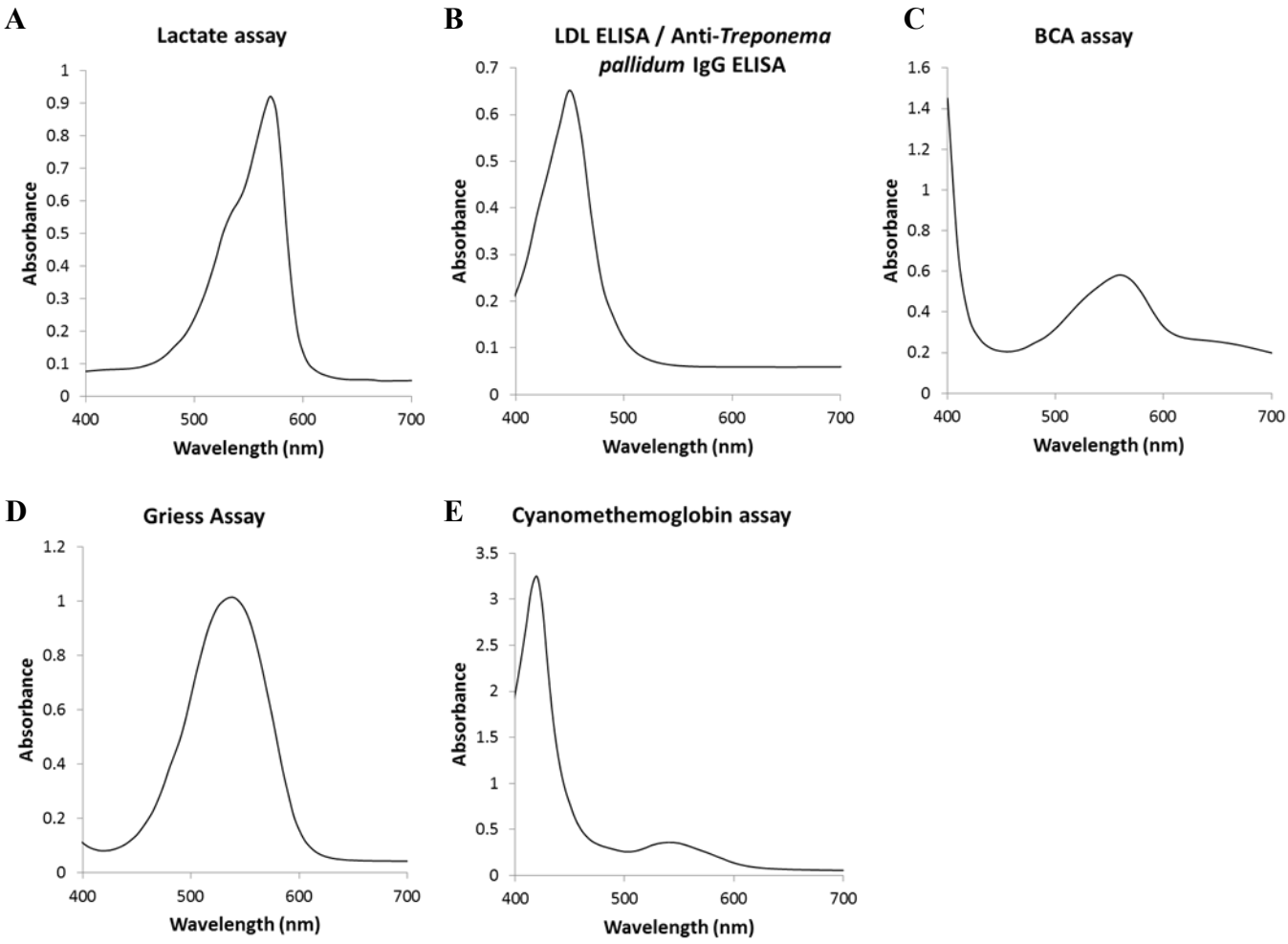
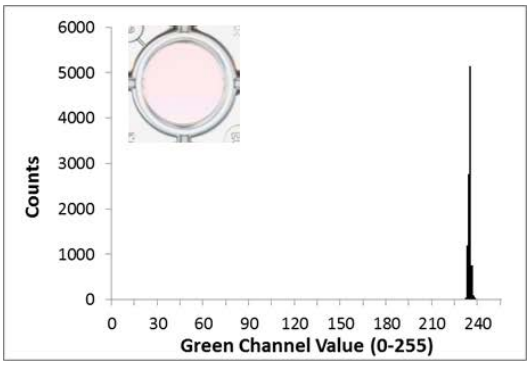
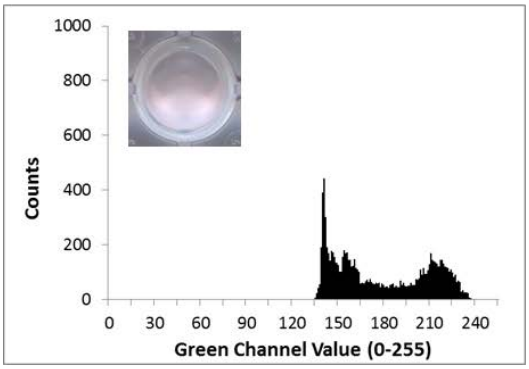


Figure S-9. Absorbance spectra of the chromogenic products of the following six assays: A) Lactate assay (lactic acid, 160 µg/mL), B) LDL / Anti-*Treponema pallidum* Ig ELISAs (blank solution / cut-off control), C) BCA assay (albumin, 40.0 µg/mL), D) Cyanomethemoglobin assay (hemoglobin, 160 mg/mL), E) Griess assay (nitrite ions, 60.0 µM)

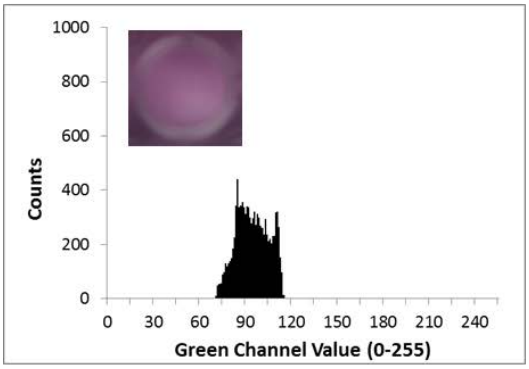
A



B



C

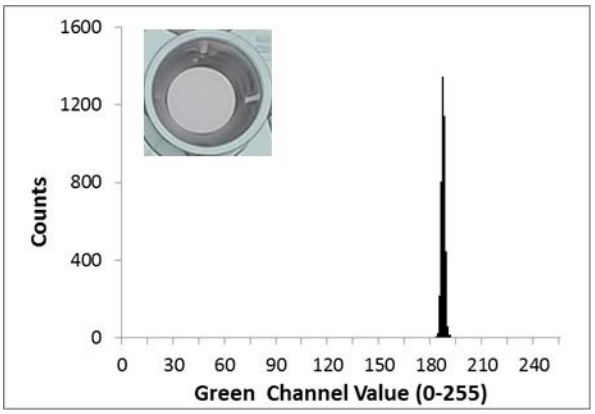


D

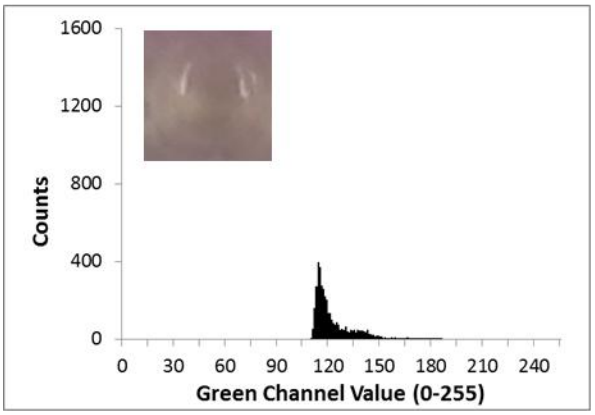
	Transmission	Reflection	Reflection (CSI)
Mean	234.6	178.2	94.8
Mode	235	141	85
Min	231	135	71
Max	238	237	117
% RSD	0.4	17.3	11.1
Total Counts	10024	10024	10024

Figure S-10. Histograms of green channel of an image of a well (inset) containing a 6.00 μM Eosin Y solution captured using the *flatbed scanner* in (A) transmittance mode, (B) reflectance mode, and (C) a scanner (using CSI technology) in reflectance mode. D) Table containing the characteristics of the distributions.

A



B



C

	Transmission	Reflection
Mean	187.2	124.2
Mode	187	114
Min	182	110
Max	192	186
% RSD	0.6	11.2
Total Counts	4060	4060

Figure S-11. Histogram of the intensity of green channel of an image of a well containing an 6.00 μM Eosin Y solution (inlet picture) captured using (A) the *camera-based photometer* and (B) the cell-phone camera. C) Table containing the characteristics of the distributions.

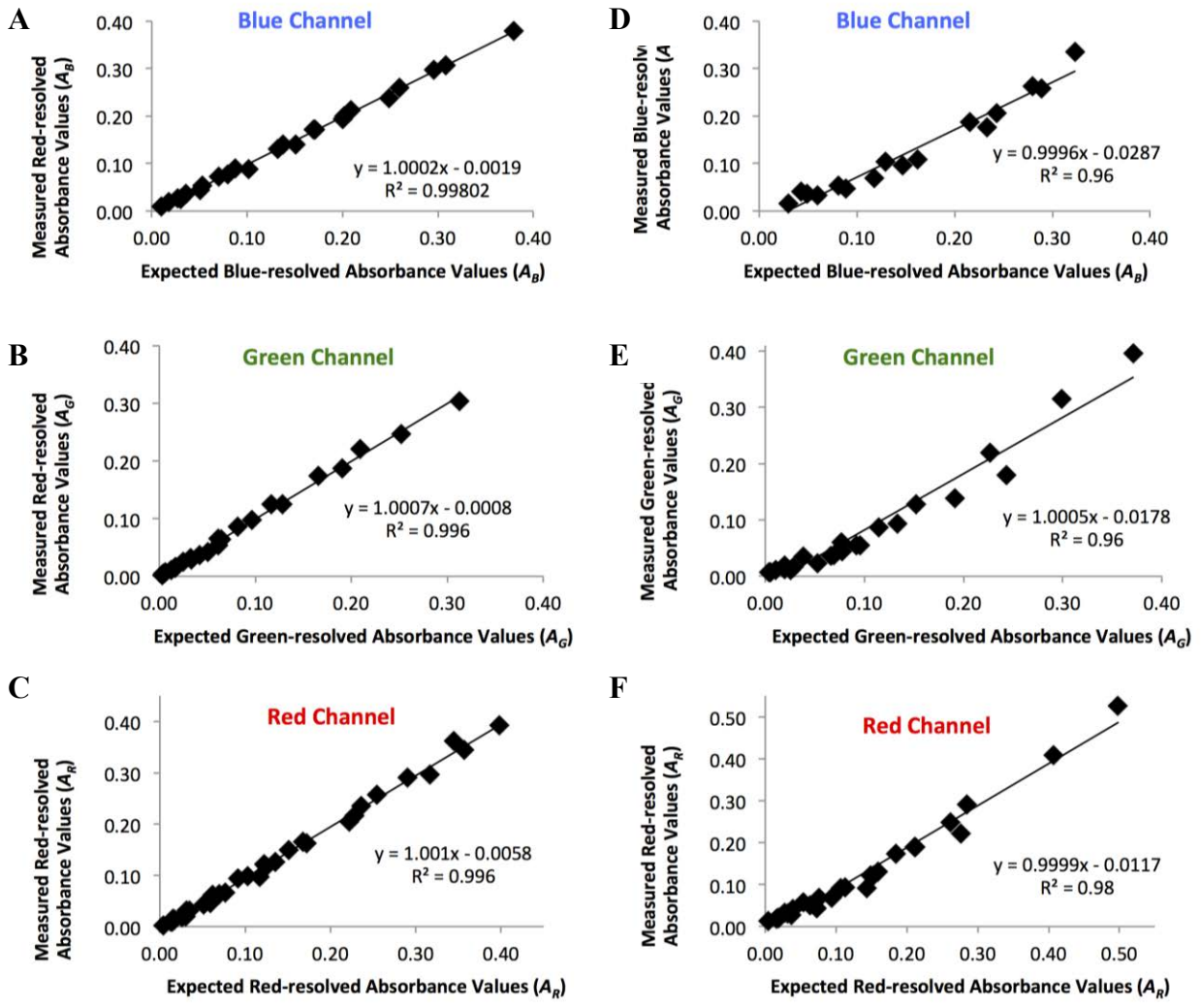


Figure S-12. Correlation plots of measured RGB-resolved Absorbance Values of solutions of 11 dyes measured using the scanner (A-C) and the *camera-based photometer* (D–F) *vs* the expected RGB-resolved absorbance values calculated using Equation S-2. Each of plots only show results from the solutions that absorb most strongly within the bandwidth of the labeled color channel: plots (A, D) show measurements from solutions of disperse orange 3, methyl orange and fluorescein; plots (B, E) show measurements from solutions of DPPH, eosin Y and rhodamine B; plots (C, F) show measurements from solutions of disperse orange 3, methyl orange and fluorescein.

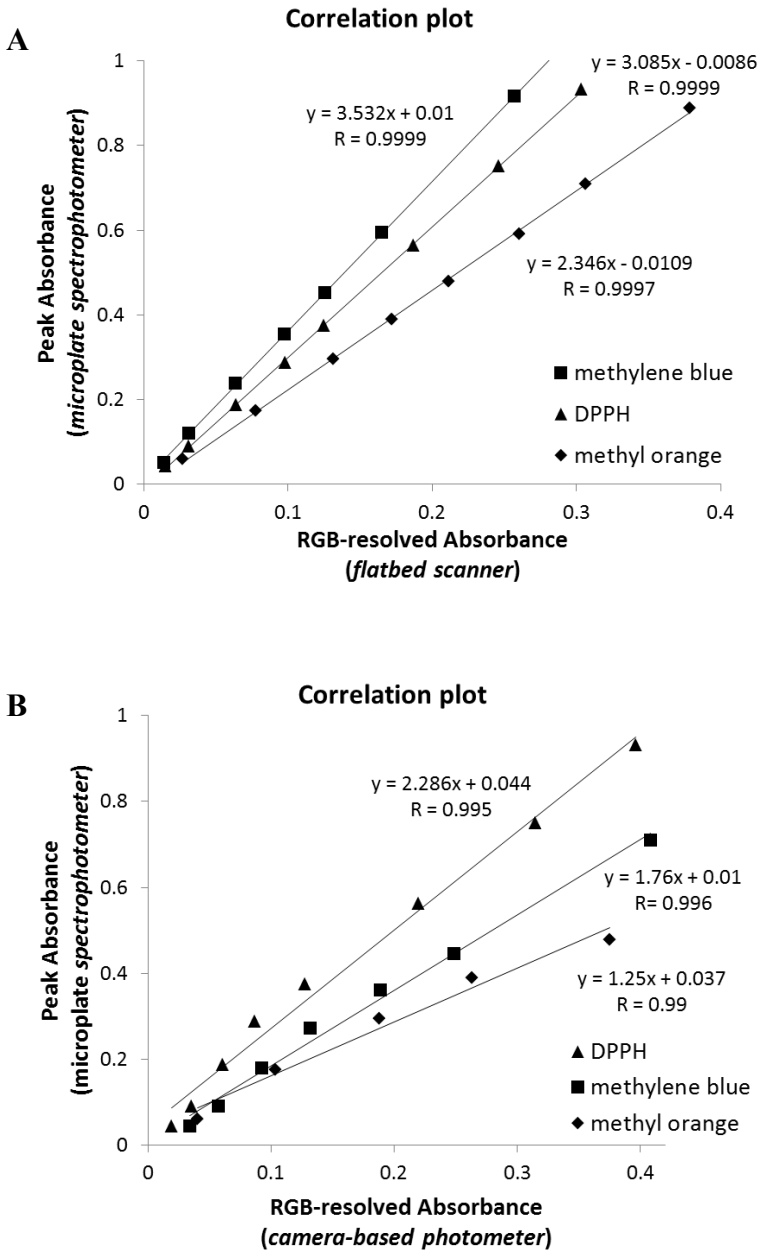


Figure S-13. Correlation plots of peak absorbance values (A_{peak}) of three chromogenic compounds (methylene blue, DPPH, and methyl orange), which absorb in a different color channel, measured using the microplate spectrophotometer *vs* RGB-resolved absorbance values measured using the *flatbed scanner* (A) or the *camera-based photometer* (B). Each data point corresponds to the mean value of seven measurements

Table S-1. Comparison of the analytical characteristics of correlation lines* fitted to the peak absorbance values (A_{peak}, measured by the *microplate spectrophotometer* as the laboratory standard) *vs.* the RGB-resolved absorbance values (A_k, measured by *flatbed scanner* or the *camera-based photometer*) for solutions of 11 different dyes.

Compound	λ_{max}† / Color channel‡	Peak absorbance vs RGB-resolved absorbance (*flatbed scanner*)			Peak absorbance vs RGB-resolved absorbance (*camera-based photometer*)		
		Intercept	Slope	Correlation coefficient	Intercept	Slope	Correlation coefficient
		$a\times10^{-3}$ ($\pm s_a$)	$b\times10^{-3}$ ($\pm s_b$)	R	$a\times10^{-3}$ ($\pm s_a$)	$b\times10^{-3}$ ($\pm s_b$)	R
Disperse orange 3	440 nm /Blue	0.0018 (±0.0009)	2.2002 (±0.0054)	0.99998	0.024 (±0.018)	1.28 (±0.10)	0.99
Methyl orange	465 nm /Blue	-0.0109 (±0.0052)	2.346 (±0.023)	0.9997	0.037 (±0.026)	1.24 (±0.11)	0.999
Fluorescein	485 nm /Blue	-0.0283 (±0.0091)	2.93 (±0.12)	0.997	0.0126 (±0.0069)	2.181 (±0.076)	0.997
DPPH	515 nm /Green	-0.0086 (±0.0028)	3.085 (±0.017)	0.9999	0.044 (±0.020)	2.286 (±0.096)	0.995
Eosin Y	518 nm /Green	-0.052 (±0.018)	16.17 (±0.60)	0.996	-0.067 (±0.029)	16.51 (±0.96)	0.99
Rhodamine B	555 nm /Green	-0.026 (±0.016)	4.43 (±0.14)	0.997	-0.005 (±0.015)	5.51 (±0.16)	0.997
Trypan blue	585 nm /Red	-0.027 (±0.013)	3.207 (±0.086)	0.998	0.050 (±0.026)	2.27 (±0.18)	0.99
Prussian Blue	605 nm /Red	-0.017 (±0.011)	2.635 (±0.069)	0.998	0.065 (±0.027)	1.316 (±0.097)	0.99
Malachine green	618 nm /Red	-0.0057 (±0.0071)	2.884 (±0.050)	0.9993	-0.05 (±0.05)	2.61 (±0.21)	0.99
Methylene blue	662 nm /Red	0.0100 (±0.0019)	3.532 (±0.011)	0.99997	0.010 (±0.015)	1.756 (±0.072)	0.996
Chlorophyll b	462 nm /Red	-0.0097 (±0.0050)	2.624 (±0.030)	0.9996	0.033 (±0.021)	1.40 (±0.11)	0.99

*All absorbance values were estimated by the mean value of $N = 7$ measurements. The regression equation we used was: $A = a + b\times A_k$.

† The spectral bandwidth is ± 5 nm

‡ The RGB color channel value used for the estimation of the RGB-resolved absorbance values of the tested solutions

Table S-2. Comparison of the analytical characteristics of calibration lines† fitted to the peak absorbance values (A_{peak}, measured by the *microplate spectrophotometer* as the laboratory standard) and the RGB-resolved absorbance values (A_k, measured by *flatbed scanner* or the *camera-based photometer*) *vs.* concentration of analyte (measured in μM) for solutions of 11 different dyes.

Compound	λmax*/ Color Channel	*Microplate spectrophotometer*				*Flatbed Scanner*				*Camera-based photometer*			
		Intercept $a\times10^{-3}$ ($\pm s_a$)	Slope $b\times10^{-3}$ ($\pm s_b$)	R^2**	LOD (μM)	Intercept $a\times10^{-3}$ ($\pm s_a$)	Slope $b\times10^{-3}$ ($\pm s_b$)	R^2**	LOD (μM)	Intercept $a\times10^{-3}$ ($\pm s_a$)	Slope $b\times10^{-3}$ ($\pm s_b$)	R^2**	LOD (μM)
Disperse orange 3	440 nm /Blue	-10.8 (± 6.3)	7.69 (± 0.18)	0.998	2.70	-5.5 (± 2.6)	3.52 (± 0.10)	0.999	2.43	-19 (± 18)	5.75 (± 0.59)	0.96	10.33
Methyl orange	465 nm /Blue	-1.6 (± 2.7)	20.35 (± 0.15)	0.9998	0.44	4.8 (± 2.7)	8.6 (± 0.11)	0.999	1.03	92 (± 27)	126 (± 13)	0.96	0.70
Fluorescein	485 nm /Blue	-3.2 (± 8.6)	26.97 (± 0.77)	0.996	1.05	4.0 (± 1.6)	8.32 (± 0.12)	0.999	0.63	-15.9 (± 6.3)	11.60 (± 0.71)	0.98	1.79
DPPH	515 nm /Green	0.5 (± 2.4)	33.31 (± 0.16)	0.9999	0.24	2.9 (± 1.6)	10.79 (± 0.10)	0.9994	0.49	-17.4 (± 8.4)	14.44 (± 0.56)	0.99	1.91
Eosin Y	518 nm /Green	3.6 (± 3.6)	63.37 (± 0.52)	0.9996	0.19	3.58 (± 0.94)	3.90 (± 0.13)	0.993	0.80	4.8 (± 1.7)	3.75 (± 0.24)	0.98	1.49
Rhodamine B	555 nm /Green	-7.4 (± 1.8)	85.75 (± 0.32)	0.9999	0.07	4.7 (± 3.4)	19.24 (± 0.58)	0.995	0.58	-0.2 (± 2.7)	15.47 (± 0.47)	0.994	0.57
Trypan blue	585 nm /Red	3.1 (± 1.8)	42.57 (± 0.21)	0.9999	0.14	10.1 (± 4.1)	13.19 (± 0.38)	0.995	1.03	-7.6 (± 7.7)	25.05 (± 0.71)	0.98	1.01
Prussian Blue	605 nm /Red	-9.6 (± 1.4)	28.13 (± 0.14)	0.9998	0.16	1.9 (± 3.5)	10.82 (± 0.23)	0.997	1.06	-7.8 (± 8.7)	14.66 (± 0.85)	0.98	1.95
Malachine green	618 nm /Red	-16.2 (± 7.4)	144.2 (± 3.6)	0.998	0.17	-2.1 (± 1.9)	49.75 (± 0.49)	0.994	0.12	-23 (± 21)	75.3 (± 5.3)	0.97	0.92
Methylene blue	662 nm /Red	-0.8 (± 4.9)	58.62 (± 0.45)	0.9997	0.28	-3.0 (± 1.2)	16.59 (± 0.11)	0.98	0.23	-31 (± 17)	23.4 (± 1.6)	0.97	2.39
Chlorophyll b	462 nm /Blue	52 (± 19)	44.2 (± 3.9)	0.97	1.41	21.4 (± 7.9)	17.40 (± 1.6)	0.97	1.49	27 (± 14)	29.10 (± 2.9)	0.96	1.58
	676 nm /Red	-84 (± 35)	33.6 (± 3.4)	0.97	3.44	0.8 (± 2.5)	4.98 (± 0.30)	0.98	1.66	3.8 (± 4.8)	5.24 (± 0.59)	0.94	3.02

†All absorbance values were estimated by the mean value of $N = 7$ measurements. The regression equation we used was: $A = a + b\times A_k$

* The spectral bandwidth is ± 5 nm

*** The RGB color channel value used for the estimation of the RGB-resolved absorbance values of the tested solutions

Table S-3. Comparison of the results for the *spectrophotometer* (laboratory standard) vs. the *flatbed scanner* and *camera-based photometer* in the following six analyses: i) Lactate assay, ii) LDL ELISA, iii)Anti-*Treponema pallidum* IgG assay, iv) BCA assay, v) Cyanomethemoglobin assay, and vi) Griess assay. The values and error bars correspond to the mean and standard deviations, respectively, of $N = 7$ measurements. The Anti-*Treponema pallidum* ELISA only permits qualitative results. The concentration of nitrite ions in the third sample tested with the Griess assay was lower than the detection limit of the method and therefore labeled as "Not Determined" (N.D.).

Assay	**λmax†/ Color Channel‡**	**Sample**	***Microplate spectrophotometer***	***Flatbed Scanner***	***Camera-based photometer***
			Concentration (± SD)		
Lactate	570 nm /Green	Serum 1	3.280 ± 0.064 mM	3.284 ± 0.042 mM	3.223 ± 0.105 mM
		Serum 2	1.078 ± 0.027 mM	1.072 ± 0.016 mM	1.084 ± 0.037 mM
		Serum 3	2.575 ± 0.052 mM	2.594 ± 0.017 mM	2.634 ± 0.071 mM
LDL ELISA	450 nm /Blue	Sample 1	3.76 ± 0.17 mg/mL	3.75 ± 0.12 mg/mL	3.74 ± 0.17 mg/mL
		Sample 2	2.35 ± 0.11 mg/mL	2.39 ± 0.12 mg/mL	2.42 ± 0.21 mg/mL
		Sample 3	4.59 ± 0.31 mg/mL	4.50 ± 0.23 mg/mL	4.61 ± 0.35 mg/mL
Anti-*Treponema pallidum* IgG ELISA	450 nm /Blue	Serum 1	positive	positive	positive
		Serum 2	positive	positive	positive
		Serum 3	negative	negative	negative
Bicinchoninic acid (BCA)	560 nm /Green	Sample 1	1.749± 0.026 mg/mL	1.755 ± 0.013 mg/mL	1.716 ± 0.080 mg/mL
		Sample 2	1.934 ± 0.034 mg/mL	1.890 ± 0.051 mg/mL	1.965 ± 0.084 mg/mL
		Sample 3	2.999 ± 0.045 mg/mL	2.977 ± 0.041 mg/mL	3.070 ± 0.081 mg/mL
Cyanmethemo-globin	540 nm /Green	Blood 1	94.1± 2.1 mg/mL	93.2 ± 1.4 mg/mL	91.2 ± 5.0 mg/mL
		Blood 2	175.6 ± 2.8 mg/mL	173.1 ± 2.1 mg/mL	172.3 ± 4.4 mg/mL
		Blood 3	179.1 ± 1.8 mg/mL	177.1 ± 1.8 mg/mL	177.2 ± 4.3 mg/mL
Griess	540 nm /Green	Water 1	35.90 ± 0.21 mM	35.94 ± 0.45 mM	34.99 ± 0.99 mM
		Water 2	2.87 ± 0.20 mM	2.78 ± 0.10 mM	2.92 ± 0.76 mM
		Water 3	ND	ND	ND

* All absorbance values were estimated by the mean value of $N = 7$ measurements.

† The spectral bandwidth is ± 5 nm

‡ The RGB color channel value used for the estimation of the RGB-resolved absorbance values of the tested solutions.

Table S-4. Characteristics of the photometric assays we performed.

Assay	Target analyte	Matrix	Type	Spectral characteristics
Lactate assay	Lactic acid	Human serum	Enzymatic assay (based on lactate oxidase)	Sharp peak (λ_{max}= 570 nm)
Low-density lipoprotein (LDL) ELISA	LDL	Human serum	Competitive immunoassay (based on horseradish peroxidase)	Sharp peak (λ_{max}= 450 nm)
Anti-*Treponema pallidum* IgG ELISA	Anti-*Treponema pallidum* antibodies	Human serum	Indirect immunoassay (based on horseradish peroxidase)	Sharp peak (λ_{max}=450 nm)
Bicinchoninic acid (BCA) assay	Total protein	Solutions of antibodies	Simple photometric assay	Very broad peak (λ_{max}= 560 nm)
Cyanomethemoglobin assay	Hemoglobin	Human blood	Simple photometric assay	Broad peak (λ_{max}= 540 nm)
Griess assay	Nitrite ions	Water	Simple photometric assay	Sharp peak (λ_{max}= 540 nm)

Table S-5. Analytical characteristics of calibration lines* for the following five assays: a) Lactate assay, b) LDL ELISA, c) BCA assay, d) Cyanomethemoglobin assay, and e) Griess assay

Assay	λmax† /Color Channel‡	*Microplate spectrophotometer*			*Flatbed scanner*			*Camera-based photometer*		
		Intercept $a\times10^{-3}$ $(\pm s_a)$	**Slope** $b\times10^{-3}$ $(\pm s_b)$	R^2	**Intercept** $a\times10^{-3}$ $(\pm s_a)$	**Slope** $b\times10^{-3}$ $(\pm s_b)$	R^2	**Intercept** $a\times10^{-3}$ $(\pm s_a)$	**Slope** $b\times10^{-3}$ $(\pm s_b)$	R^2
Lactate assay (μM lactic acid)	570 nm /Green	19.3 (± 3.1)	4.991 (± 0.023)	0.9999	18.2 (± 4.1)	1.39 (± 0.031)	0.998	-3.75 (± 4.8)	1.49 (± 0.036)	0.998
LDL ELISA (μg/mL LDL)	450 nm /Blue	606 (± 48)	-234 (± 25)	0.99	244 (± 10)	-101 (± 6.4)	0.992	331 (± 35)	-135 (± 21)	0.95
Cyanomethe-moglobin Assay (mg/mL hemoglobin)	540 nm /Green	1.9 (± 2.5)	1.797 (± 0.020)	0.9995	1.9 (± 1.5)	0.698 (± 0.012)	0.998	-4.1 (± 1.7)	0.735 (±0.013)	0.998
BCA assay (μg/mL albumin)	560 nm /Green	-1.3 (± 4.7)	12.07 (± 0.22)	0.999	0.1 (± 1.5)	4.819 (± 0.073)	0.9993	0.1 (± 3.4)	4.79 (± 0.16)	0.997
Griess Assay (μM nitrite ions)	540 nm /Green	-4.3 (± 1.6)	15.84 (± 0.05)	0.9999	5.2 (± 3.5)	5.47 (± 0.11)	0.999	-3.9 (± 5.6)	6.00 (± 0.17)	0.997