

Design and Development of a Color Picker System to Integrate in POC Device Systems.

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Abstract: Nowadays is increasing the demand for miniaturized, user-friendly, automated, and portable sensing systems able to provide a fast and reliable response. In this context, colorimetric detection has emerged for its intrinsic advantages, such as simplicity and rapidity, but also for the outstanding development of novel materials, such as plasmonic nanoparticles, and new technologies. Here, the Color Picker system, a system reproducing in the ba has been developed and tested on a plasmonic paper. The aim is to provide a tool for a colorimetric detection that can be successively integrated in next generation diagnostic devices for real world applications.

Keywords: Color Sensor, RGB Display, AuNPs, Plasmonic Paper.

1 Introduction

During the last few years an exponentially growing attention has been focused towards the easy and fast identification of many biomarkers for several diseases so that many lives can be saved thanks to the detection of a disease at an early stage as well as facilitating the management of more personalized and efficient therapies, aiming to develop and improve personalized medicine. With this in mind, fervent activity is directed towards the advancement of Point-of-Care (POC) devices [1]: these tests enable analysis using very small sample volumes with no or little preparation, providing results in shorter times compared to routine lab tests. They offer the additional advantage of being interpreted and implemented without any special equipment or training, while they are administered externally to hospitals and clinics practice, in a home environment [2]. Furthermore, they are cost-effective and can be easily transported. In this scenario, also stimulated by the development of a large number of novel materials, colorimetric detection has blossomed due to its advantages: sophisticated or expensive instrumentation is not required because the color changes can be read by

naked eyes, so they are characterized by simplicity, practicality and low cost [3]. The key issue in colorimetric detection is to convert the event of detection into a color change so that several smart materials, such as for example noble metal nanoparticles, 2D nanomaterials as graphene or reduced graphene, and hybrids composed by plasmonic particles and bidimensional material, have been developed to be exploited as sensing platform in colorimetric assays [4]. Since the earliest days of nanoparticle synthesis, the engineering of metal nanoparticles has progressed dramatically, with significant contributions in multiple fields, such as chemistry, physics, engineering, medicine and biology but including also completely new fields as microelectronics and boosting their applications [5]. The growing interest is originated not only from the bright, visible colors that can be obtained, but also because it is possible to control these colors by the fine tuning of various parameters, such as particle shape and size, state of aggregation, or the nature of the surrounding medium. Nanoparticles of noble metals, such as gold, silver, and copper display strong localized surface plasmon resonances, (LSPRs) that are oscillations of d-electrons cloud driven by an electromagnetic radiation [6] and are the source of their

unusual optical properties. The resonance frequency strongly depends also on the dielectric constant of the medium around the metal nanoparticles, so that according to the changes of the refractive index the position of the LSPR is displaced. In this scenario, the sensitivity towards the variations in the refractive index is used to identify biorecognition events occurring onto the particle surface, providing a great potential for novel sensing platform [7]. Anyway, the detection of molecules using the LSPR of metal nanoparticles is commonly based on spectroscopic measurements, requiring bulky instrumentation and trained personnel. To transfer colorimetric detection based on metal nanoparticles to applications in routine diagnostic, there is still a strong request of user-friendly devices. In the present work a system able to reproduce the color detected by the TCS34725 Color Sensor in the backlight of an I2C RGB Display, the Color Picker system, has been developed with the aim to provide a tool that can be integrated in POC devices for the next generation of sensing platforms enabling a real-time, highly sensitive and accurate diagnostics. The Color Picker system, in addition, has been tested to measure the changes in the colors of a plasmonic paper (paper with AuNPs incorporated in its fiber network), arising from the different refractive index around the particle. In a further step, the sensing platform based on those novel materials, will be integrated in POC systems relying in new technologies such as lab-on-chip (LOC) hardware and remote connections based on Internet-of-Things (IoT) infrastructure.

2 Materials

2.1 Color Picker

The principal components of the Color Picker are the Color sensor I2C V2 (TCS34725FN) – Seeed and I2C 16x2 Arduino LCD with RGB Backlight Display V2.0 from DFRobot. The TCS34725 sensor and the RGB backlight display are connected by means of a breadboard and controlled by an Arduino Uno Microcontroller. The TCS34725 is a digital Light-to-Digital Converter, meaning it converts light from the visible spectrum to a digital signal that is read by an external microcontroller. One of the main components is a 3x4 photodiode array, composed of red-filtered, green-filtered, blue-filtered, and clear (unfiltered) photodiodes that are coated with an IR blocking filter minimizing the IR spectral component of the incoming light and allows color measurements to be made accurately. Also, the amplified photodiode currents are simultaneously converted to a 16-bit digital value by four integrating analog-to-digital converters (ADC). Upon completion of a conversion cycle, the results are transferred to the data registers, which are double-buffered to ensure the integrity of the data. All the internal timing, as well as the low-power wait state, is controlled by the state machine. Communication of the TCS34725 data is accomplished over a fast, up to 400 kHz, two-wire I2C serial bus. The LCD1602 RGB Backlight Module is a 16x2 LCD screen with an

adjustable RGB backlight color, up to 16M (2563) backlight colors in theory. That display uses an I2C communication interface meaning that it only needs 2 communication lines for the communication and backlight control and only 4 pins for the connection with a microcontroller: VCC, GND, SDA, SCL. Also, it is provided with an onboard AiP31068L LCD driver chip (LCD controller) and a PCA9633 RGB control chip (RGB controller), can display 2x16 characters and support functions like scrolling-displaying, cursor movement and backlight color adjustment. By programming the LED brightness with a PWM controller, it is possible to tune the color of the backlight display. The functional block diagrams of TCS34725 color sensor and DFRobot 1602 RGB backlight have been reported in Fig. 1A and 1B.

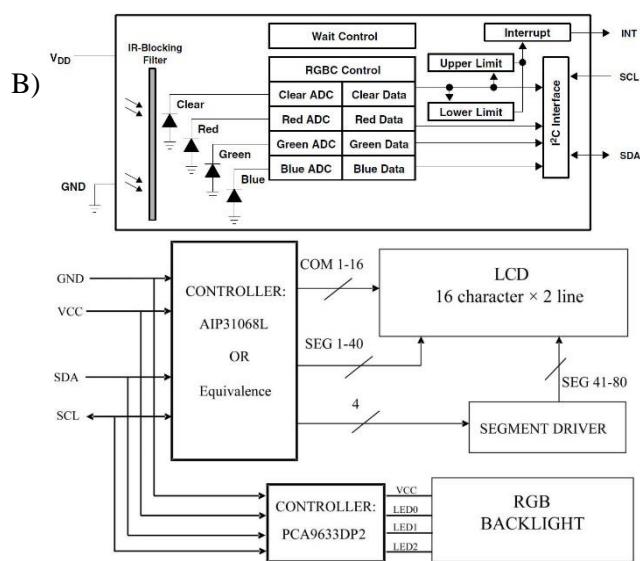


Fig. 1: A) Functional block diagram of TCS34725 sensor; B) Functional Block diagram of DFRobot 1602 Backlight display.

Also, the calibration and the tests have been performed using the Spyder Checker 24 from Datacolor and using MATLAB software to calculate the Moon Penrose inverse and the transformation matrix.

2.2 AuNPs and Plasmonic Paper

All the chemicals and reagents used in this study were analytical grade and used as received without further purification. Tetrachloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), trisodium citrate dihydrate and glycerol were purchased from Aldrich. The citAuNPs are adsorbed on laboratory filter paper (Whatman grade No. 1, 180 μm thick).

3 Methods

The Color Picker has been tested for first measuring the RGB values of the Sunflower patch of the Spider Checkr 24 at different integration Times. In this case, the values returned from the sensor are normalized to the clear. Successively, a further test has been carried out following the TCS 34725 calibration. The calibrated

RGB values returned from the sensor have been calculated according to the following formula 1

$$\begin{pmatrix} R \\ G \\ B \end{pmatrix} = (M) \times \begin{pmatrix} R' \\ G' \\ B' \end{pmatrix} \quad (1)$$

Where:

-R, G, B are the RGB values in the sRGB Color Space

-M is the transformation matrix

-R' G' B' are the responses from the RGB channel of the sensor normalized to the clear.

The transformation matrix is the following:

$$(M) = \begin{pmatrix} R_r^* & R_g^* & R_b^* \\ G_r^* & G_g^* & G_b^* \\ B_r^* & B_g^* & B_b^* \end{pmatrix} \times \begin{pmatrix} R'_r & R'_g & R'_b \\ G'_r & G'_g & G'_b \\ B'_r & B'_g & B'_b \end{pmatrix}^{-1} \quad (2)$$

Where:

$$R_r^* \quad R_g^* \quad R_b^*, \quad G_r^* \quad G_g^* \quad G_b^* \quad \text{and} \quad B_r^* \quad B_g^* \quad B_b^*$$

are the reference sRGB values for red, green, and blue Color Checker Patches,

$$R'_r \quad R'_g \quad R'_b, \quad G'_r \quad G'_g \quad G'_b \quad \text{and} \quad B'_r \quad B'_g \quad B'_b$$

are the measured values of the red, green, and blue Color Checker Patches normalized to the clear, and the matrix

$$\begin{pmatrix} R'_r & R'_g & R'_b \\ G'_r & G'_g & G'_b \\ B'_r & B'_g & B'_b \end{pmatrix}^{-1} \quad (3)$$

is the Moon Penrose pseudoinverse.

After the calibration, the RGB values of the Sunflower patch of the Color checker have been measured for different Integration Times (IT).

3.1 Synthesis of AuNPs

The AuNPs have been prepared by heating 98 mL of milliQ water under reflux while stirring, and at the boiling point 2 mL of trisodium citrate dihydrate solution (1 wt%) have been added. After 5 minutes, a tetrachloroauric(III) acid trihydrate (HAuCl₄.3H₂O) solution has been added with a final concentration in the system of 1mM and left boiling for 5 minutes. Finally, the solution turned into a red color, indicating the presence of AuNPs (CitAuNPs) and cooled down.

3.2 Plasmonic Paper preparation

A common filter paper for laboratory use has been used for CitAuNPs absorption. One strip of paper (about 20x20 mm) has been immersed in the solution of the pre-synthesized AuNPs and left overnight at 4 °C, as proposed in the work of Singameni [8]. The paper strip was taken out and washed with MilliQ water. As final step, the paper has been dried for about 45 minutes at 44 °C into the oven.

3.3 Test of refractive index sensitivity

To test the sensitivity towards variations in the refractive index, the plasmonic paper has been wetted in water and glycerol and then the RGB values measured with the Color Picker system.

4 Results and Discussions

4.1 citAuNPs Characterization

The citAuNPs have characterized by UV-Vis spectroscopy (Fig. 2):

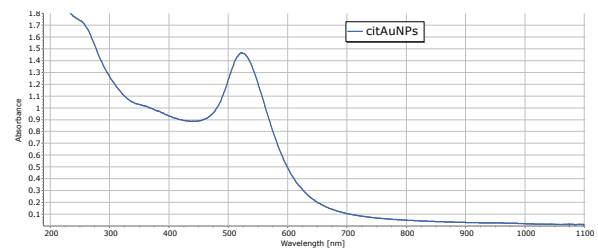


Fig. 2: UV-Vis. Spectra of the citAuNPs used to prepare the plasmonic paper.

The LSPR is centered at about 524 nm and from spectral data a size of 15-20 nm is evaluated for citAuNPs.

4.2 TCS34725 Sensor calibration

The data obtained from the first test have been reported in table 1:

Table 1. RGB values measured for the Sunflower patch of the Color Checker normalized to the clear.

	Red	Green	Blue	Clear
IT 24 ms	110	91	46	125
IT 120 ms	110	93	46	622
IT 614 ms	110	93	46	3188

In this test, the RGB data returned by the sensor have been normalized to the clear, so that, as it is possible to observe, they are independent from the Integration Times and are almost the same. The color space of the RGB data normalized to the clear is not defined, so that is not possible a comparison with the reference values that are defined in the L*a*b*, sRGB and Adobe RGB color spaces, losing some information. To acquire a more accurate estimate of the RGB values and to define the color space of the data returned from the sensor, a calibration of the TCS34725 Color Sensor has been performed so that the RGB output values are in the

sRGB color space. The data obtained in this test have been reported in table 2:

Table 2. RGB data obtained with the TCS34725 calibration.

	Red	Green	Blue	Clear
Sunflower patch Color Checker (sRGB Color Space)	238	158	25	
IT 24 ms	148	81	35	125
IT 120 ms	150	81	36	623
IT 614 ms	151	81	35	3218

From the data reported in table 2 it is possible to observe that at increasing of the Integration Time, the difference between the measured values in the sRGB color space is quite small. Also, the measured values are different from the reference values for the Sunflower patch of the Color Checker. The tiny difference in the measured values for different Integration times may be motivated by the normalization to the clear. In fact, the data used in Eq. 1 for the calibration, are normalized to the clear, so that they are independent from the Integration time. Also, the difference with the reference values of the Color Checker may be justified with the different illuminant used to measure the references for the Color Checker and the color of the Sunflower patch in the experiment. In the case of the Color Checker, the reference values have been measured using a D50/2 degrees illuminant, whereas the illuminant used in the experiment to measure the sRGB values of the Sunflower patch is the led integrated in the TCS34725 sensor. In Fig. 3 are reported A) the spectral power distribution for CIE D50 light source (line 1) [9], and B) the measured spectrum of the LED integrated into the TCS34725 sensor.

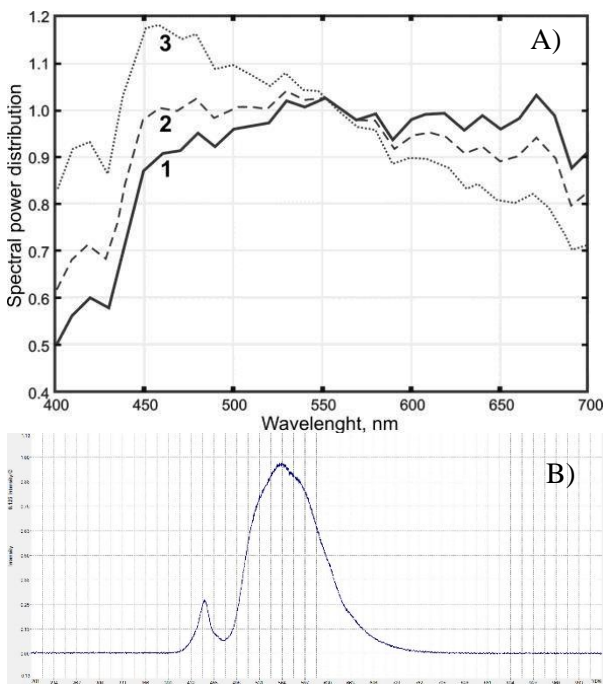


Fig. 3: A) Spectral power distribution of illuminants CIE D: 1 – D50, 2 – D55, 3 – D65 [9]; B) Measured Spectra of the light emitted by the LED incorporated into the TCS34.

It is possible to observe that whereas the spectral power distribution for the CIE D50 illuminant is a broad band,

the spectrum of the LED presents two peaks, whose characteristics are reported in table 3:

Table 3. Data of the spectrum emitted from the LED integrated into the TCS 34725 sensor

	CENTROID WAVELENGTH [nm(AIR)]	PEAK WAVELENGTH [nm(AIR)]	PEAK LEVEL [INTENSITY]	FWHM [nm(AIR)]	ΔPEAK POSITION [nm(AIR)]	OFFSET PEAK POSITION [nm(AIR)]
FIRST PEAK	451.321	451.464	0.275	15.529	0.000	0.000
SECOND PEAK	566.961	562.959	0.972	107.819	111.495	111.495

Currently the majority of LEDs use phosphor conversion (phosphor-converted LEDs or pcLED). White light is generated by mixing a portion of the blue light emitted directly from an LED with a Gallium Nitride (GaN) substrate (smaller peak at 451 nm) with the light converted to yellow emitted by the phosphors (broad peak at 567 nm). The phosphors are located on the emitting surface of the LED, inside the encapsulant, or away from the LED (remote phosphors). The differences in the spectrum are then reflected in the different sRGB values detected. Although the output values are different from the reference sRGB, after calibration the sensor acquired an increased precision producing the same output for the same input: measuring for example the Sunflower patch (but also other objects such as, for example, the cover of the notebook) the sensor always returned the same values even after multiple measurements. In Fig. 4A the picture of the overall Color Picker system has been reported, whereas in Fig. 4B is shown the picture of the Color Picker while sensing the blue cover of a notebook. It is possible to note how the backlight of the DFRobot RGB display turned blue as the cover. Finally, in Fig. 4C the Spyder Checkr24 picture with the patches used for the calibration and the test has been provided.

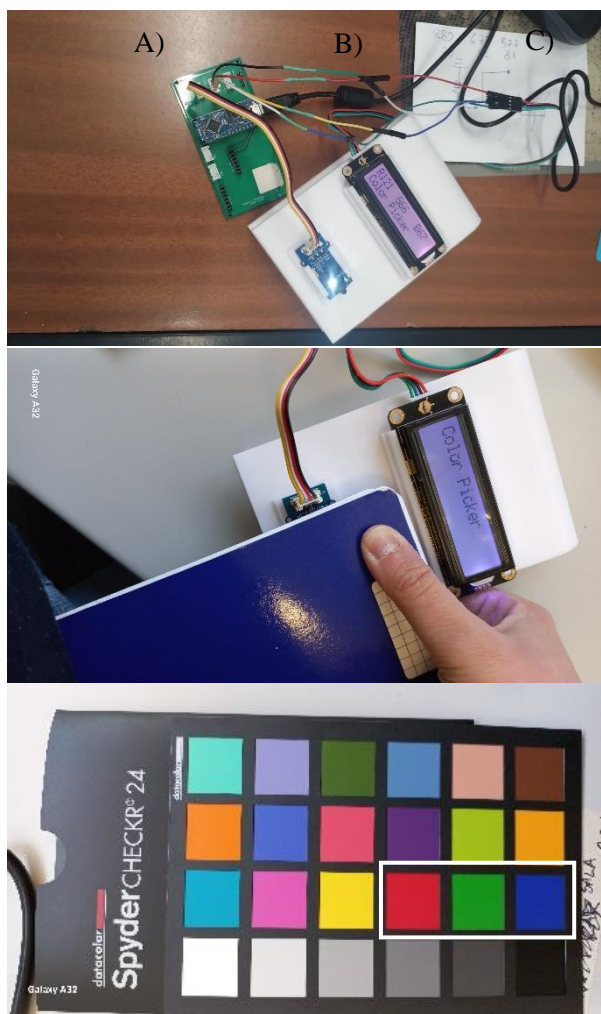


Fig. 4. A) picture of the Color Picker system with the TCS34725 sensor, the RGB backlight DFRobot display, and the microcontroller Arduino Nano assembled onto a board; B) testing the Color Picker with the blue cover of a notebook; C) the Spyder Checkr 24 used to calibrate and to test the Color Picker system. In the picture the primary red, the primary green and the primary blue patches used for the calibration along with the Sunflower patch used to test the device have been indicated.

4.3 Test on Plasmonic Paper

The Color Picker device with the calibrated TCS34725 sensor successively has been employed to test the changes in the color of a plasmonic paper strip arising from the different local dielectric environment. In figure 5 the picture of the plasmonic paper A) in its dry state and wet with B) water and C) glycerol is shown. In figure 6 is illustrated the display of the Color Picker device with the color of plasmonic paper in the backlight.

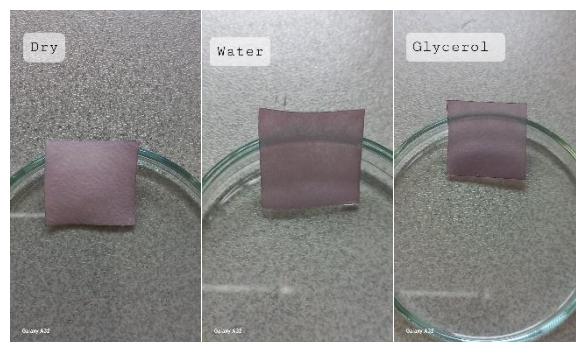


Fig. 5. Plasmonic paper A) dry B) wet with water C) wet with Glycerol



Fig. 6. Backlight of the I2C 16x2 LCD Display reproducing the color of the plasmonic paper detected by the TCS34725 color sensor.

The RGB values obtained from the sensor, along with the refractive index of the solvent embedding the plasmonic paper have been reported in table 4.

Table 4. RGB values for plasmonic paper

	Refractive Index of the embedding medium	R	G	B
Dry Plasmonic Paper	Air (1)	85	83	78
Paper in Water	1.33	90	80	79
Paper in Glycerol	1.47	93	81	78

From the values measured by the sensor it is possible to observe a different trend for the separated RGB channels. Whereas the values of the red channel increase when the refractive index of the medium increases, the values of the green and blue channels do not show a specific trend, are almost identical for the different refractive indices. On this basis, it has been possible to demonstrate that the red channel is responsive to the changes in the refractive index of the local dielectric environment around the surface of a plasmonic AuNPs grafted onto the paper structure.

5 Conclusions

In summary, a device able to reproduce in the backlight of a RGB display the color detected by the TCS34725 color sensor, the Color Picker system, has been developed aiming to create an output tool to be integrated into advanced sensing platforms for a more accurate and rapid diagnostic. The Color system has

been tested before and after calibration, finding an improved precision after calibration providing the same values of the sRGB values even after numerous measurements. In addition, the TCS34725 sensor calibration offers the additional advantage to define the Color Space of the output values (sRGB). Differences in the measured sRGB values with the reference of the color checker have been found, depending on the different light source used for the measurements. After calibration, the Color Picker System has been tested to measure the differences in the color of plasmonic paper when immersed in solvents with different refractive index. Whereas the values of the red channel increase at

increasing of the refractive index, the values of the green and blue channel are almost identical, thus proving that the Color Picker system is able to detect changes in color of plasmonic substrates arising from variations in the dielectric environment around plasmonic nanoparticles. In further step, the Color Picker device will be employed to detect differences in the color of plasmonic nanoparticles arrays arising from binding events between analytes and receptor localized onto the nanoparticle surface for a colorimetric detection, with the aim to be incorporated into POC devices for an easy and real time diagnostic.

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