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DIGITIZATION OF COLORIMETRIC MEASUREMENTS FOR QUANTITAVE ANALYSES

USING A SMARTPHONE

By

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A Master Thesis submitted to the
Department of Computer Science
in partial fulfillment of the
requirements for the degree of
Master in Multimedia Applications

2017

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

A programmable smartphone connected to a built-in image sensor is a portable and low cost instrument for analysis based on chromogenic reagents. The RGB values collected from an image are used to quantify concentrations in colorimetric tests. Measuring color demands a sufficient and constant illumination to report results with great accuracy and repeatability. However, a smartphone capturing an image is involved in issues related to ambient light variability. A smartphone application may be supported by a wide variety of devices, employing different camera technologies. The color pixels values are expressed in different ways depending on the software used for processing the image. This research is attempting to identify and evaluate existing image processing techniques in order to design a Mobile Sensing System (MSS) to quantify the concentration of an analyzed substance by collecting the color information contained in the image pixels.

CHAPTER 1

INTRODUCTION

1.1   Motivation

The smartphone applications developed for quantitative analysis purposes based on colorimetric detection are increasing in the latest years. These applications are based on correlations between the RGB values of an image and the concentrations of a substance in a specific environment. Basically this type of analysis use a membrane containing a chromogenic reagent that experiments a color change while the concentration of a specific substance is varying, typically classified as optical sensors.

This research is motivated by a previous personal experience working on optical sensors. The results of these previous works, made in collaboration with other authors, are summarized in two published papers [1] [2]. As described in these publications, the presence or absence of substances such us heavy metals and sulphide in water can be quantified by collecting the color pixels from a digital image. This quantification method simplifies the analytical procedure through allowing analyses of colorimetric tests in situ and out of lab. The images analyzed are captured using a digital camera. Then, the images are processed by an ad hoc developed PC software that allows the user to quantify the concentration.

This procedure opens the door to the design of a mobile sensing system capable to quantify a concentration by gathering the color information of an image captured by a smartphone camera. Therefore, there is a need to further research this topic to explore its use employing a smartphone as a color measuring device. Their advantages include the portable capability of the artefact and the reduction of costs. However, a smartphone application may be used by a wide variety of devices. The mobile devices technology vary in depend on price, date of acquisition, brand, operative system, and so on. This fact requires applications with inter-phone repeatability. Moreover, capturing images from a mobile device involves a great variability on parameters such as pixel resolution, ISO number, white calibration, and every configurable setting in a color camera. This variability has a direct and profound impact on color quantification.

1.2   Objective

This research is attempting to identify the existing algorithms used for color image processing and evaluate them through designing a prototype able to quantify the concentration of an analyzed substance by a correlation to the color information of the image pixels. A color quantification is required, the application needs to be able to obtain colorimetric values that describe the measured color and determine the concentration of a substance after the reaction of an optical sensor.

1.3   Research questions

The main questions that required responses are:

- Is a smartphone a capable device for quantifying the concentration of a substance in colorimetric tests?

- How an image can be processed in order to report results with inter-phone repeatability?

1.4   Research methodology

Color image pixels and substance concentration are the two variables in the research data generation. The pixels collection provides numerical data that can be used in a quantitative analysis. The result obtained after correlate the two variables provides relevant information for designing the artefact. The improvement of the artefact requires both, the manipulation of the pixel data and the evaluation of the results obtained after the action.

1.4.1   Research Strategy

The research is involved in the following process:

- Analyze the quantification procedure in a colorimetric test.

- Design a prototype to solve the problems described.

- Evaluate the design.

- Provide results.

1.4.2 Evaluation

The application needs to be evaluated to demonstrate that the artefact designed meets the objective established. In this way, the results will show if the artifact is able or not to solve the problem described. The accuracy of the artefact will be qualified by the comparison with a reference measuring instrument. On the other hand, the inter-phone repeatability will be qualified trough a comparison among images captured from different smartphones technologies.

1. Evaluate the accuracy of the quantification: the reaction of the chromogenic reagents are measured using both, a spectrophotometer as a measuring instrument and the smartphone application. The data is compared to qualify the artefact and evaluate the accuracy.

2. Evaluate the inter-phone repeatability: different images obtained from different devices are tested in an equal environment in order to evaluate the behavior of the artifact facing problems described such as image size or device technologies variability.

CHAPTER 2

STATE OF ART

2.1 Colorimetry

2.1.1 What is color?

Color is a sensation created in response to excitation of our visual system by electromagnetic radiation or light. In other words, color is the perception generated in the brain when the light reach the retina. The light perceived is constrained by the visible region of the electromagnetic spectrum with wavelengths in an approximated range from 400nm to 700nm. Color can be describing accurately by measuring the physical power or radiance of the incident light. The result is a spectral power distribution (SPD), commonly divided into 31 areas, each representing a 10nm band (figure 2.1). [3]
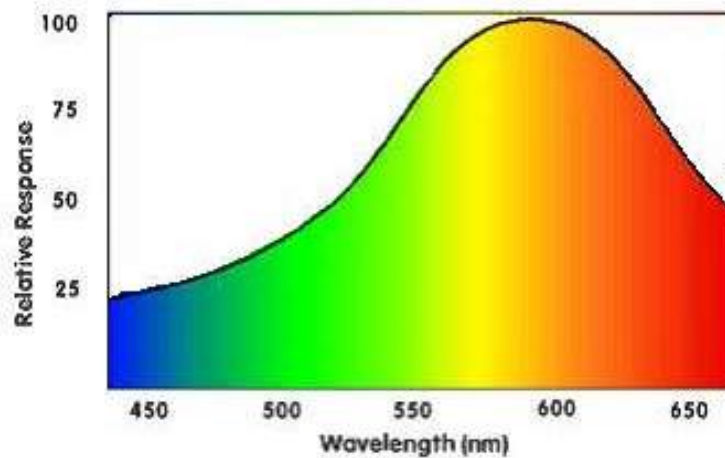
Figure 2.1: The spectral power distribution

The human retina contains cones, three types of color photo-receptor cells which respond to radiation at different spectral sensitivity peaks in short (S, 420 nm to 440 nm), middle (M, 530 nm to 540 nm), and long (L, 560 nm to 580 nm), represented in figure 2.2. A fourth type of

photo-receptor cells, called rods, are also present in the retina. Rods cells are more numerous and sensitive than cone cells. However their functions are focused on vision with low light intensity, having almost no role in color vision.
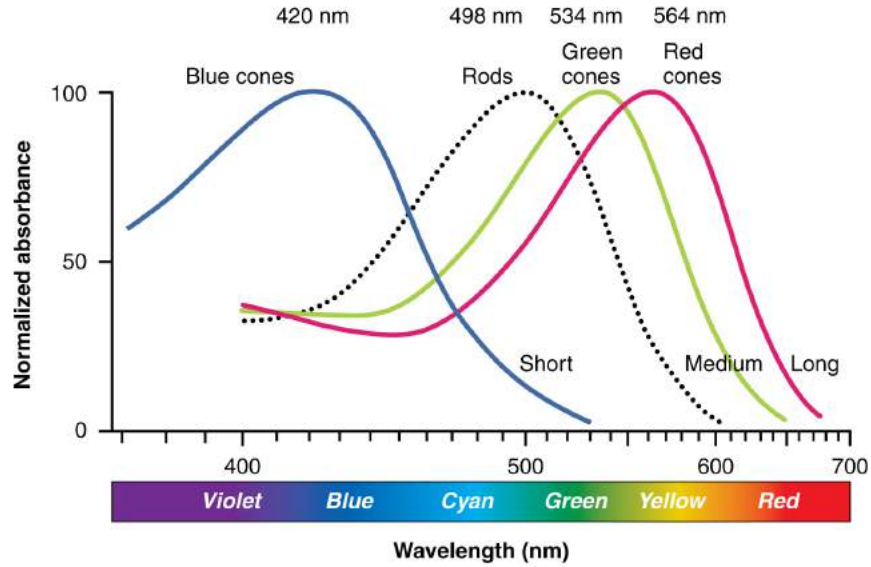
Figure 2.2: Human spectral sensitivity to color

2.1.2 Colorimetry

In color science, colorimetry is the area responsible of giving a description and a specification of a color. Based on the fact that the human eye use three types of color photo-receptor cone cells, three numerical values are needed to describe a color. The figure 2.3 shows the Commission internationale de l'clairage (CIE) chromaticity diagram, where any visible color can be mapped by defining the tristimulus value [4] [5].
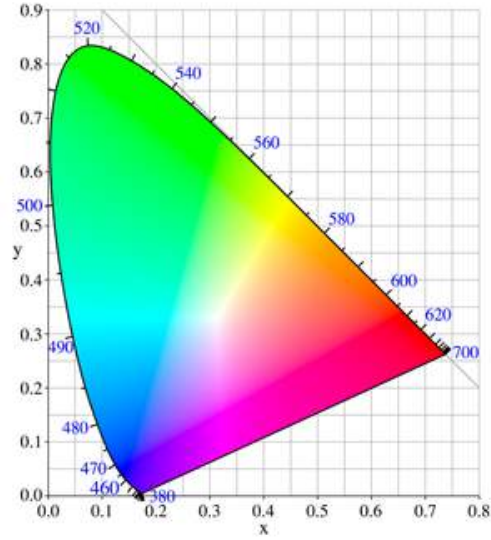
Figure 2.3: The C.I.E. Chromaticity Diagram

Nonetheless, a color can be described by the combination between brightness and chromaticity. For instance, a color white is a bright version of a color grey, being equal in chromaticity and different in brightness. In the CIE XYZ color space the Y parameter is a measure of luminance. The chromaticity of a color is then specified by the two derived parameters x and y, two of the three normalized values being functions of all three tristimulus values X, Y, and Z [6]

$$x = X/(X + Y + Z) \tag{2.1}$$

$$y = Y/(X + Y + Z) \tag{2.2}$$

$$z = Z/(X + Y + Z) = 1 - x + y \tag{2.3}$$

2.1.3 Color Space

A color space or color model is formed by a set of numerical values able to define a singular color. The three components of a color can be defined as many different ways as color spaces existing. Color in multimedia applications demands a suitable method for representing the color signal perceived by a device. Selecting a color representation must to take into account how the

signals are generated and which information is needed from these signals. As represented in the figure 2.4, the color spaces are constrained by their volume of colors or gamut. The color models may be used to define colors, discriminate between colors, judge similarity between colors and identify color categories for a number of applications [4].

Figure 2.4: Examples of color spaces

In the additive color reproduction, the colors are represented by combining the primaries and the fraction of each mixed primary. In image and video devices the spectra from red, green and blue light beams are physically summed at the surface of the projection screen (figure 2.5).

Figure 2.5: Additive color reproduction RGB

2.2   Devices for measuring color
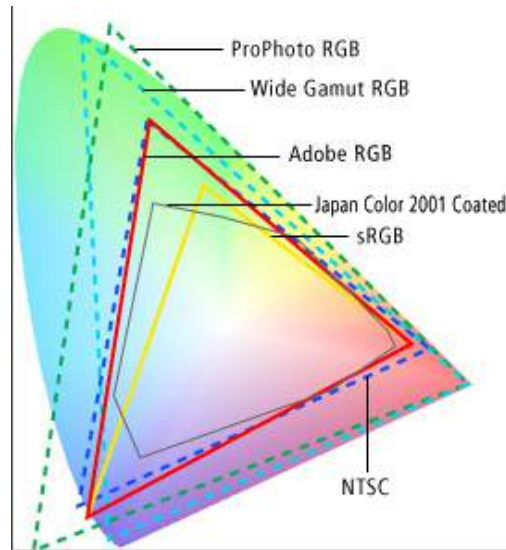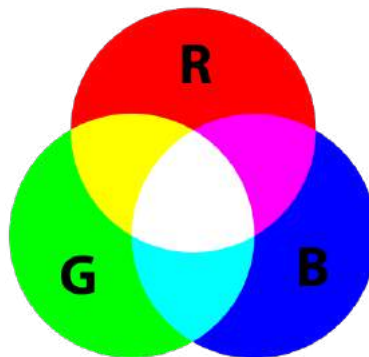
2.2.1   Ultraviolet-visible spectrophotometer

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region, using light in the visible and adjacent (near-UV and near-infrared) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved [7].

2.2.2   Colorimeter

A colorimeter is a device used in colorimetry. The device measures the absorbance of particular wavelengths of light by a specific solution. This device is most commonly used to determine the concentration of a known solute in a given solution by the application of the Beer-Lambert law, which states that the concentration of a solute is proportional to the absorbance. [8].

2.2.3   Image sensors

The spectacular growth of the mobile application development converts a smartphone in a multitask device. Cameras, GPS, microphone, accelerometer... are embedded sensors that bring an opportunity to develop new applications useful to society. Nowdays we can find apps used for social networks, healthcare, entertainment, education, safety, data analysis, not only using the built-in sensors but also external ones. The advance of technology allows to reduce the unit build cost of the embedded sensors and the devices is becoming in a low cost way to report or take actions by managing the behavior of a sensor[9]. All these circumstances give rise to the area of mobile phone sensing research. A MSS requires a user level application running on the phone for reading an internal phone sensor, or external sensors, reporting sensed data. For this purpose, the phone operating system must offer an Application Programming Interface (API) to manage the data reading and reporting [10]. During the latest years smartphone cameras have been used across a wide variety of domains in computer vision researches trying to emulate the basic functions of the human vision system. The analysis of the user environment through images and videos is a very interesting point in safety applications [11] and also in applications for users suffering from visual impairment [12].

Smartphone cameras can be used as well for colorimetric detection through processing the RGB values from the pixels of an image. In that case, mobile devices can be used in bioanalysis with a

wide variety of Cellphone-based quantitative analysis [13]. Today smartphones come with at least one camera to get images and videos. The method used to digitize images is a Bayer filter mosaic, a color filter array for arranging RGB color filters on a square grid of photosensors. Its particular arrangement of color filters is used in most single-chip digital image sensors installed in cameras and scanners to create a color image. The figure 2.6 is representing the pattern formed by 50 percent of green, 25 percent of red and 25 percent of blue, hence is also called RGBG, GRGB, or RGGB[14].
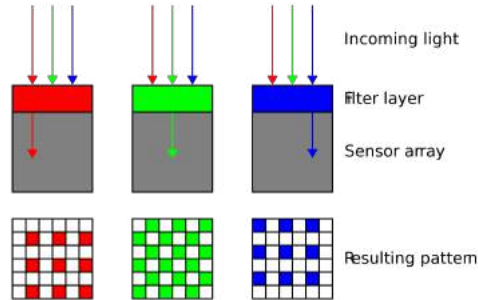
Figure 2.6: Bayer filter used in Complementary Metal-Oxide Semiconductor (CMOS) image sensor

2.3   Color Image processing

2.3.1   Introduction

The common used smartphones operative systems provide a software to manage different camera settings such as white balance, focus, zoom, flash and in some cases (smartcameras) time exposure and aperture parameters. Once the image has been captured is digitalize and compressed usually in a JPG format with an embedded sRGB ICC profile represented in figure 2.7. Images are formed by a matrix of pixels, each pixel contain values for each canal R G B.
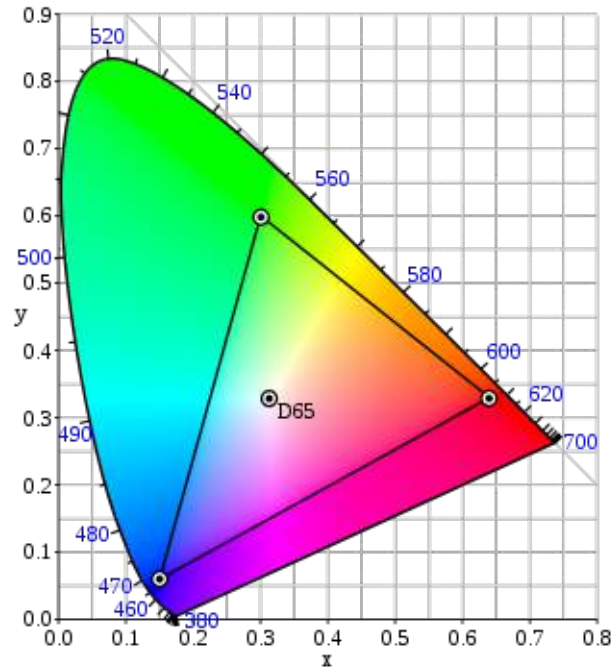
Figure 2.7: sRGB space

2.3.2   RGB color space

Basically, an RGB color model is a set of colors defined by the chromaticity of red, green and blue. It can be represented in a cube containing as many colors as possible primaries combinations, where the brightest point corresponds to the coordinate (1,1,1) and (0,0,0) to the darkest one. Each color contains the intensity of each channel and are expressed from 0 to 1 (when the values are normalized), integer values (i.e: 0-255) and percentage per channel among others.
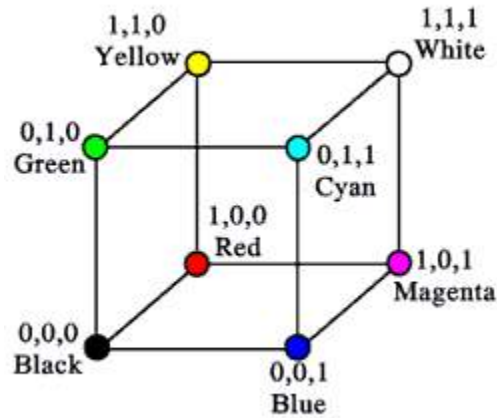
Figure 2.8: RGB color space

A digital image can be described as a set of digital values or pixels. A pixel is the smallest individual element in an image containing the color information for each position inside a two dimensional array (figure 2.9). The amount of pixels inside an image vary in depend on the image acquisition resolution. The resolution of an image is expressed as an NxM matrix, where N is the number of pixels columns (image width) and M is the number of pixels rows (image heigth).
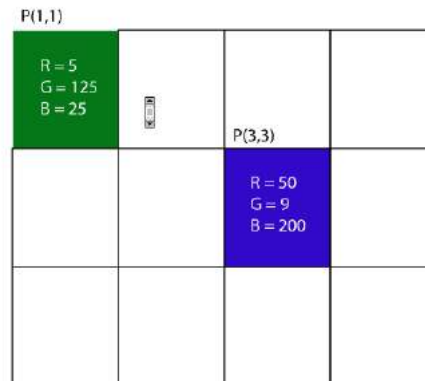
Figure 2.9: A 4x3 pixel array

2.3.3 Normalized RGB

Normalized RGB is a representation which is easily obtained from the RGB values by a simple normalization procedure:

$$r = R/(R + G + B) \tag{2.4}$$

$$g = G/(R + G + B) \tag{2.5}$$

$$b = B/(R + G + B) = 1 - r + g \tag{2.6}$$

Since the sum of the three normalized components is known, the third component can be omitted, reducing the space dimensionality. The normalized values or pure colors, do not take into account the brightness parameter, they are only defining the chromaticity of r and g. Normalizing RGB values is an interesting point to avoid the effects of illuminant variability on matte surfaces[15][16].

2.3.4 HSV color space

HSL, HSV, HSI, or related models are commonly used in computer vision and image analysis for colorimetric detection or image segmentation. It is very useful in applications focused on object detection, object recognition and image analysis among others. The R, G, and B intensities in a digital image are depending on the intensity of light reflected by an object. For that reason, image analysis are better described using parameters such as hue, lightness and saturation. The hue is the parameter representing the chromaticity of the color perceived (yellow, red, blue...), lightness is the brightness relative to the brightness of a similarly illuminated white and saturation is the perceived intensity of a specific hue (figure 2.10) [1] [17] [18].
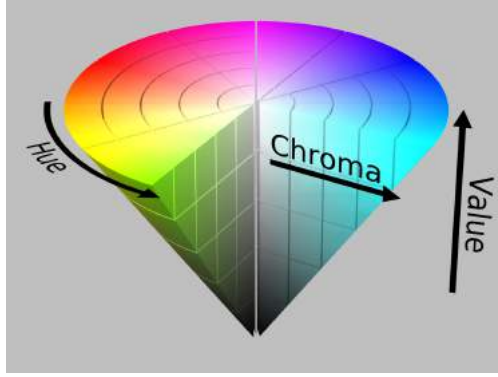
Figure 2.10: HSV color space [17]

2.3.5 White calibration

J.A. Toque et al.[19] compensate the effect of the illuminant variability using the equation 2.7. This white calibration is trying to mitigate the uneven reflection of light from the surface of the object.

T'_i = i value of a pixel of the image

X_b = average pixel value of the white background

B_i = i value of a pixel of the white background

T_i = new i value of a pixel of the image adjusted

$$T'_i = Ti(\frac{X_b}{B_i})$$
(2.7)

Through establishing a reference color in the image the rest of color values can be calibrated.

2.3.6 Thresholding and edge dectectors

The simplest thresholding methods replace each pixel in an image with a black pixel if the image intensity I, is less than some fixed constant T, then:

$$Iij < T$$
(2.8)

Color images can also be thresholded by separating the RGB channels of an image. After that, channels can be used individually, or combined with an AND operation. Edge detectors techniques are often used in application for image segmentation and object detectors. Sobel, Canny or Laplacian are the most used for this purposes [20].

2.4   Optical sensors

There is an increasing demand to measure the physical and chemical properties of an environment. For that reason there is a need to change the classical analytical methods with more robust and modular sensors that simplify the analytical procedure. A sensor is a device capable of recording directly, continuously, and reversibly a physical parameter (physical sensor) or the presence or concentration of a chemical species (chemical sensor). A chemical sensor system consists of a sensitive layer, a transducer, a data-acquisition process, and an evaluation. This research is focused on optical sensors that basically consist in the interaction of electromagnetic radiation with a sample after a recognition event that modifies different optical parameters that may be recorded [21]. The increasing interest in chemical analysis based on color changes bring advantages such as simplicity of use, facility to combine with portable and widely distributed imaging devices and friendly analytical procedures in out-of-lab applications for in situ and real-time monitoring [18]. The main inconvenience analyzing images from a portable device is the variability of the environment where the picture is captured. Variable conditions such as focal distance, angle, illumination from the camera to the object have to be taken into consideration in order to obtain the best accuracy and repeatability for color measurements.

Ariza-Avidad et al [1][2], employ computer vision-based analytical procedures to determine a concentration of substances such as heavy metals or sulphide in water. The change in color measured from the image of a disposable membrane acquired by a digital camera using the hue coordinate (H) of the HSV color space as the analytical parameter is able to sense the substances in aqueous solution. The chemical sensor is photographed before and after the reaction. Then, the images are processing using thresholding techniques that allow to isolate the region of interest (ROI). The H parameter is obtained from the image pixels and is correlated to a concentration. Although the color space used is the HSV, only the information related to chromaticity is used (H), ignoring the saturation and brightness values that are closely related to the intensity of light. Furthermore, the images are captured using a cube light box (figure 2.11) that ensures an optimal image capture in terms of illumination, angle and focal distances.

Figure 2.11: Cube Light Box [2]

This method for analyzing optical sensors simplify the classical methods employed in a lab (where results are typically obtained using a spectrophotometer), reducing costs and allowing an in-situ analysis. However the method require an external software running on a computer to process the images, reducing the portable capacity of the instrumentation.

Aree Choodum et al [22] employ an Iphone as a device for a rapid quantitative analysis of trinitrotoluene (TNT) in soil. In this work the concentration of TNT are correlated to the RGB values of the image captured after the reaction (figure 2.12).
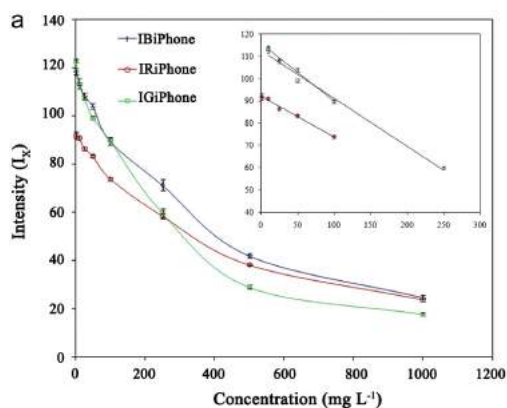

Figure 2.12: Correlation between TNT concentration and RGB values [22]

To evaluate the accuracy of the color measured using the smartphone, a comparison between an image captured by an Iphone and another one captured by a DSRL camera was carried out, obtaining satisfactory results that allows an user to determine the concentration of TNT in soil using an Iphone. However, in this research there is no information about the image capture conditions and there is no evaluations explaining the behavior of the application running in different devices.

The camera software of the smartphones tries to compensate the ambient light. They are basically designed to improve the image quality, but do not take into account the color fidelity of the photographed object. Therefore, this kind of investigations are suitable for an unique device.

Attempting to avoid the capture of images under ambient light and trying to maintain the portability of the instrumentation, the research carried out by Ahmet F. Coskun et al [23] brings a possible solution to the problem described. As represented in figure 2.13, an opto-mechanical attachment installed at the back of the cellphone eliminates possible image artefacts due to the hand motion of the user, creating a more repeatable, reliable and sensitive platform for personal use.
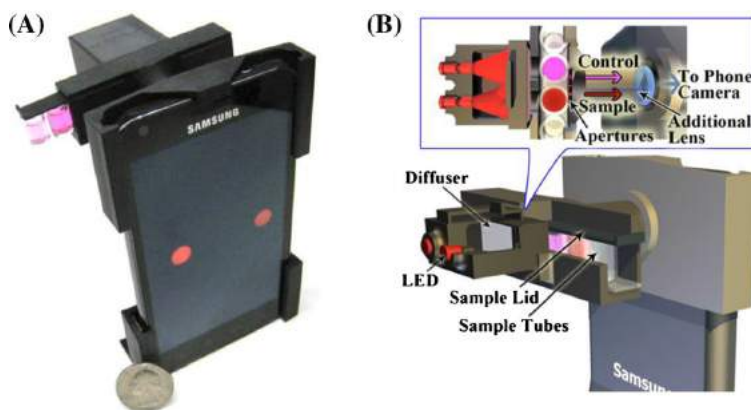
Figure 2.13: An opto-mechanical attachment installed at the back of the cellphone [23]

Obviously, this solution requires the manufacture of one attachment for each existing device model. Therefore, the given solution is not focused on the use of multiple devices but to convert a smartphone in a reliable tool for colorimetric detection.

An approach for an application able to ensure the inter-phone repeatability for the analysis of colorimetric sensors is described in the research carried out by Ali K. Yetisen et al [24]. The

designed algorithm considers that the application developed for sensing glucose, protein and pH in urine, is able to work in a wide variety of devices and operating systems and also that the image capture takes place under different environmental conditions. To evaluate the application, the authors use two different operating systems (Android and iOS) on both, high and low-end smartphones (i.e. iPhone 5, 8 MP camera; and Samsung I5500 Galaxy 5, 2 MP camera) and the images are taken under ambient light. The RGB values obtained from a portion of the image are converted to tristimulus values, X, Y, Z. The Z parameter is ignored and the color is expressed as 2D chromaticity values.

CHAPTER 3

ARTEFACT DESIGN

3.1 Printed sulphide optical sensor

In order to find out which are the requirements for designing an artefact able to quantify the concentration of a substance using an optical sensor, a set of experiments were carried out employing a sulphide sensing membrane, following and replicating the same procedure than in previous work published by Ariza-Avidad et al.[1].

The experiments were carried in collaboration with the ECsens research group [27], in the labs of the Analytical Chemistry Department, University of Granada. As described in the related paper, a chromogenic reagent that experiment a hue shift in the presence of sulphide in water can be used as colorant, taking part in an ink formulation. The ink can be printed by inkjet printing technologies as described in figure 3.1. The printer processes a digital file containing a pattern previously designed.
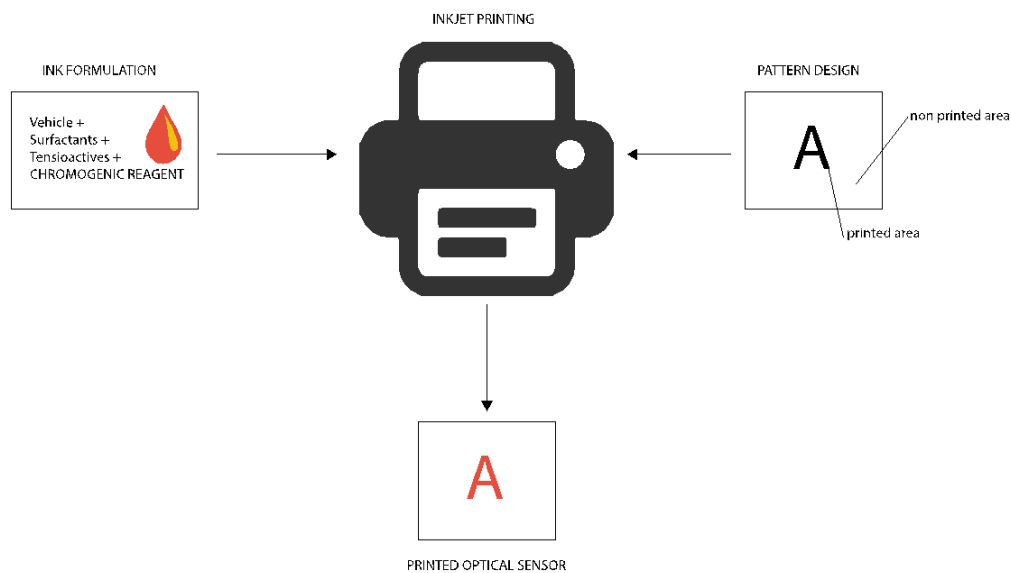
Figure 3.1: Printed Sensors workflow

3.2 Pattern design

The pattern was designed using Adobe Illustrator CS6 software (Adobe Systems Inc., San Jose, CA, USA). It consists in a circular shape with a diameter of 3mm with four repetitions. The digital file generated is a monochrome bitmap file of 1-bit per pixel (1bpp). That means that the values of those pixels defining the printed area is 1, being 0 for the ones defining the non-printed area (figure 3.2).
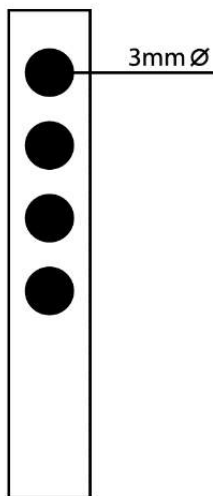
Figure 3.2: Pattern design

3.3 Printing optical sensors

The sulphide disposable sensing membranes were printed using a Fujifilm Dimatix DMP-2831 printer (Fujifilm Dimatix, Inc., Santa Clara, CA, USA) on a white Biodyne A nylon membrane (Pall Corporation, Port Washington, New York,USA). The cartridge of the printer is containing the chemical sensor ink and the pattern designed is processed by the printer to generate the final plot (figure 3.3).
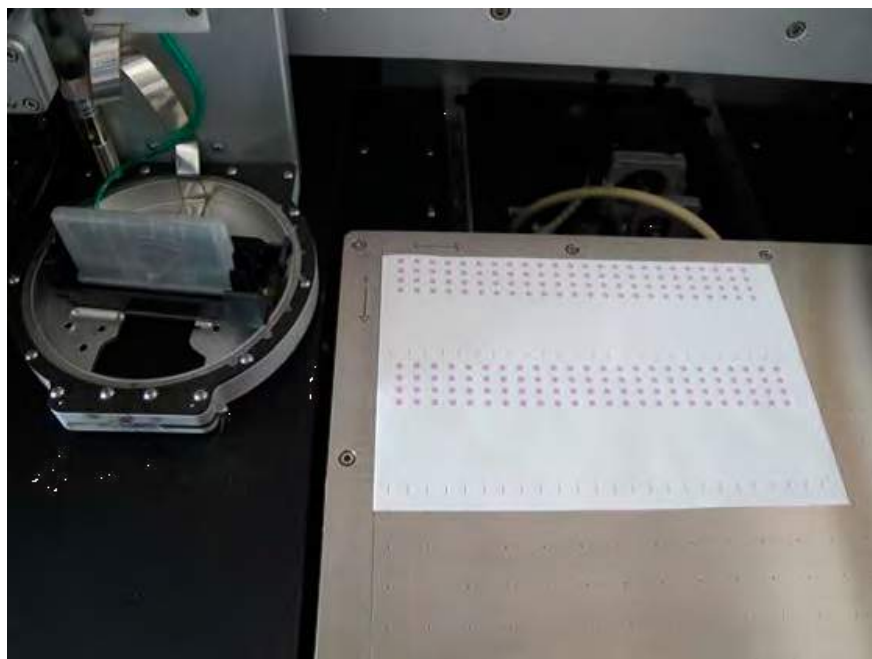
Figure 3.3: Printing Optical Sensors

3.4 Optical sensor reaction

The response of sensing membranes to sulphide was evaluated from different sulphide standard solutions from $10^{-7}M$ to $10^{-4}M$ by adding the volume of each standard to a 10 mL polyethylene plastic tube with pH adjusted 2 mL of 0.5 M buffer phosphate solution pH 7.4. The membrane was introduced for 5 min into the tube without shaking (figure 3.4). Nine concentrations were tested and the color information was collected for four replicates each one. The corresponding concentrations from 1-9 were the following: $10^{-7}M$, $5*10^{-7}M$, $10^{-6}M$, $2.5*10^{-6}M$, $5*10^{-6}M$, $7*10^{-6}M$, $8.5*10^{-6}M$, $10^{-5}M$ and $10^{-4}M$ respectively.
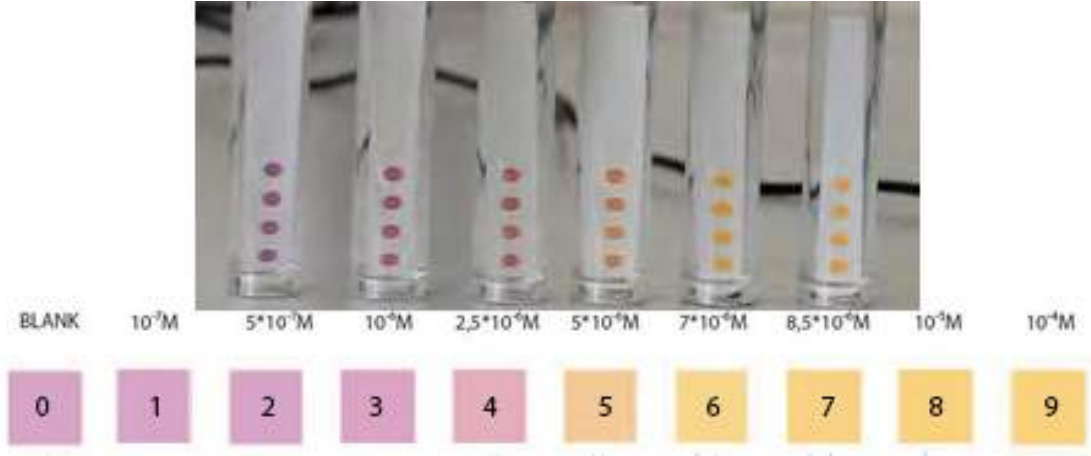
Figure 3.4: Optical sensor reaction

3.5 Evaluation of the reaction using a spectrophotometer

A Colormunki spectrophotometer from X-Rite (Grand Rapids, Michigan, USA) was used as reference measuring instrument in order to measure the reflectance of the sensing membranes before and after the reaction. The illuminant was D65, with an observer angle of 2 degrees. The range of the spectrum was 380 nm to 730 nm and the spectral interval was 10 nm. We collected the spectral sensitivity curves of three linear detectors providing the CIE tristimulus values X, Y and Z, known as the CIE standard observer.

With reference to the standard observer, the equations 3.1, 3.2, and 3.3 represent the tristimulus values for a color with a spectral power distribution where is the wavelength of the equivalent monochromatic light (nm).

$$X = \int_{380}^{730} I(\lambda)\overline{x}(\lambda)d(\lambda) \tag{3.1}$$

$$Y = \int_{380}^{730} I(\lambda)\overline{y}(\lambda)d(\lambda) \tag{3.2}$$

$$Z = \int_{380}^{730} I(\lambda)\overline{z}(\lambda)d(\lambda) \tag{3.3}$$

21

To achieve a universal measurement and in order to compare it to images captured by a smartphone, a transformation from the tristimulus XYZ values to sRGB color space (equation 3.4) was needed.

$$\begin{bmatrix} R_linear \\ G_linear \\ B_linear \end{bmatrix} = \begin{bmatrix} 3.2406 & -1.5372 & -0.4986 \\ -0.9689 & 1.8758 & 0.0415 \\ 0.0557 & -0.2040 & 1.0570 \end{bmatrix} \begin{bmatrix} X \\ Y \\ Z \end{bmatrix} \quad (3.4)$$

The RGB values converted from the XYZ values collected before and after the reaction are shown in table 3.1.

Table 3.1: RGB values for each reaction using a spectrophotometer

SULPHIDE CONCENTRATION	Red intensity	Green Intensity	Blue Intensity
Blank (no reaction)	213	168	206
1	211	164	205
2	211	165	201
3	213	165	197
4	225	174	184
5	244	202	163
6	254	214	154
7	254	211	148
8	255	213	148
9	255	217	157

These RGB values corresponding to each sulphide concentration are represented in figure 3.5.
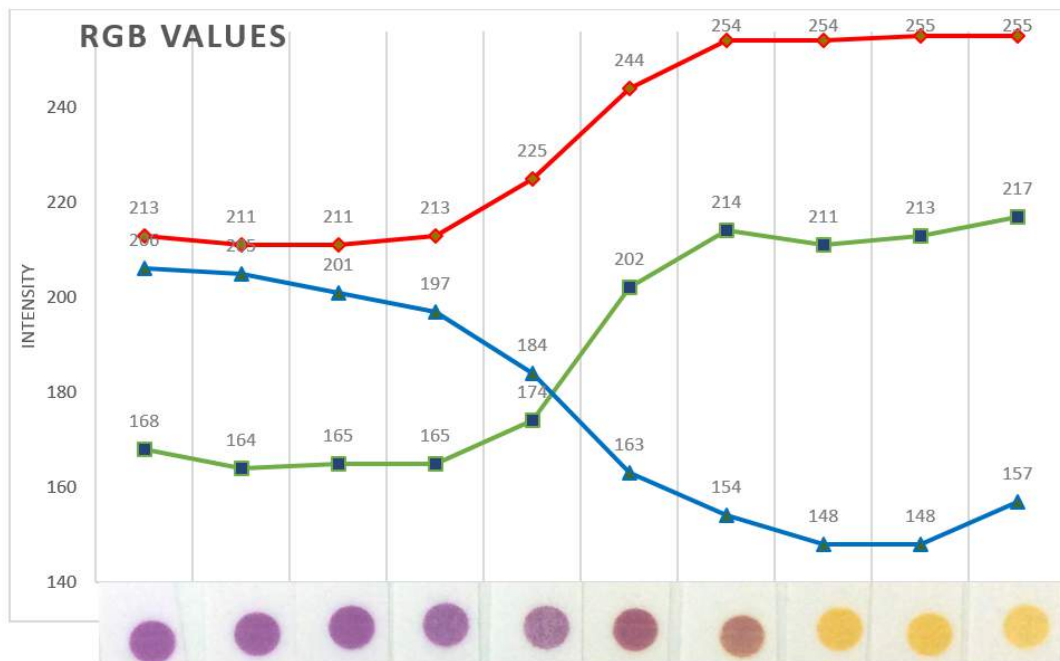
Figure 3.5: RGB values for each reaction using a spectrophotometer

As we can observe, the color of the chromogenic reagent experiments a hue shift from violet to yellow while the sulphide concentration in water is increasing. In a closer look at the case we can notice that the intensity of the blue channel is decreasing while the intensity of red and green increases. In an additive color mode sRGB the sum of R and B results in color yellow. In other words, violet color is complementary to yellow color (figure 3.6).

23
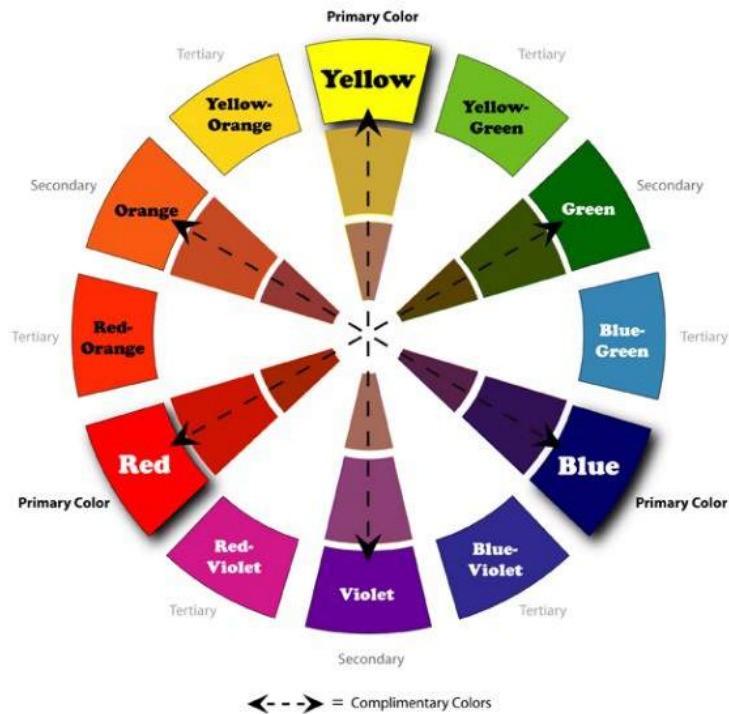
Figure 3.6: Complementary colors diagram

This approach leads to a suitable artefact calibration. The blue channel can be used as analytical parameter. A system able to correlate the blue intensity to a sulphide concentration in water could be designed by entering the data of the calibration into the system, represented in table 3.2.

Table 3.2: Blue intensity for each reaction

SULPHIDE CONCENTRATION	Blue Intensity
Blank (no reaction)	206
$10^{-7}M$	205
$5*10^{-7}M$	201
$10^{-6}M$	197
$2.5*10^{-6}M$	184
$5*10^{-6}M$	163
$7*10^{-6}M$	154
$8.5*10^{-6}M$	148
$10^{-5}M$	148
$10^{-4}M$	157

For instance, B_i = *Blue intensity measurement of the optical sensor by the spectrophotometer*

If $B_i < 201$ and $B_i > 197$ then

Concentration $< 10^6$ M

However, the repeatability of the system has a strong dependence on the measuring instrument employed in the process. In this case, the instrument is a spectrophotometer that can be successfully calibrated, also the intensity and color temperature of the illuminant are controlled and furthermore, the distance to the object and angle are known. The use of a smartphone as a color measuring instrument involves a great variability as mentioned in the introduction and as we will see in the following experiments carried out.

3.6   Analysis of the requirements

Once the case has been analyzed using a traditional color measuring instrument, the requirements for designing the artifact can be defined:

- Capture an image using a smartphone: After the optical sensor reaction, the application must allow the user to capture the image of the sensing membrane. For this purpose, the camera software of the device will be used.

- ROI setection: In order to both, isolate the ROI of the image and reduce the image size.

- Color image processing: Collect and process the color values of the ROI.

- Quantification: Correlate the concentration of the substance to the color values gathered previously by the device.

- Share results: Allow the user to know the concentration of the sulphide in water.

CHAPTER 4

RESULTS

4.1   Image capture

Three different smartphones were used for capturing images of the optical sensor through replicating the previous process, an iPhone 5s (Apple Inc., Cupertino, CA, USA), a Samsung Galaxy S4 Zoom (Samsung Electronics, Suwon, South Korea) and a LG Optimus L5 (LG electronics, Seoul, South Korea). A light box was used in order to both, isolate the sensing membranes of the ambient light and place the smartphones in an equal position while capturing the image. In this way, the distance from the smartphone camera to the optical sensor was 5 cm. The illuminant inside the box was provided by an array of D65 led (figure 4.1). The images were captured using the automatic mode available in the camera software of the devices. The dimensions expressed in pixels of the images acquired by the three smartphones were 2448x3264 (iPhone), 4608x3456 (Samsung) and 1920x2560 (LG). The color space embedded in the image is the sRGB for the three cases.

4.2   Image processing

For selecting the ROI, Adobe Photoshop CS6 software (Adobe Systems Inc., San Jose, CA, USA) was used.
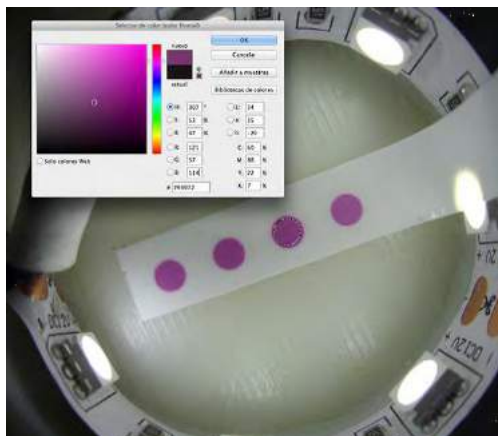
Figure 4.1: Light box and ROI

The color information from the images captured after the reaction was collected. The average of the RGB values of the ROI was obtained and are shown in tables 4.1, 4.2 and 4.3.

Table 4.1: RGB values and number of pixels for each reaction (Samsung)

SULPHIDE CONCENTRATION	Red intensity	Green Intensity	Blue Intensity	No. Pixels
1	129,18	85,75	127,2	5450
2	127,98	84,75	124,25	5530
3	125,95	81,09	123,04	5346
4	126,29	90,13	123,56	5486
5	126,45	94,22	118,01	5501
6	125,91	84,51	96,57	4969
7	130,97	98,26	82,97	4877
8	144,36	132,3	44,9	4855
9	141,51	131,1	47,33	4867

Table 4.2: RGB values and number of pixels for each reaction (LG)

SULPHIDE CONCENTRATION	Red intensity	Green Intensity	Blue Intensity	No. Pixels
1	126,44	91,01	143,84	2326
2	126,75	89,92	143,91	2204
3	124,28	89,06	142,11	2214
4	126,37	96,01	140,85	2227
5	129,27	101,64	132,81	2266
6	125,55	87,33	109,23	2139
7	133,69	101,71	96,74	2041
8	153,99	130,08	60,39	2148
9	153,66	130,57	65,97	2464

IPHONE

Table 4.3: RGB values and number of pixels for each reaction (Iphone)

SULPHIDE CONCENTRATION	Red intensity	Green Intensity	Blue Intensity	No. Pixels
1	99,87	78,94	102,75	4500
2	98,44	78,31	99,54	4425
3	96,13	76,26	97,88	4433
4	99,05	86,97	100,99	4579
5	104,19	97,74	98,12	4657
6	100,69	79,43	75,9	4170
7	112,13	100,32	74,12	4214
8	140,93	143,69	63,87	4638
9	141,97	145,2	64,97	4705

A comparison among the values gathered by the three smartphones and the spectrophotometer is represented in the figure 4.2.
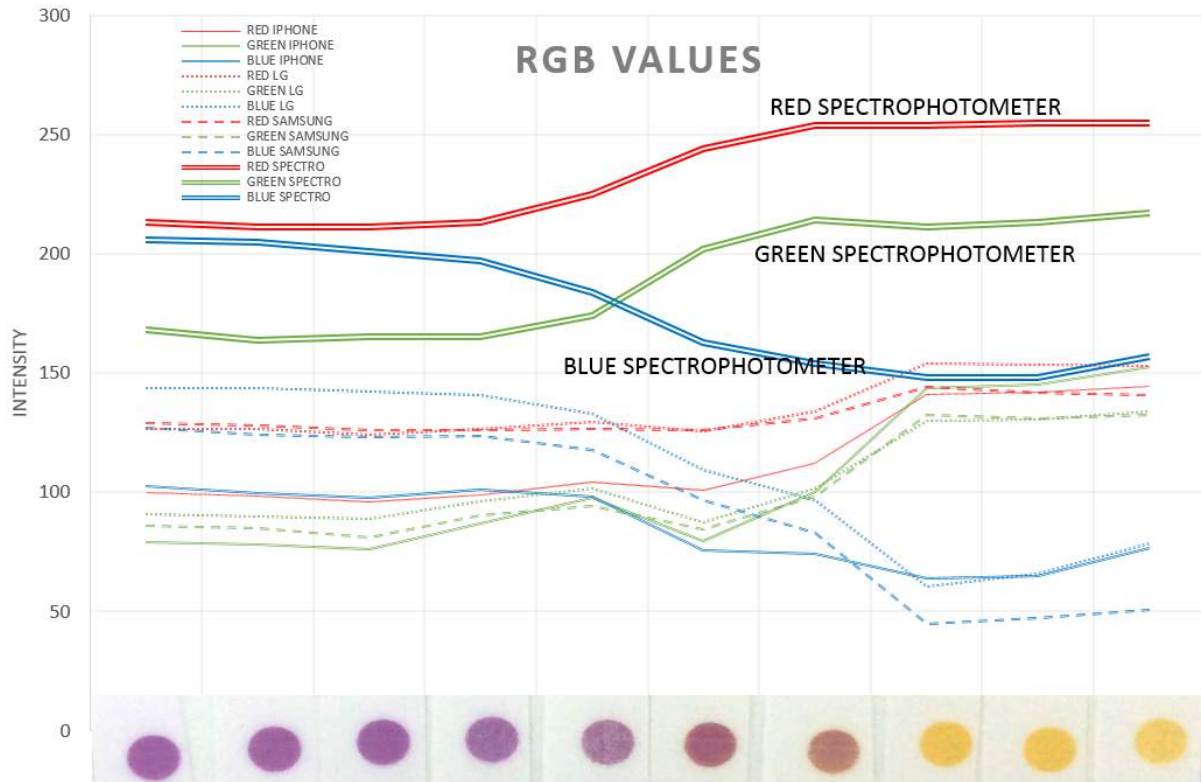
Figure 4.2: RGB values comparison among devices

The intensity of the RGB values collected by the spectrophotometer are significantly higher than the smartphone ones. This is because of the illumination provided by the instrument. It is specifically designed for this purpose and it results in a better performance of the color reflected. Moreover, the intensity of the values collected by the smartphones are different among them. Although the images were captured under equal circumstances by using a light box, the results are strongly affected by the color interpretation from different devices technologies. Furthermore, the automatic mode used for capturing images employs algorithms designed mainly to improve the image quality, but not to preserve the color fidelity of the environment.

4.2.1   Normalized RGB

As we are interested in the chromaticity of a measured color, we can overlook the brightness value (that is closely related with the intensity of light perceived) through normalizing the RGB

values. All the previous values from each one of the four devices were normalized following the equations 4.1, 4.2 and 4.3

$$r = R/R + G + B \tag{4.1}$$

$$g = G/R + G + B \tag{4.2}$$

$$b = 1 - r - g \tag{4.3}$$

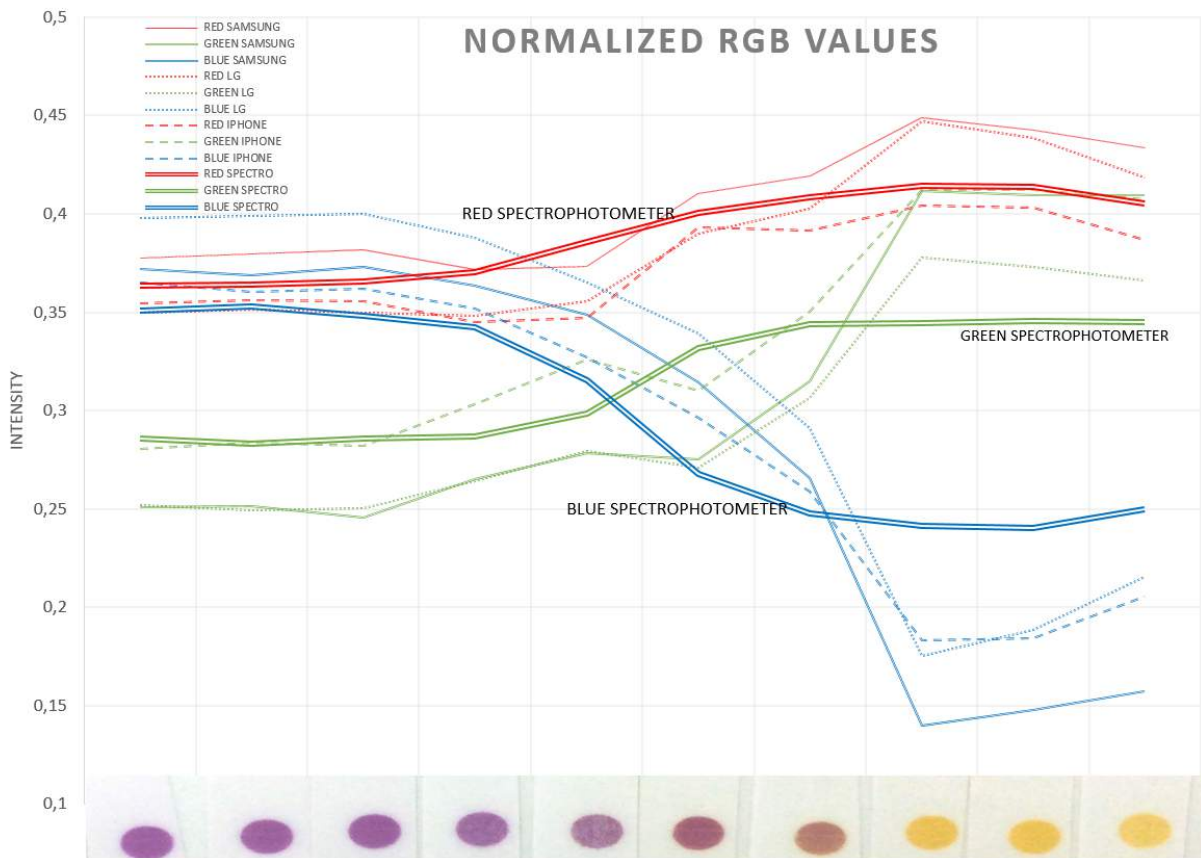The results are represented in figure 4.3

Figure 4.3: Normalized RGB values comparison among devices

After normalizing RGB values the intensity responses are more similar. However the results are not sufficiently satisfactory to create a common calibration data for the three smartphones.

The design needs to improve the performance of the artefact in order to ensure the inter-phone repeatability.

4.2.2 HSV color space

The HSV color space defines a color in hue, saturation and brightness. As observed before, the optical sensor experiments a hue shift, from violet to yellow. In this kind of analysis the hue parameter provides more useful information than brightness and saturation. The values representing brightness and saturation can be ignored. Firstly because of their dependence on the amount of light and secondly to reduce the space dimensionality. The RGB values gathered by the three smartphones were converted into the HSV color space (ignoring the saturation and brightness values) following the equations 4.4 - 4.7.

$$Cmax = max(R, G, B) \tag{4.4}$$

$$Cmin = min(R, G, B) \tag{4.5}$$

$$\Delta = Cmax - Cmin \tag{4.6}$$

$$
H = \begin{cases}
0 & if \quad Cmax = 0 \\
(60 * \frac{G-B}{\Delta} + 360) mod 360 & if \quad R = Cmax \\
60x \frac{B-R}{\Delta} + 120 & if \quad G = Cmax \\
60x \frac{R-G}{\Delta} + 240 & if \quad B = Cmax
\end{cases} \tag{4.7}
$$

The H values are shown in the table 4.4.

Table 4.4: H values collected by the three smartphones

SULPHIDE CONCENTRATION	H Samsung	H LG	H Iphone
1	302,74	280,24	292,74
2	305,18	280,93	296,89
3	303,89	279,83	295,14
4	304,53	280,62	291,70
5	315,71	293,19	356,47
6	342,52	325,62	8,54
7	19,11	8,07	41,36
8	52,72	44,67	62,07
9	53,37	44,20	62,42

The H parameter values are represented in a circular range from 0 to 360 (figure 4.4). That explains for example, the reaction no. 6 where the color is close to 360 (a red color varying from 342 to 8).
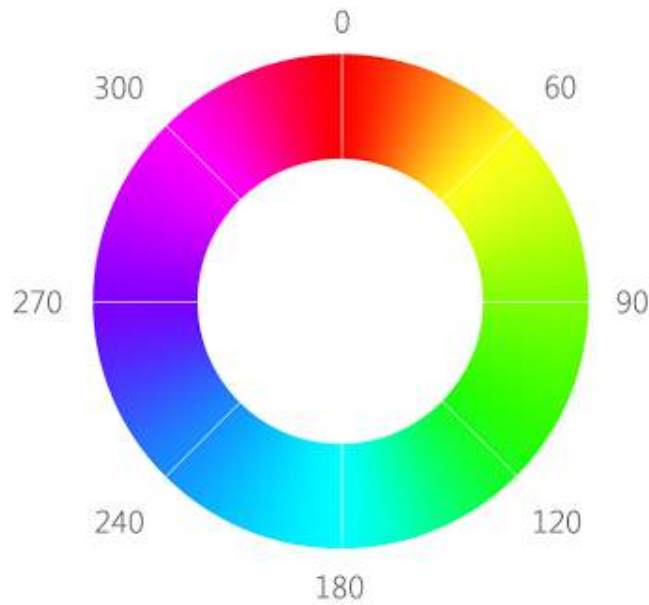

Figure 4.4: H parameter representation

The comparison is represented in figure 4.5
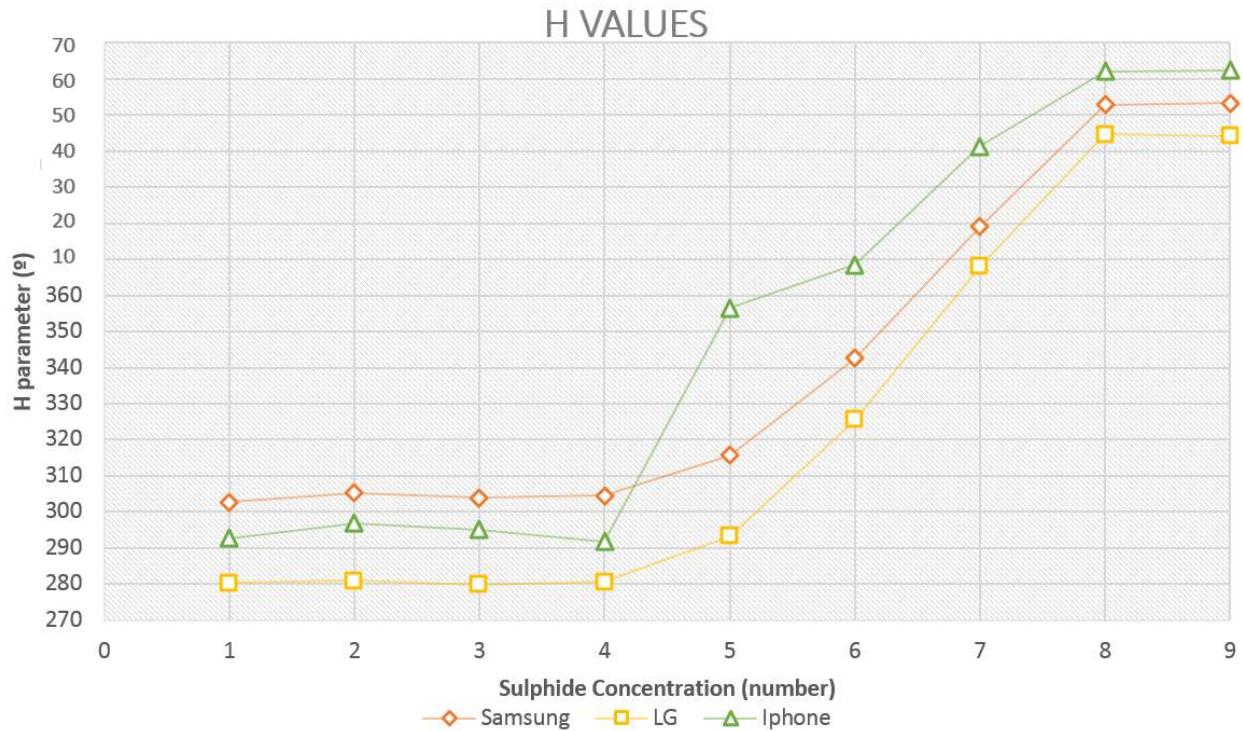
Figure 4.5: H parameter comparison

As it was already in the case of normalized RGB, the values gathered are not enough valid to design a system with inter-phone repeatability. We can observe that the differences among the colors measured by the three smartphones are not only caused by variations in lightness and saturation. Actually, one of them is significantly different in hue from the rest ones, making it impossible the use of a single calibration running in different devices.

4.2.3 White background calibration

The normalized blue collected by the three smartphones for each reaction is represented isolated in figure 4.6

NORMALIZED BLUE VALUES

Figure 4.6: Normalized blue for each reaction

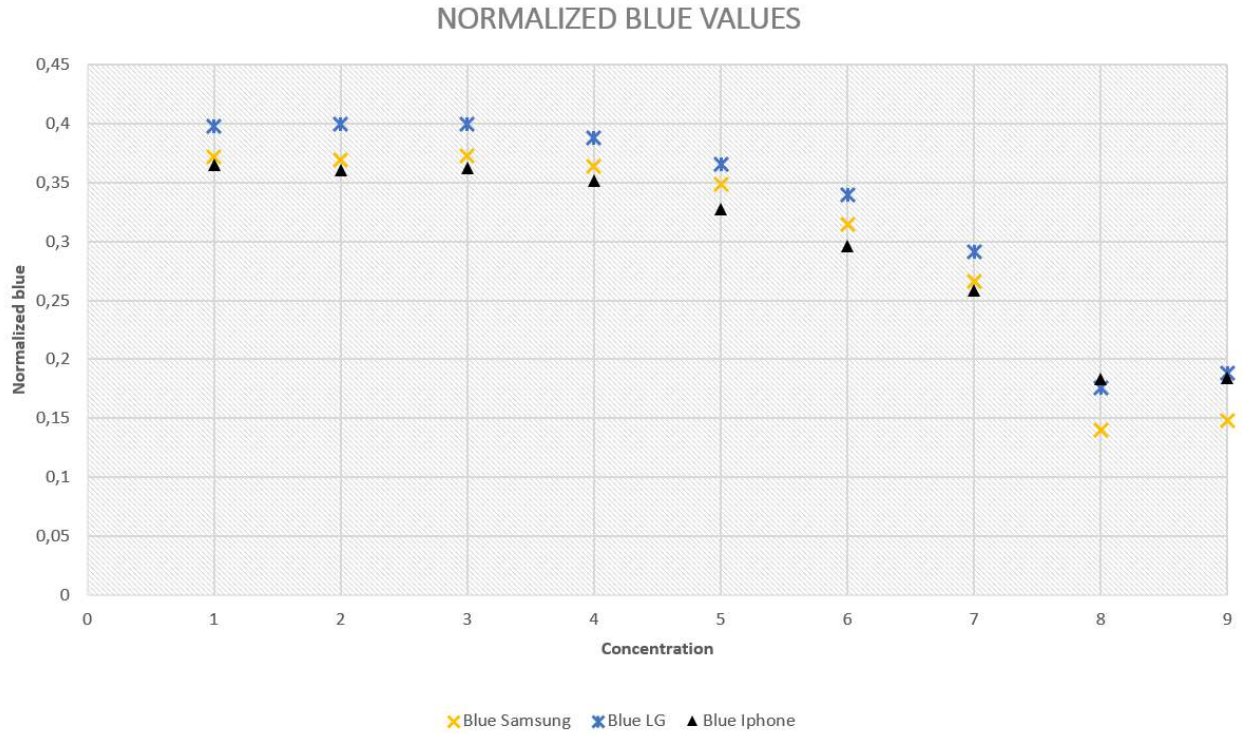In order to improve the accuracy of the artefact, the color values of the image background was also collected. The optical sensors are printed on a white nylon substrate which color is an almost neutral color. It could be used for calibrating the device. The color information of this white background was measured by the four devices (the three smartphones and the spectrophotometer) and are shown in the table 4.5.

Table 4.5: RGB values of the nylon substrate

Device	Avg. Red Background	Avg. Green Background	Avg. Blue Background
Spectrophotometer	204	201	208
Iphone	150	184	151
LG	171	170	171
Samsung	157	168	160

The RGB values obtained using the spectrophotometer are defining an almost neutral color, slightly bluish. The measurements collected using the LG smartphone are also defining a neutral

34

color. However, the iPhone and the Samsung are defining a greenish color.

The normalized blue of the white background was used as reference value for the calibration. The content of blue of the optical sensor was obtained by establishing a ratio between the blue intensity of the white background and the blue intensity of the sensor.

Blue ratio = AVG. Normalized blue of the background / AVG. Normalized blue of the sensor
This ratio was obtained using the measurements of all the reactions for each of the smartphones. The results are showed in table 4.6.

Table 4.6: Blue ratio of each reaction

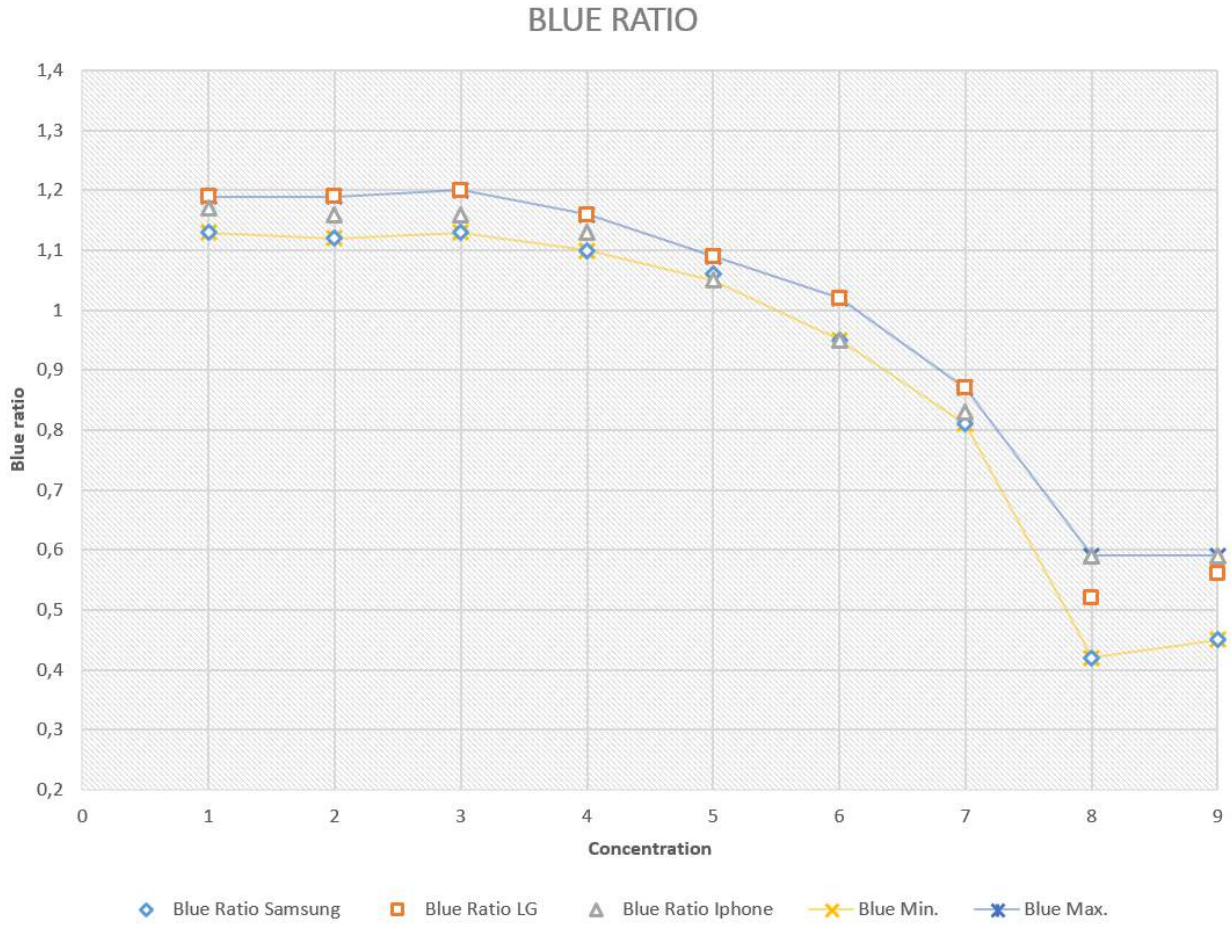| Concentration	Blue Ratio Samsung	Blue Ratio LG	Blue Ratio Iphone	Blue Min.	Blue Max.
1	1,13	1,19	1,17	1,13	1,19
2	1,12	1,19	1,16	1,12	1,19
3	1,13	1,20	1,16	1,13	1,20
4	1,10	1,16	1,13	1,10	1,16
5	1,06	1,09	1,05	1,05	1,09
6	0,95	1,02	0,95	0,95	1,02
7	0,81	0,87	0,83	0,81	0,87
8	0,42	0,52	0,59	0,42	0,59
9	0,45	0,56	0,59	0,45	0,59

The data is represented in figure 4.7

Figure 4.7: Blue Ratio values from the three devices

CHAPTER 5

CONCLUSIONS

A smartphone can be used as a color measuring device for quantify the concentration of a substance in colorimetric tests. In this research a set of experiments were carried out in order to evaluate the artefact designed. The RGB values comparison among the spectrophotometer and the three smartphones shows how different is the intensity of the reflected light perceived. This fact, is affecting the interpretation of color by each device. The color measured for each reaction is indicating a lack of accuracy in terms of color values. Nevertheless, quantification accuracy is important, but not the accuracy of the color measured with regard to the spectrophotometer. As described in the previous work [1], the suplhide optical sensor used in the experiments can be analyzed employing a digital camera and using the H parameter of the HSV color space. However this method is valid only when the image is captured by a singular device. The comparison of the results obtained from images captured by multiple devices is indicating a great range of error in the quantification. This results are not satisfactory for an application with interphone-repeatability. As observed in the analysis phase the blue channel can be used as analytical parameter in the quantification. This fact, reduce the space dimensionality and ease the data processing of the device. Through normalizing the blue channel, the range of error among the three smartphones is reduced. Furthermore, taking advantage of the neutral color of the surface of the nylon membrane, the color pixels can be calibrated, compensating the effect of the undesired color dominance in an image, reducing even more the range of error, leading to a suitable application design with interphone-repeatability. Finally, the research carried out is describing a method to digitize color measurement in quantitative analyses and should be applied to multiples analysis of colorimetric tests.

BIBLIOGRAPHY

[1] M. Ariza-Avidad, M. Agudo-Acemel, A. Salinas-Castillo, L.F. Capitan-Vallvey. "Inkjet-printed disposable metal complexing indicator-displacement assay for sulphide determination in water". Analytica Chimica Acta, Volume 872, 4 May 2015, Pages 55-62

[2] M. Ariza-Avidad, A. Salinas-Castillo, M. P. Cuellar, M. Agudo-Acemel, M. C. Pegalajar, and L. F. Capitan-Vallvey, "Printed Disposable Colorimetric Array for Metal Ion Discrimination". Anal. Chem., 2014, 86 (17), pp 8634-8641 DOI: 10.1021/ac501670f

[3] Sharma, G., Trussel, H.J. (1997): Digital color processing. IEEE Trans. on Image Processing, 6(7): 901-932.

[4] K.N. Plataniotis and A.N. Venetsanopoulos. "Color Image Processing and Applications Engineering", February 18, 2000 Springer-Verlag.

[5] http://hyperphysics.phy-astr.gsu.edu/hbase/vision/cie.html#c2

[6] CIE (1932). Commission internationale de l'Eclairage proceedings, 1931. Cambridge: Cambridge University Press

[7] Jeong-Yeol Yoon (2012): "Spectrophotometry and Optical Biosensor2. Introduction to Biosensors pp 121-139

[8] https://global.britannica.com/science/colorimetry

[9] Nicholas D. Lane, Emiliano Miluzzo, Hong Lu, Daniel Peebles, Tanzeem Choudhury, and Andrew T. Campbell, "A Survey of Mobile Phone Sensing" Comm. Mag., vol. 48, pp.140 - 150 September 2010

[10] Elsa Macias , Alvaro Suarez and Jaime Lloret. "Review Mobile Sensing Systems ".Sensors 2013, 13, 17292-17321; doi:10.3390/s131217292

[11] E. Koukoumidis, M. Martonosi and P. Li-Shiuan, "Leveraging smartphone cameras for collaborative road advisories", IEEE Trans. Mobile Comput., vol. 11, no. 5, pp. 707-723, 2012

[12] Ruxandra Tapu, Bogdan Mocanu, Andrei Bursuc, Titus Zaharia, "A Smartphone-Based Obstacle Detection and Classification System for Assisting Visually Impaired People". IT/Telecom SudParis, ARTEMIS DEpartment, UMR CNRS MAP5 8145, Evry, France.

[13] Sandeep Kumar Vashist, Onur Mudanyali, E. Marion Schneider, Roland Zengerle, Aydogan Ozcan, "Cellphone-based devices for bioanalytical sciences" Analytical and Bioanalytical Chemistry May 2014, Volume 406, Issue 14, pp 3263-3277

[14] http://www.bluebugle.org/2013/03/smartphone-camera-technology-analysis.html

[15] W. Skarbek and A. Koschan. "Color Image Segmentation - A Survey -". Technical report 94-32, Computer Science Department, TU Berlin, 1994.

[16] Vladimir Vezhnevets, Vassili Sazonov, Alla Andreeva. "A Survey on Pixel-Based Skin Color Detection Techniques" Graphics and Media Laboratory. Faculty of Computational Mathematics and Cybernetics Moscow State University, Moscow, Russia

[17] Joblove, George H.; Greenberg, Donald (August 1978). "Color spaces for computer graphics". Computer Graphics. 12 (3): 2025. doi:10.1145/965139.807362

[18] Luis Fermin Capitan-Vallvey et al. "Recent developments in computer vision-based analytical chemistry: A tutorial review" ArticleinAnalytica chimica acta 899 October 2015 doi:10.1016/j.aca.2015.10.009

[19] Computer Vision, Imaging and Computer Graphics: Theory and Applications: International Joint Conference, VISIGRAPP 2009, Lisboa, Portugal, February 5-8, 2009. Revised Selected Papers

[20] Debosmit Ray. "Edge Detection in Digital Image Processing" June 06, 2013.

[21] Mara Ariza Avidad, "Color and nanomaterials in optical sensors". PhD Thesis.University of Granada, July 2014, ISBN: 978-84-9083-168-7 pp.25 - 53

[22] Aree Choodum, Proespichaya Kanatharana, Worawit Wongniramaikul, Niamh NicDaeid, "Using the iPhone as a device for a rapid quantitative analysis of trinitrotoluene in soil". Talanta 115(2013) 143 - 145.

[23] Coskun AF, Wong J, Khodadadi D, Nagi R, Tey A, Ozcan A. "A personalized food allergen testing platform on a cellphone". Lab on a chip. 2013;13(4):636-640. doi:10.1039/c2lc41152k.

[24] Ali K. Yetisen, J.L. Martinez-Hurtado, Angel Garcia-Melendrez, Fernando da Cruz Vasconcellos, Christopher R.Lowe, "A smartphone algorithm with inter-phone repeatability for the analysis of colorimetric tests". Sensors and Actuators B: Chemical. Volume 196, June 2014, Pages 156 -160. doi:10.1016/j.snb.2014.01.077

[25] David J.You,TuSanPark,Jeong-YeolYoon, "Cell-phone-based measurement of TSH using Mie scatter optimized lateral flow assays".Biosensors and Bioelectronics 40(2013)180-185.

[26] Byoung-Yong Chang, "Smartphone-based Chemistry Instrumentation: Digitization of Colorimetric Measurements". Bull. Korean Chem. Soc. 2012, Vol. 33, No. 2 549 http://dx.doi.org/10.5012/bkcs.2012.33.2.549

[27] http://ecsens.ugr.es