

## UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Físicas y Nanotecnología

## TÍTULO:Generation of False Color Images of Pigments and Colorants in Heritage Artworks

Trabajo de integración curricular presentado como requisito para

la obtención del título de Físico/a

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Jhonatann Carlos Almeida Cuatín C.I. 1720246717 To my wonderful mother Miryan for the Universe of love and effort that she gives to me. To my grandparents Albita & Rafael, for their unconditional love and blessings. To my Love, after all this time, always, for you all.

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## Resumen

Esta investigación tiene como objetivo generar un dispositivo que permita la obtención de fotografías de dos bandas diferentes del espectro: visible (400-700nm) e infrarrojo cercano (940nm) para construir una imagen en Falso Color mediante edición fotográfica; estas imágenes serán de muestras tomadas de cuadros de menos de 100  $\mu$ m, para ello el sistema utilizó un microscopio de luz transmitida, que fue adecuado para funcionar como un microscopio de luz incidente. Esto se lleva a cabo mediante la adaptación de componentes según las necesidades de cumplir condiciones optimas de funcionamiento. Además, se establecen algunos pigmentos base en conjunto con el personal del Instituto Nacional de Patrimonio Cultural (INPC) con el fin de construir la primera base de datos de información de Falso Color para futuros análisis. Los pigmentos se prepararon con dos aceites comunes utilizados por pintores históricos y se encapsularon para la posteridad. El sistema resultante (MMSI) es un dispositivo capaz de proporcionar imágenes esenciales para la generación de imágenes FC en un corto tiempo, que se compone de parte intercambiables y mejorables en cualquier momento. Además, MMSI Pigment Checker es una base de datos que contiene ocho pigmentos que son útiles para identificar rastros de pigmentos de al menos  $5\mu$ m de muestras obtenidas de pinturas antiguas. La aplicación del sistema en tres muestras de prueba proporcionó información necesaria para discernir e identificar los pigmentos utilizados en pinturas del museo de la Iglesia de San Francisco en Quito-Ecuador.

Palabras clave: Falso Color, Pigmento, Color, Microscopio, Luz Infrarroja, Luz Visible, Patrimonio Cultural.

## Abstract

This investigation aims to generate a device that assembles photographs from two different bands of the spectra: visible (400-700nm) and near-infrared (940nm) in order to construct a False Color image through photo editing; these images were taken from samples of less than  $100\mu$ m, for this purpose, the system used a transmitted light microscope, that was adapted to work as an incident light microscope. This was carried by adapting components according to the needs of fulfilling conditions for optimal performance. Furthermore, some base pigments are selected in conjunction with the staff of the INPC in order to construct the first database of FC information for future analysis. The pigments were prepared with two common oils used by historical painters and encapsulated for posterity. The resulting system (MMSI) provides the essential photographs for FC image generation, which is composed of interchangeable and upgradeable components anytime. Also, the MMSI Pigment Checker is a database that contains eight pigments that are useful to identify traces of pigments of at least  $3\mu$ m from samples retrieved from ancient paintings. The system's application in three test samples provides information to discern and identify pigments used in paintings in the museum of the San Francisco Church in Quito-Ecuador.

Keywords: False Color, Pigment, Color, Microscope, Infrared Light, Visible Light, Cultural Heritage.

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# List of Abbreviations

 $\mathbf{D}\text{-}\mathbf{F}\mathbf{C}$ Disorder-False Color

 ${\bf FC}\,$  False Color

 ${f GP}$  Gigapan

**ILM** Incident light Microscope

**INPC** Instituto Nacional de Patrimonio Cultural

 ${\bf IR}~{\rm Infrared}$ 

 ${\bf IRFC}$  Infrared-False Color

 ${\bf LEDs}~{\rm Light}~{\rm Emitting}~{\rm Diodes}$ 

 $\mathbf{MD} \ \mathrm{Multidimmer}$ 

 ${\bf MMSI}\,$  Micro Multi Spectral Imaging

 ${\bf NIR}~{\rm Near-Infrared}$ 

 ${\bf RGB}~{\rm Red}\mbox{-}{\rm Green}\mbox{-}{\rm Blue}$ 

 ${\bf TLM}\,$  Transmitted light Microscope

 ${\bf UV}\,$ Ultra Violet

 ${\bf UVFC}\,$ Ultra Violet-False Color

**VIS** Visible

# Chapter 1 Introduction

Due to the desire of the National Institute of Cultural Heritage in Ecuador (INPC for its acronym in Spanish) to dispose of an additional method in its repository to detect and categorize pigments in a playful, fast and non-destructive way, the design and application of a system are presented to obtain microscopic photographs in the Visible (VIS) and Near-Infrared (NIR) ranges. The current system that uses the INPC consist of capturing general images of artistic works in the Visible (VIS) and Infrared (IR), after those images are transformed to false color (FC) (figure 1.1). This system provides excellent results to differentiate pigments; however, this is expensive and require special components such as a modified reflex camera to be full-spectrum (360-1100nm) [1]; another limitation is that it only provides information about the superficial pigments and remnants of sketches before using pigments, this due to the transparency of some of them under IR light.

The system's development was carried out through some modifications to an optic microscope, such as implementation of probes of light, live image capture device, adjustments to optimal operating conditions, and others. The main goal is to analyze different layers of paint for the detection of own or intruder pigments from the epoch of the piece of art in a non-destructive and quick manner. The system prioritizes the conservation of the painting since it is not necessary a big sample (less than a millimeter square), and also,



Figure 1.1: "San Agustin entre la Sangre y la Leche" by Miguel de Santiago; image taken at the San Agustin Church, Quito-Ecuador.



Figure 1.2: Procedure of channel changing in order to generate a FC image. [2]

this sample can be typically obtained from the edges of the frame. The analysis technique is the false color (FC), this is a common technique that is used to differentiate regions of materials, components, or bodies that are not distinguished from everything that is observed; it is made through the changing of the color layers of photography, it is shown in figure 1.2 [2]. The FC is a technique that uses the sensitivity of the materials to a portion of the IR spectra [3]. In the area of art the FC analysis is a powerful method to identify a large bunch of pigments for different epochs. The success of the method lies in the chemical composition of the pigment and its interaction with the IR light. Some pigments could look similar under the human eye but look different under FC; this means that the VIS spectra of a two pigments could be similar, however the IR spectra will be significant different. In the figure 1.3 we can see the comparison of a bunch of pigments of the  $17^{th}$  century for the visible range with the FC technique [4].



Figure 1.3: Pigment checker for VIS and IRFC images [4].

This work is focused on the most common pigments present in part of cultural heritage of Ecuador in  $17^{th}$  century. An example of a relevant artist of this epoch is Miguel de Santiago; a Quiteño painter (1620-1706) who is the author of countless works [5], an example is the Series of the Christian Doctrine (1670) sheltered in the museum of the San Francisco Church [6], being one of these paintings object of study in this writing. A set of pigments such as lead white, azurite, yellow ochre, vermilion, verdigris and others compose the pigment checker at micro-scale following the requirements of the INPC; those were prepared with established procedures in historical treatises for authors such as Palomino, Pachecho or Cennini [7, 8], some of this information could be found in the recovery work of Parrilla [9].

## 1.1 Scope of Research

This study scopes to develop a system which allows obtain photographs in the visible and infrared range of microscopic samples in order to generate false color images. The system will be focused to collect a bunch of information about pigments of 17<sup>th</sup> century present in paintings. The stratigraphic analysis of a sample from a picture will show important information about repaintings, restorations, authenticity, and other aspects. It will land into the establishment of a pigment checker of the most important colors of the epoch mentioned above. Now, cultural heritages of all the countries are invaluable objects that have to be preserved through the time but also has to be certificated as originals. However, non-destructive analyses are not always available or are really expensive. For that reason is important to develop new techniques to allow the investigation of low cost and without the need of special training.

## 1.2 Objectives

#### **1.2.1** Principal Objective

The main objective is to obtain false color images at micro-scale of pigments from  $17^{th}$  century in the cultural heritage of Ecuador through the adaptability of simple stuff into a light microscope.

#### **1.2.2** Specific Objectives

- To design the components of a normal multi-spectral system for a microscope.
- To calibrate the system with the best possible configuration.
- To create a pigment checker for specific colors of  $17^{th}$  century.
- To establish an adequate transformation procedure of images to false color.
- To apply the system on painting consider as cultural heritage in Ecuador.

## 1.3 Outline

This research work contains five chapters. The first one is a brief introductory information about False Color, history of pigments and the Instituto Nacional de Patrimonio Cultural (INPC) role. The chapter two contains deep information about False Color (FC), light microscopy, Multi-Spectral Imaging system (MSI) and color models. The third chapter involves the experimental Micro Multi Spectral Imaging (MMSI) through three stages and the pigment checker construction. The fourth chapter comprise the resulting MMSI device and pigment checker, application, MMSI capacity and discussion. Finally, conclusions and recommendations in chapter 5.

# Chapter 2 Theoretical Background

## 2.1 False Color (FC)

The FC image analysis, or pseudo color image analysis, is a well know technique that is applied to make a photography more compressible and to find out invisible details, it means that the FC helps to shows hidden information that is not perceptible with the human sight. This is due to that a material is able to reflect light in the infrared (IR) or ultraviolet (UV) spectrum which is not possible to see with the human eyes. So, In order to make useful the information in those spectrum ranges the FC use these to distinguish regions of colors that are similar or that even do not appear different on the real color image [10]. Finally, it is important to mention that this technique is mostly qualitative than quantitative. Certainly, the FC is widely applied in several areas such as in military, astronomic, geological, digital, artistic and others fields. For example, in the military field it is possible to construct an FC image in gray scale (fig. 2.1a) using a pair of detectors in order to segment some details with facility [10]. Another example of FC image is presented in figure 2.1b, which shows the observation of Carina Nebula in a visible and FC color composition that was also constructed with two detectors in VIS and IR [11].

#### 2.1.1 How to make a FC image

In this field of image analysis it is possible to find several kinds of FC transformations. Most commonly the way to transform a current image into a FC image is to change the color channels (Red-Green-Blue (RGB)) into certain order using a image taken with IR, UV or just VIS probes, it is necessary at least two of them. This procedure has a huge amount of manners to construct the FC, since we can choose how to organize the channel using any kind of source. For example, it is possible to use an IR image to replace the red channel and let out the blue channel, in other way is available to use a Ultra Violet (UV) image to replace the blue channel skipping the red channel; finally it is probable to choose just disorder the channels in order to have different colors. However it is necessary to take into account some aspects in order to choose how to transform a photograph. First it



(	(a)	Aerial	image	for	VIS	and	FC.	[10]	
			0						



Figure 2.1: Examples of FC images in different fields of study in order to show the wide applicability of the FC analysis; in the figure (a) the process was through the assignment of layers with different information in the VIS and IR [10], the figure (b) the

FC image was constructed using the data of the band coded by Smith as MSX (6.8–10.8  $\mu m)$  [11].

is necessary to establish the equipment available and the conditions in which is possible to take the photographs and also is really important to have a data base with identified components in the photograph that allow the comparison between the experiment and the known previous. Nonetheless, the figure 2.2 shows the comparison of three types of FC color transformation for two similar pigments (Malachite and Cadmium Green) [12]; the transformations are: infrared false color (Infrared-False Color (IRFC)), ultraviolet false color (Ultra Violet-False Color (UVFC)) and a not conventional FC transformation that move the channels in a different order (Disorder-False Color (D-FC)). The procedure to change the color channels is summarized in the table 2.1.

Table 2.1: Some examples of FC transformation procedures.

FC Transf.	Images Involved	Procedure	Result
IRFC	VIS-IR	$G \to B, R \to G, IR \to R$	IRGB
UVFC	VIS-UV	$G \to R, B \to G, UV \to B$	GBUV
D-FC	VIS	$\mathrm{G} \rightarrow \mathrm{R},  \mathrm{B} \rightarrow \mathrm{G},  \mathrm{R} \rightarrow \mathrm{B}$	GBR

The photographs (VIS, IR and UV) of the whole pigment checkers were downloaded from the web of Cosentino [4] and those were transformed into the correspond FC images (the process will be detailed in the next section). For the case of the two pigments



Figure 2.2: Examples of FC transformations in two common pigments of the 17<sup>th</sup> century (1.VIS, 2. IRFC, 3. UVFC, 4. D-FC) [4].

presented in the figure we can see that the powerful method is the IRFC transformation which shows the highest differentiability; this is the goal of the FC: bring out details of each region of color that are not distinguishable with a regular photography.

## 2.2 Light Microscopy

One of the most affordable field of microscopy is the light microscopy due to its relative low cost, high applicability and handy operation procedures. For logistic purposes of this investigation will be consider just two types of microscopes; the first one is the transmitted light microscope (Transmitted light Microscope (TLM)) and as its name suggests it allows to pass the light through the sample; on the other hand is the incident light microscope (Incident light Microscope (ILM)), which irradiate light on the surface of the sample [13]. Considering that the proposed system needs to have its own sources of light the useful structure of a microscope are all the lenses that compose its column, so in the figure 2.3 is presented the lenses for each type of light microscopes. The reason to mention both microscopes is because the INPC requires the application of the system in a TLM, however a target of this write is to see the surface of the sample and this is a characteristic of an ILM. Consequently it is important to know and understand both types because it is necessary to transform a TLM into a ILM. The microscope provided by the INPC is a Olimpys Bh2 with objectives for 4x, 10x, 20x, 40x and 100x magnifications and 10x ocular magnification.



Figure 2.3: Lens configuration for two kinds of light microscopes: TLM, which make pass the light through the sample and ILM, wich irradiate the surface of the sample being a Reflector the special part of the ILM, this focus the beam into the sample.

## 2.3 Background Information: Multi-Spectral Imaging (MSI)

The MSI system is a common structure implemented in several fields of investigation such as biology, food safety and quality or art [16, 17, 18, 19, 20, 21]; in general those are composed by a detector, sources of light, samples and software. So, for the purposes of this work, the system is based in the MSI system that is currently used in the INPC. This system was acquired from Cultural Heritage Science Open Source (CHSOS) organization in Italy owned by Antonino Cosentino (https://chsopensource.org). Consentino provided to the laboratory of the INPC the whole equipment and training to use the MSI in the investigations of cultural heritage in Ecuador since 2013.

#### 2.3.1 Components

All the components are detailed in the table 2.2 and are presented in the Attachment A. The core item in the system is the modified reflex camera which is a Digital Single-Lens Reflex (DSLR) camera without the IR cut-off filter that is near the sensor[1], the process to modify this camera can be found in the Life Pixel web page (www.lifepixel.com).



Figure 2.4: Range of detection for the Nikon D800 which is able to detect from the UV (360nm) until the IR (1100nm) and InGaAs camera that is specialized in the detection of the furthest part of the NIR (1700nm) [12].

The characteristics of this specific camera make possible the generation of extra-high quality images; first the modification allows, in conjunction with the InGaAs camera, the detection of all the spectrum (IR-VIS-UV), as shown in figure 2.4; each nomenclature in the figure represent the usefulness of the band ( $R \rightarrow Reflectography$ , FC $\rightarrow$ False-Color and F $\rightarrow$ Fluorescence) [12]. Furthermore, the term "Digital" suggest the possibility to tethered the camera to a computer in order to operate features as shutter time, aperture, ISO, focus and to take pictures without touching the camera that should be very still during all the process. It is important to mention that always is crucial to use the color checker, which will provide a color, a frame and a gray scale frame of reference.

The Rest of the equipment will be necessary on specific occasions. For example, another essential part of this system is the Gigapan (Gigapan (GP)), it holds the camera and move the focus area, however if it is necessary just one photo to see the whole artistic picture with enough resolution, the GP will rest. The use of either of the two lenses will depend on the size of the portrayal. Also, the attachment of a certain filter and the use of a probe will bank on the required range of spectra that will be captured.

#### 2.3.2 Photo-taking

The process to generate a photography to analysis is not complicated but is laborious. Following the process of Cosentino, learned in the INPC, the first step is to determine if the painting is big enough to use the GP. If it is needed the GP, the base tripod is placed at approximately 2 meters (20mm lens) or 4 meters (200mm lens) from the object to photograph, it is important to adjust the tripod in a way that the camera will finally be at the center of the frame (Attachment B). Next, the GP is attached to the camera

Component	Specification
Camora Nilton D800	36 MP
Camera Nikon Douu	Modified Sensor for full spectrum detection.
InGaAs camera	BOBCAT-320-Star
GigaPan EpicPro	SN:P006813ZRL
Nikon Longog	Nikkor 200mm 1:4
INIKOII LEIISES	Nikkor 20mm 1:3.5.
	UV filter 403nm
Filters	Band-pass VIS filter $(400-700 \text{nm})$
	IR filter $(1000nm)$
Lampa	Incadecent Smith-Víctor Q60
Lamps	UV lamp XeLED N1 3UV-R4-AC.
Tripoda	Manfroto 190XDB
mpous	Smith-Víctor Raven RS8.
Color Checker	10cm
Digmont Chooleang	Different binders (gum arabic
r igment Uneckers	egg tempera, linseed oil and fresco.)[22]

Table 2.2: Multi-spectral image system components (Showed in Attachment A).

and it is programmed to align the camera with certain area of the painting to take a photography of just that region; the establishment of the camera capture conditions, the focus and the shot are made from a computer with the software provided by the camera's manufacturer. The GP will move the camera with high precision along all the canvas, as showed in figure 2.6, following the numerical sequence (for further information about the performance of the GP visit https://gigapan.com). All photo pieces are joined to have a super-high quality image for about 10k x 10k pixels.

Once the system is ready, it is the moment to take the photographs for a single portion of the spectra. For a VIS or IR image, it is placed the VIS or IR filter, respectively, in the lens and the incandescent lamps aim to the painting; for the UV image, it is necessary to bath the picture with the UV probes along all the area and next let those fixed in the tripods to take a photograph. In the figure 2.5 is shown an example of the light path for the case of IR photography.

#### 2.3.3 Image Processing

The usage of the system requires two previous treatments before the FC transformation. The first one corresponds to the alignment of the bunch of images using the program Hugin Panomara [24]; this provides useful tools to join and balance all the photographs



Figure 2.5: Set configuration for a IR photograph. [23] Using halogen lamps is possible to irradiate a frame with VIS and IR light, in case to need a VIS image it is just necessary use a VIS filter and for a IR photo is used the IR filter, it is important to mention that for the FC analysis the detector must keep it position for every photo.



Figure 2.6: Schematic view of each portion of canvas to capture with help of the GP. The GP is the device that allows the precise motion of the camera in order to photograph a part of the paint for the previous construction of high quality image of the whole frame.



Figure 2.7: Methodological FC image generation process using PS.

mostly automatically. The next step corresponds to the white balance of the whole image using or not the color checker; it is carried out with the program UFRaw [25], and it also permits the change of file type (.RAW $\rightarrow$ .jpg) that will be needed to begin the FC transformation. The white balance helps to control how the colors are captured since those could be a little bit different if there is a distinct amount or type of light [26]. In the same way, the steps to balance the photo are presented in attachment C. These processes are the same for the VIS, IR, and UV images.

The last step is to move the color channels in the transformed images in order to generate the false color. This process is realized with the program Adobe Photoshop Cs6 (https://www.adobe.com/la/products/photoshop.html) and it is presented in the figure 2.7 [27]. Finally, It is important to mention that, for reasons later mentioned, just the process using PS will be applied for the final system and analysis.

## 2.4 Color Analysis

There exists several kinds of models to register numerically certain color such as RGB , CMY YIQ, HSI or CIE-L\*a\*b\* color models. One of the most used is the RGB model and consist into assign a number between 0 to 255 for tree coordinates in order to compose a

color; in the 0,0,0 corner is considered the absence of color and 255,255,255 corner (the opposite corner) is called white, the rest of the edges correspond to a certain color R for red, G for green, B for blue and their combinations. This model could be appreciated visually as a perfect cube in the figure 2.8a [28]. The RGB model is one of the most used for screens of technological devices and also it will be used in this investigation to collect the color information.

In the other hand, the CIE-L\*a\*b\* system is useful in the color science in order to compare two colors that, for the purposes of this write, will be an important aspect. This system works with the following values: L\* that correspond to the lightness of the color and could be between 0 (black) and 100 (white), a\* correspond to a position between -128 (negative means green) and 128 (positive means red) or green and b\* correspond to a position between -128 (negative means blue) and 128 (positive means yellow). This system can be represented as a sphere (fig. 2.8b) [29]. In order to compare two colors first is calculated the variance of each L\*a\*b value of both colors and next the Color Difference ( $\Delta E$ ); this value is obtained using the following equation. Finally, according to Mokrzycki ([30])  $\Delta E$  can be from 0 to 100 where the first one correspond when two colors are completely equal and the second means a completely divergence of two colors; so it is important to establish a limit in  $\Delta E$  in dependence of needs of the analysis that, in the case of this write, will be establish in the next chapters.

$$\Delta(E) = \left[ (\Delta(L^*))^2 + (\Delta(a^*))^2 + (\Delta(b^*))^2 \right]^{\frac{1}{2}}$$
(2.4.1)

### 2.5 State-of-the-Art:

The main character that is postulated as reference in this work is Cosentino, who has a vast trajectory in the analysis of pigments using multi-spectral images. A recent report shows the application of transmitted multi-spectral imaging (MSIT) images in a canvas with a bunch of pigments; it provides significant results in the detection of different layers of colors that could help with the detection of sketches, repaints or hidden information [31]. The figure 2.9 shows the apparition of some pigments that are under a layer of a whitish color (titanium white). This method can be joined with the proposal device in the writing since the MSIT could detect areas with inconsistencies meanwhile the other



Figure 2.8: Main Color Models for the MMSI. (a) is the model that will be used to the sampling of pixels in the generated image, this will provide a set of 3 numbers, next this information will be transformed in the model in (b) that will be useful for the data analysis.

could deeply analyze those regions. Also, Cosentino shows a microscopic image (fig.2.10) of a cross-section of a composition of blue pigment (Egyptian blue) with a white one (titanium white); it gives a projection of what will be seen with the proposed system.

More examples of FC applicability in Cultural Heritage are presented by Barbu, Aguilar, Dolezynska, and Delaney in their different investigations, all of them driven by the characterization of pigments. Barbu used the results of IRFC and X-Ray fluorescence spectrometry analysis to identify a total of 240 standard pigments; she also adapted a portable microscope with IR and VIS probes in order to collect photos of specific areas to transform into FC to the fast and preliminary identification of pigments, this work was performed in the picture from Doamnei church in Romania [32]. Aguilar et al. also studied the behavior of pre-hispanic and colonial pigments from Mexico under FC analysis using the non-destructive method just by taking VIS and IR photographs and mixing the color channels; they conclude with the recommendation of a wide database of pigments and also the posterior confirmation of results using XRF measurements and Raman spectroscopy [33]. Dolezynska and Klisinska worked in recognition of the palette of colors of an influential Polish painter Rafał Hadziewicz; also, they used IRFC, XRF, FTIR and join the SEM-EDS technique; for the last one bring out the possibility to analyze a cross-section area of the pigments [34]. Delaney et al. scoped to the visualization of preparatory sketches, the identification of non-original materials, and, as always, the



Figure 2.9: Transmitted Multi-Spectral imaging example. The frame was prepared with different pigments and a base above those, the image was taken putting the source of light behind the frame and it shows the possibility to obtain information of hidden areas of paint. [31].



Figure 2.10: Stratigraphic image of blue and white pigments to show the model of a batch of pigments arranged one on top of the other [31].

mapping of authors pigment; they used two hyperspectral cameras (400-900nm and 950-1700nm); in this case, the analysis was applied into Picasso and  $13^{th}$  century paintings and they conclude saying that the mixing of techniques will improve the quality of the results exponentially [35]. Finally, in the figure 2.11 show an example of an FC image for each author cited before.

Furthermore, Cosentino constructs several transmitted spectra for pigments such as Vermilion, Madder lake, and Alizarin 2.12 using an ASEQ LRI spectrometer; these plots



(a) Painting from Doamnei church [32].



(b) Painting from a Teotihuacan painting [33].



(c) the Portrait of a Young Woman [34].



(d) Madonna and Child with Four Angels [35].





Figure 2.12: Transmittance spectra of a batch of red pigments in order to see the similarities and differences of those in the IR band (above 750nm) [31].

bring out the differences in the collected light (transmitted in this case), which are the details that the FC will use to discern two similar pigments. The figure allows the analysis of the differences of three red pigments, more or less in the VIS range the colors are similar; however, it is necessary to look for big differences: around 675nm Vermilion and Madder lake have the bigger differentiation, and around 900nm Alizarin differs from the other two pigments [31]. These important regions are called spectral bands and are useful in the selection of probes and filters in the MSI in order to obtain the best FC transformation.

Hayem et al. [36] realized a deeper study of the spectral bands; her main objective was to construct some FC procedures on the dependence of the pigments analyzed; as mentioned before, the construction of spectra is fundamental in order to obtain an acceptable FC. First, Hayem established a database of 20 pigments from the  $17^{th}$  Century and were prepared with linseed oil; also, she clarifies that the pigments were not prepared with ancient recipes. Once they obtain the canvas with each pigment, the procedure was the color channel changing with reflected photographs taken in the 400-1000nm spectra. The spectra were constructed through the measure of reflectance information in the mentioned range for each pixel in the photo. So they analyze the spectra looking for the bigger differences between a family of pigments and choose three spectra bands to use in the R, G, and B channels. For example, in a set of blue pigments, the best spectral bands are in B= 483nm, G= 735nm and R= 986nm (fig. 2.13); those were chosen to look for the bigger discrepancies in the spectra between the pigments, i.e., 483nm band permits the differentiation of Smalt and Lapis, on the other hand, 735nm band allows to differentiate



Figure 2.13: Establishment of spectral band in a set of blue pigments in order to determine the best bands for the construction of a FC transformation for the adequate differentiation of the blue pigments [36].

Azurite and Lapis from the other two and finally 986nm band will make differ Azurite and Indigo from Smalt and Lapis. All these groups of affirmations will make possible the discernment between these blue pigments.

# Chapter 3

# **Experimental Procedure**

The objective is to adapt the MSI system into the microscope to obtain the necessary images to have FC photos but, for a beginning experiment, just to IRFC. It is important to test and to establish the optimal components and conditions, in dependence with the needs and possibilities in which the Microscopic Multi-Spectral Imaging (MMSI) system provide the best results. Before to starting the application of the MSI into the microscope Olympus BH2, it was important to verify the state of this; due to it was a while without use, the microscope pass through a quick maintenance which was established and supervised by the laboratory. The relevant components for the purposes of the MMSI are: ocular, extension tube with projection eyepiece, prisms and objectives (Attachment D). So, the experimentation with the MMSI is described in three stages below.

## 3.1 MMSI Stage I

For this first try the MSI is put into action using the BH2 using a standard sample of a Koran Book which contains red and green pigments.



## 3.1.1 Column

Figure 3.1: Column for the MMSI stage I, using a DSLR camera, microscope and halogen illumination.

Specific Phase	Components
Computer	Software: ControlMyNikon V4.2
	Nikon D800
Detector	Tripod
Detector	Horizontal arm
	Lens
	Vis filter $(400nm - 700nm)$
Filter	IR filter $(1000nm)$
	Filter Adapter
	Ocular
Microscope	Objectives $(4x, 10x, 20x, 40x, 100x)$
	General Structure
	Holegen: Goose-neck, bulb
Drohad	IR: 3x LED Clusters (BL016-15-28)
Frodes	Lego Structure
	Universal AC-DC adaptor

Table 3.1: Components for MMSI Stage I.

According with the figure 3.1, the column for the stage I is composed by the items of the original MMSI as the modified camera Nikon D800 with a external filter, additionally the microscope and the sources of light that hits the sample at any specific angle or place. The filters and the sources will be placed in dependence of the photography that is being taken: VIS or IR.

#### **3.1.2** Components and Procedure

The stage I will precisely needs all the items detailed in table 3.1 to work properly. Before starting it is important to place the battery (charged) and the lens with the filter; in addition is important to put a protection (ex: ring of cotton) in the ocular to avoid scratches in the filters due to closeness between filter and ocular. In the case of the probes, those require a manner to hold the lamps near the sample; the halogen bulb will use a goose-neck that will be attached to a table, this is powered directly from the 110V plug. The IR probes will be attached to the microscope via a structure constructed with metallic Lego pieces (fig. 3.2a), those need DC current of 6-8 volts, for this it is used a universal AC-DC adaptor with dimmable voltage (1.5 to 12 V).

The process to assemble this stage starts with the positioning of the Nikon D800 into a horizontal arm and this, in turn, is attached into the tripod; next, in the computer, it is necessary the live-image function provided by the camera in order to line up properly the light path that ends in the camera. Next, the probes are placed near the sample in the mechanical stage with the respective power supply and the procedure can start. The first photography is going to be for VIS spectra; with the halogen bulb turned on and seeing the screen of the computer the microscope and the camera's lens are manipulated to get a well focus image with any objective lens. After that, the IR probes will be turned on and the IR filter is attached, this process must be realized very carefully in order to do not move the sample and any configuration in the system, finally the IR photo is taken with the best focusing in the camera lens and microscope. The first stage in showed in the figure 3.2b.

#### 3.1.3 Conditions

The first condition to guarantee a good photography, in general, is a well defined illumination directly into the sample with both sources of light; next a well focus image of the sample looked in screen that, as mentioned before, is achieved with the lenses features in the microscope and camera. In addition, the capture's conditions in the camera are important too, those are the aperture, shutter speed and ISO. For the first one, as the word suggest, correspond to how much the lens in the camera is open to the pass of light; the shutter speed is value that give options to capture a object that is in movement, it refers to the time that the shutter will be open; finally, the ISO value correspond to a measure of detector's sensitivity and will define the grain size in photo. The three values are correlated in order to get a good photo, however for the MMSI system is recommendable to use the maximum aperture possible (lower f-number) and for the other two conditions is pertinent to try several combinations to look for the best photo.

#### 3.1.4 Image Preparation and FC Transformation

It was not necessary to capture several photos to construct a bigger image, therefore the utilization of Hugin Panorama editor was not necessary, but since the photos were taken with the Nikon D800 those were generated in .RAW format, so it is necessary the white balance and .jpg format transformation with UFRaw. Once the acceptable file for PS is generated the transformation into FC begin; this is followed as mentioned in figure 2.7.



(a) (a) Support frame for IR probes constructed with Lego pieces, (b, c)attached into the microscope using screws and nuts and (d) focused to the sample.



(b) Component configuration of stage I using the Column I from the figure 3.1

Figure 3.2: MMSI stage I

### 3.1.5 Final Remarks for MMSI stage I

In the figure 3.3 is presented photographs take with MMSI stage I in order to test the magnification of the image and their quality; since the light irradiate in different ways for
each objective the system presents its first limitation with this configuration: for 40x and beyond magnifications it is not possible get a enough resolution due to the short distance that the sample and objective let to light irradiation (< 1mm); for 4x magnification the small details in the sample are not perceptible to consider useful for an analysis.



Figure 3.3: Photographs of a Koran book sample for 4x, 10x, 20x, 40x objectives.

About the FC transformation, in the figure 3.4 is showed the VIS, IR and FC photographs for the Koran book sample taken using the MMSI stage I using the camera's features: ISO-320, 1/10s for shutter time and exposure F/1.8. The image preparation with UFRaw and PS are adequate in this stage and generate a well known behavior seen in the Antonino's pigment checker (fig. 1.3) for, at first glance, a pigment called Malachite for the green top layer and for the second layer it is just possible begin to discern that this pigment correspond to an Alizarin or a Cadmium red. It is important to denote the bottom missing part in the IR photo as a result of the motion of the camera to change the filter; this misalignment should be consider to improve the MMSI.



Figure 3.4: VIS, IR and FC images of a Koran book sample for 10x objective using the MMSI.

In another try with a different sample (fig. 3.5 from the painting "San Agustin se presenta en una vision a Santa Gertrudis" that comes from a yellow background it is possible to appreciate a better configuration of camera's settings together with a better lighting that in the Koran Book photographs were not correct, it is important to check the photographs in order to make a quick evaluation of quality. In this particular case the camera was configured with ISO-100, 1s for shutter time and aperture F/1.8. The images were treated in the same way as the previous ones.



Figure 3.5: VIS, IR and FC images a yellow fund of "San Agustin se presenta en una vision a Santa Gertrudis" for 10x objective using MMSI.

As a final remarks for the stage I, the sources are not completely efficient, it is related mainly with the incidence of light in the sample thought the small remaining space. For the VIS bulb, it is not enough just to have one direction of radiance in the sample, this provoke a formation of shadows and do not provide the necessary reflected light for the procurement of adequate saturation. In the case of the IR sources, the radiance of light is adequate since this comes from 3 different points but in certain situations it will be better to focus the sample in another angle, in this case to the Lego structure do not allow the individual movement of each probe, there is necessary change the holding system of the IR bulbs. Finally, since the camera Nikon D800, the filters, tripods and other stuff belong exclusively to the MSI system it is necessary adequate fixed components for the MMSI.

## 3.2 MMSI Stage II

This stage focuses on experimenting with another kind of detector due to the impossibility to use the professional camera; the important consideration in order to choose certain camera is to know if this device provide a live image function in the computer. Most of the standard or digital cameras will no have this function; this just let the possibility to use a regular webcam.

### 3.2.1 Column



Figure 3.6: Column for the MMSI stage II, that use a Webcam, the microscope and halogen illumination.

The only change in the column (fig. 3.6 for this stage will be the detector. The mentioned changes in sources will be reconsidered at the moment that a detector will be the definitive. Also, the filters now will be part of the microscope because those will rest in the ocular.

### **3.2.2** Components and Procedure

In table 3.2 are listed the component needed to use MMSI stage II. Before the assembling the system the webcam needs a little modification similar but simpler than the modification performed in the Nikon D800; in general, the webcams have a tinny IR filter that in certain cases has to be removed. So, it is important to mention that this modification it not always necessary or even is better to let the filter in its place; this because in some webcams the lack of filter could produce an overexpose of the sensor that will damage it permanently. The only way to determine if the filter is necessary or not is removing this and test the existence of overexposure. The figure 3.7 represent the general process to remove such a filter. Also the software to use a webcam could be any commercially available in internet, in this case is being used the free trial of YouCam 9 Deluxe obtained directly from the web page of CyberLink Corp.

Specific Phase	Components				
Computer	Software: Cyberlink YouCam 9				
	WebCam				
Detector	Universal Support				
	Klamp				
Filtor	Vis filter (400nm-700nm)				
Filter	IR filter (1000nm)				
	Ocular				
Microscope	Objectives $(4x, 10x, 20x, 40x, 100x)$				
	General Structure				
	Holegen: Goose-neck, bulb				
Probos	IR: 3x LED Clusters (BL016-15-28)				
TTODES	Lego Structure				
	Universal AC-DC adaptor				

Table 3.2: Components for MMSI Stage II.

The structure begin, as before, with the attachment of the webcam into the universal support in the manner that this looks down. The next step is to align the camera such that it is possible to see an image in the screen of the computer. Next, place the filter over the ocular with a protection between those as in the stage I. The configuration of this stage is practically the same as the stage I but with different detector. Finally the probes are positioned for each kind of image. The photographs are taken in the same order as before, the first step is the image focusing using the microscope and the VIS image is taken with the VIS filter and halogen bulb; next, the filter is changed and the IR bulbs are turned on, at the end the image is taken. In figure 3.8 is presented the stage II of the MMSI.

### 3.2.3 Conditions

Due to the webcam and the filters are not attached the distance left between those components allows the interference of the environment light, for this reason is necessary work in the darkest possible place. Also, since the camera is not too sophisticated the capture conditions are not available anymore, so the images will be taken with the standard configurations of the camera.



Figure 3.7: Removal process of the webcam IR filter for a. identification of camera, b. removal of screws on the coverage, c. identification of the little lens, d. separation of len from the support, e. identification of the IR filter, f. removal of the IR filter, g. relocation of the lens and coverage.

### **3.2.4** Preparation and FC transformation

Following the predisposition of the white balance in the first MMSI, this process was tested with a pure sample of White Lead. Since the images are produced directly in a .jpg format it is not possible use UFRaw for this purpose, so feasible software for this procedure are PS or Gimp (https://www.gimp.org) which is an alternative image manipulation program. For the first one the process start with the image opened and in the option (*Imagen > Ajustes > Curvas...*); in the next opened window are chosen the whitest, a grey and the darkest point with the sampler from the photo. In the other case, Gimp offer and automatic white balance in the option (*Colores > Auto > Balance.de.blancos*). The comparison of the two balances and without this process is presented in the figure 3.9. The FC transformation was carried out in the same way as always.

### 3.2.5 Final Remarks for MMSI stage II

As a first recommendation for the next stage it is important to ensure a good quality of image, it means that the detector has to provide good resolution images, figure 3.10 is a example of the quality of image with the same Koran Book sample of the stage I; the



Figure 3.8: MMSI stage II prototipe configuration using the column II of the figure 3.6.



Figure 3.9: Comparison of different white balances and FC transformation for a White Lead sample.

resolution is not enough to begin a trustworthy analysis. It should be mentioned that is not necessary a quality of a Nikon D800 but is essential to overcome the resolution of the last mentioned figure; it will be adequate a resolution between the provided by both devices. Also, this webcam does not provide a whole image of the ocular, it could represent a lose of information and must be changed.

On the other hand, it is important to remark that the purpose of the filter is cut off all the light that is not desired but knowing that the IR sources emit light with maximum intensity at 940nm (Attachment E), that the IR filter allows pass light above 1000nmand that any other kind of light is hitting the sample is pertinent assert that the filter is



Figure 3.10: Resolution test of the MMSI stage II of a Koran Book sample.

not necessary anymore but is important to ensure that nothing but the light reach the sample. In that case, the system will need another VIS probe that emit light only between 400 to 700*nm* approximately in order to remove also the VIS filter. In the figure 3.11 is presented the comparison an IR image of White Lead using the IR filter with another IR image of the same sample without filter using the MMSI stage II. This modification is a big advance because the cost of the MMSI is greatly diminished due to how expensive are the high quality filters.



Figure 3.11: White lead sample images testing a IR filter (1000nm).

Talking about the preparation the images in the figure 3.9 is presented the results of the FC transformation without white balance and for two white balances. The VIS photos do not show a big difference after the balance, however the infrared images are different and those are which produce the divergence in the FC pictures. Comparing the FC images and knowing that the White Lead do not change under FC according with figure 1.3 the best option is do not make the white balance. This option is also valid because the purpose of the white balance is to standardize the color for different temperatures of light [26] but in the case of the MMSI at the darkness the light that irradiate the sample is always the same.

Finally, it is important to optimize each component in order to make the process faster, it means that, for example, the tripods or the universal support must be removed due to those components are prone to moving or, in other case, the well illumination of the VIS source is not adequate for the small space in the mechanical plane and the big bulb.

## 3.3 MMSI stage III

This is the final prototype of the MMSI system, it consider all the details of the two previous stages in order to get the most optimal system.

### 3.3.1 Column

In the figure 3.12 is presented the column for the final stage of the MMSI, each literal in the figure correspond to the configuration of the VIS and IR sources respectively. For the case of VIS source it corresponds to a construction of cold LEDs around the sample in order to irradiate the sample from 4 different points. The IR probes hit the samples from 3 different locations as always.

### **3.3.2** Components

### Detector

The detector use the same software of the stage II (YouCam 9) to the live image and for capture the images. This is composed by two parts: a webcam and the Webcam support designed to fit exactly in the extension tube for cameras of the microscope BH2. The webcam shows a excessive sensor's sensibility of light without the tiny filter that was removed following the figure 3.7, so, in this, case the webcam needs this stuff to avoid possible damages. In the figure 3.13 is showed the modeling and 3D impression of this component; this was intended to allow the attachment of any webcam (exact dimensions in Attachment F). The model has some pits in the base of the webcam and in the fixing tube: the first are to hook up some Lego pieces to accommodate the webcam in a fixed position; the second ones are in case of need to adjust the support in the extension tube,





(b) Components configuration for IR images

Figure 3.12: Column for the MMSI stage III, using a webcam, microscope, IR and VIS sources of light.

this could be realized using nuts and screws. Finally the Webcam Support was printed in black color to isolate the webcam from intrusive light due to that the filters are not part of this stage.

### Sources

The VIS probes are now a composition of LED's in parallel that emit visible light between 400 and 700nm approximately (Attachment G); those are adhered in a circular structure to surround the sample. This structure is another 3D impression that was constructed to embrace exactly all the objectives of the BH2 because those are the support of this stuff



(a) 3D model of Webcam support.



(b) 3D impression Webcam support for different views.

Figure 3.13: Detector support structure for the quick location of the Webcam into the microscope using Lego pieces for the well attachment.

and of the LED's, in turn; the layout of this was planned to move along the objectives with facility by just tightening and loosening a screw. The idea of this is the irradiation of the VIS and IR sources for each kind of photography by only changing the VIS probe position. In the figures 3.14a and 3.14c is presented 3D modeling and the posterior impression (exact dimensions in Attachment H), it was made in a white color in order to permit the visualization and the maximum reflection of light in the sample. For the case of the IR probes, those are the same of the previous two stages but now each one have a goose-neck to an independent mobility.

Both sources are powered by a AC-DC universal transformer and also are connected

to a circuit that contains a double switch to turn on a certain source and a dimmer. The circuit is presented in the figure 3.14b, it directly allows the connection of the transformer in one side and in the other side both sources are powered; this whole stuff will be called The MultiDimmer (Multidimmer (MD)). Since the probes need different voltages this configuration is managed in the transformer. About the electronic characteristic of the sources: the VIS LED's are 4 Epistar chip LED's of 3W that works at a maximum of 4V; the IR sources are 3 infrared LED clusters made of GaAlAs of 320mW/sr that use maximum 8V. The construction of the MD is presented in the 3.14d.

The last component added in this stage correspond to a complete covering of the system, this is composed by a wooden cube frame that will be the support of a felt in a cubic form; this will be called Black Box (BB). The purpose of this is prevent any kind of light reach the sample more than the expected (VIS and IR) but this also allows the manipulation of the microscope though a breach at one side of the BB. The measurements of the frame are 55cmx45cmx60cm and there is necessary  $3.27m^2$  of felt in a cube form to cover this (Attachment I).



(a) 3D VIS support modelling for different views





(c) 3D VIS support impression with the VIS LED's and in the microscope



(d) MD construction

Figure 3.14: VIS Support and MultiDimmer constructions for the MMSI stage III.

Specific Phase	Components				
Computer	Software: Cyberlink YouCam 9				
	WebCam				
Detector	Webcam Support				
	Lego structure				
	Ocular				
Microscope	Objectives $(10x, 20x)$				
	General Structure				
	VIS: 4x chip LED's (GP-3WW6-J45T),				
	VIS support,				
	screw and nut				
Probes	IR: 3x LED Clusters (BL016-15-28),				
	3x goose-necks				
	Multidimmer				
	Universal AC-DC adaptor (MW79)				
Covering	Black Box: Cubic Wood Structure				
Covering	and Double layer black felt.				

Table 3.3: Components for MMSI Stage III.

### 3.3.3 Procedure

For the Webcam Support, it is necessary to align the camera with the image provided by the microscope through the ocular, this in order to fix permanently the position of the webcam. Once the camera is well aligned and fixed the Webcam Support can always be removed and put in with an instant alignment. The next step is locate the VIS Support in the 10x objective in the highest possible position and the screw is tighted. Now, it is able to harbor the system with the Black Box. After that, the three goose necks are attached in the wood box with the IR clusters oriented to the position of a sample. Finally, the AD-CD adaptor is connected into the 110V plug and fixed for 1.5V, next is connected the Multidimmer to the adaptor with the switch turned off, at the end plugs of the VIS and IR probes are connected in any of the assigned positions for sources.

Now, the system is ready to take photographs. In the computer is the image provided by the detector using the software. The sample is placed under the objective with the VIS probe; turn on this source. Next, it is necessary to situate the VIS LED's flushed with the tip of the objective (Fig. 3.15). Move the dimmer of the Multidimmer if the image looks with overexposure. Locate the sample in the screen and get the best resolution with the coarse and fine focuses; now, a VIS image is taken. The next step is move the VIS support at the misaligned position with the LED's turned off, it is important to do not



move the sample in this process.

Figure 3.15: Vis Support position for the aligned and misaligned state to allow the positioning of the two states of the column III (fig. 3.12) for VIS and IR images.

For the second photo, the transformer will be at 7.5V and the IR probes, oriented at the sample, are turned on. The resolution in this image is not too distinguishable but is important to obtain the best definition of the contour of the sample using, as before, the mechanism of the microscope. The figure 3.16 shows the comparison of IR images of different intensities of light, it is important to obtain a well balanced image; this can be also managed with the dimmer. Once these considerations were taken into account the photograph is captured.

It is important to mention that is feasible take all the necessary photographs with the same objective; once all the images were taken the process to remove the VIS Support begin with the positioning of the objective in another stage of the turret; next, loosen the screw and remove the support, finally the process start over with another objective.

### 3.3.4 Conditions

The principal condition that must be fulfilled is the isolation of the sample from any other type of light, this is realized with the BB ensuring all system coverage, also this can be reviewed using the webcam; if in the screen is possible to see something is because the BB is not working correctly. Also, an important condition is the alignment of the VIS Support with the space left between the objective and the sample to ensure the well irradiation of the sample. Finally, the practice in the MSSI will provide the knowledge to



Figure 3.16: Intensity comparison for poor, good and over exposure in IR images.

No.	Pigment	Ident. Number	RGB	Chemical Formula
1	White Lead	46000	200 197 192	$2PbCO_3 * Pb(OH)_2$
2	Azurite	10200	39  51  67	$CuCO_3$
3	Red Lead	42500	$195 \ 80 \ 0$	$Pb_3O_4$
4	Vermilion	10610	$115 \ 46 \ 41$	HgS
5	Yellow Ochre	40010	$158 \ 107 \ 0$	$Fe_2O_3 * H_2O$
6	Verdigris	44450	27 131 156	$Cu(CH_3COO)_2 * 2Cu(OH)_2$
7	Malachite	10300	$75\ 119\ 94$	$CuCO_3.Cu(OH)_2$
8	Burnt Sienna	40430	61 40 39	Iron Oxides

Table 3.4: Summary of pigment bases [1, 9].

understand how to control the MD and the intensity of the light.

## 3.4 MMSI Pigment Checker

### 17<sup>th</sup> Century Pigments

The pigments that constitute this checker were established according the requirement of the laboratory in the INPC. Those are common colors that could be seen in several paintings that are mainly in churches. In the table 3.4 are presented the pigments to prepare the samples, those were provided and prepared by personal of the laboratory following ancient recipes reported in Parrilla writing [9] or are commercially acquired. In the first column of the table is the regular name, this is following by the identification number of the pure pigment, the next is the RGB measurement taken from the pigment checker of Cosentino (fig. 1.3) using the eyedropper tool in PS, finally there is the chemical formula of each one in the list. These colors are commonly stored in a dust form and need a treatment to be applied in a canvas.

### **Pigments Preparation**

For the purpose of this investigation the pigment's preparations are not necessary to be specific and complex, since it will not be for to restore or to paint a piece of work. So, the preparations of the pigments are, in this case, with linseed and walnut oil in order to obtain a different consistence of the material to make possible the color's application. The amount of oil vary for each color but it is not specific, just it is appropriate to obtain a consistent pasty mixture to be applicable in the canvas. The pigments were applied in a region of the little canvas separated with sticky tape, once each color was applied it had a drying time of at least two days. Until this point the process was done by personal of the laboratory. In the figure 3.17a is presented the canvas with the eight different pigments prepared with both oils.

In order proceed with the sampling for the eventual sample mounting this is made thinking that is necessary see the stratum of the pigment. The figure 3.17b describes the procedure to take a sample in the pigments base canvas. It is important to mention that the pigments treated with linseed oil result to be more elastic and easy to take, however, with the walnut oil, the pigments result less consistent and tend to crack making possible just collect little fragments or granules.

### Acrylics Preparation

The next step is the mounting of samples in acrylics. In order establish the best type of sample holder some white lead samples were mounted in the three different kinds of acrylics available in the laboratory: transparent, white and pink; in attachment J is presented the comparison of each one of those. The selected one is the transparent acrylic due to this allows to pass away light that is not touching the sample.

Before begin, it is necessary to have ready transparent acrylic powder, methacrylate, a sample mold, a sample and some basic tools; the process to a sample is in figure 3.18. First, a acrylic polished cube must be ready with half of the height of the mold, next the sample is taken from the container and placed it in a solid surface. After that, using a tweezers, the cleanest cut of the sample is chosen, placed over the mentioned acrylic (that is on the mold) and located near the long side of cube. Finally, it is covered completely first with the acrylic powder using tinny shovel and next with methacrylate using a dropper.



(a) Canvas of pigments for -a- linseed and -b- walnut oil for the pigments presented in the table 3.4.



(b) Process to take a sample of pigment from the canvas and its collecting. Figure 3.17: Pigments preparation.

The acrylic is ready after 20 minutes and now the polishing process start. The final stage is realized with a mineralogical polisher Struers DP-U2 using sandpapers number 150, 400 and 1200 in that order. The first one is for remove the excess of acrylic and part of the covering over the sample, the next one is to expose the sample and, at the end, the purpose of last one is to remove big scratches of the previous sandpapers. In this procedure is really important check the progress each 2 or 3 seconds of polishing to do not lose the sample; this is realized using a stereoscope Olympus SZH. This process is followed with each pigment in figure 3.17a obtaining a total of 16 acrylics.

### Photo-taking

Whit all the acrylics ready the final stage of the MMSI is applied in order to obtain the images for the pigment checker at micro scale. The process was related in the section 3.3.3 and this is done with 10x magnification and next with 20x; for each pigment the VIS image goes first and after the IR photo. The FC transformation process is the usual (fig. 2.7). In the figure 3.19 is presented an example of the MMSI application in the pigment 2a (Azurite with linseed oil) for 20x.



Figure 3.18: Acrylics preparation process using the pigment samples and the encapsulation using white acrylic and methacrylate.



Figure 3.19: MMSI image of Azurite for VIS, IR and FC images for 20x objective.

## Chapter 4

# **Results and Discussion**

## 4.1 Final MMSI System

All the experimentation with the system explained in the previous chapter gives, as a first result, a device to produce adequate photographs to generate a microscopic FC image in order to identify certain pigment. This was done taking into account previous remarks of two stages of experimenting and one stage of construction; in figure 4.1 is presented the final MMSI system with all the stuff ready.



Figure 4.1: Presentation of the first Final MMSI system with all the components of the table 3.3 in the respective location.

This system is not complicated in two main aspect: first, the MMSI is a device that someone, with a light microscope, could construct even at home because it has not special or rare components (i.e. hard to find), just is necessary take into account the considerations related in this writing, also the cost is low in comparison with a lot of analysis devices in the industry (MMSI cost in Attachment); secondly, there is not necessary a special previous training to set up or use it, it is enough play with the system and the software to understand its functioning and to start with the pigment identification.

Also, the "simplicity" of the system breaks the limitation to have certain microscope,

it is not necessary a specific model or type of light microscope. In this line, the system was tested in another microscope available in the laboratory: Olympus BX53. This microscope is a little more modern with its own camera (DP80) made by Olympus itself; the application was realized with all the same stuff as before without any problem but it is important to mention that for each microscope the VIS support, for example, needs different measures according with the objectives and the nosepiece of the microscope in order to make it optimal and comfortable. The figure 4.2 presents the results for this application and it is pertinent to mention that the microscope 's camera shows a low receptivity for the IR spectra, this could means that this kind of cameras also will need a similar treatment of IR cut-off filter removal such as in the Nikon D800 and webcams. The difference of intensity of the IR images taken with the BX53 is because it was used tree different time exposures, the last one is for the highest possible; however it does not reach the receptivity of IR light of the webcam. Finally, even through the DP80 camera is more sophisticated than a regular webcam the resolution did not change a lot, this could be checked comparing the figures 4.2 and 3.19.



Figure 4.2: Images generated with the MMSI using another microscope (BX53) in order to test the reproducibility of the components in the MMSI.

Finally, the system has as a principal limitation the impossibility to work with 40x and 100x objectives due to the limited space available between this and the sample; this is because the light does not reach properly the sample in a transmission microscope, this

could be solved applying the system in a reflection microscope but it is important to consider that those are less common and will need some other considerations of configuration. Also, the resolution provided by the actual webcam of the MMSI could not be the best at the moment but provide enough information to an analysis, however this component always can be replaced by another device with better characteristics in function of the budget.

## 4.2 Pigment Checker Results

The importance to construct an own pigment checker for a MMSI is crucial because the conditions of light and/or reception of information will be different if, for example, a component is changed or if it is made another MMSI from zero, even if the samples are the same. So, the 16 acrylics prepared in the experimental part were submitted to the MMSI stage III (fig. 4.3); the database that contains all the images is available in a Drive Folder (Check the figure 4.3 caption). The RGB measurements of each pigment for VIS and FC images are presented in the next 8 tables; those were taken using the color swatch tool of PS. This was configured to make a RGB average in an area of 3x3 pixels for just to give one RGB value which is register; following this procedure 4 different points were taken dispersed in the sample, which are the same in the VIS and IR images. It is important to mention that, at this point, the method becomes semi-quantitative because the points are chosen by criteria of the operator that will look for the biggest crystals or regions of solid color that correspond to the know pigment.

In the last table can be appreciated the change of color channels realized in PS. It is evident that the values for R and G channels move aside to give space to the infrared information proportioned by the red channel of the IR image, letting away the B channel. That is a desired result for each pigment, i.e. the transformation of a color to another were the information of other part of the spectra, invisible for the human eye, taken part and form a characteristic color.

Now, for instructions of the laboratory in the INPC the pigments to consider for comparing with test samples are going to be those prepared with linseed oil but for in case of the Red Lead will be used the pigment with walnut oil. This is because the linseed oil pigments are common in the paintings due to its stability in contact with air, with other ambient conditions or for the reaction with oil itself [37]; with the Red Lead is



Figure 4.3: MMSI Pigment checker for VIS, IR and IRFC images for (a) linseed and (b) walnut oil preparations according with fig. 3.17a for 20x objective (to obtain all the images in the best quality please visit:

https://drive.google.com/drive/folders/18fb6gH291BP0a2qoOLTtRDmiA5KJlSaT?usp=sharing).

(1) White Lead													
		VIS			$\mathbf{FC}$				VIS			$\mathbf{FC}$	
	R	G	В	R	G	В		R	G	В	R	G	В
	252	255	254	252	252	254		209	224	255	247	205	221
Linseed	255	255	255	252	255	254	Walnut	210	224	250	242	200	212
	251	255	255	249	250	254		211	222	246	254	204	216
	255	255	255	254	255	254		216	228	240	251	202	212
Average	253	255	255	252	253	254		211	224	248	248	203	215
(2) Azurite													
		VIS			FC				VIS			FC	
	R	G	В	R	G	В		R	G	В	R	G	В
Linseed	2	46	140	255	2	47	Walnut	3	12	171	253	3	11
	6	24	149	252	6	24	() alliat	2	9	164	254	1	8
	6	24	149	252	6	24		1	2	174	254	1	3
	3	23	139	255	3	23		1	19	165	255	0	18
Average	3	32	141	254	3	31		2	10	168	254	1	10
(3) Red Lead													
		VIS			FC				VIS			FC	
	R	G	В	R	G	В		R	G	В	R	G	В
Linseed	51	47	37	198	51	47	Walnut	255	139	20	160	255	140
Linbood	41	37	36	198	41	38	() alliat	255	145	35	187	254	144
	44	48	58	190	44	48		254	145	31	186	254	145
	48	42	42	228	45	45		245	129	43	154	243	129
Average	46	43	43	203	45	44		252	139	32	172	251	139
					(4)	) Ver	milion						
		VIS			FC	1			VIS	1		FC	
	R	G	В	R	G	В		R	G	В	R	G	В
Linseed	115	26	44	181	115	27	Walnut	135	76	87	250	136	76
200000	112	29	44	187	112	28	( ) all of c	117	66	76	238	117	66
	119	33	62	204	119	34		118	65	78	226	118	65
	123	25	52	224	123	26		145	79	89	234	145	78
Average	117	28	50	199	117	29		129	71	82	237	129	71
	r			1	(5) ]	Yellov	v Ochre	1					
		VIS			FC				VIS			FC	
	R	G	В	R	G	В		R	G	В	R	G	В
Linseed	110	43	0	239	110	44	Walnut	145	110	67	245	144	109
	105	40	1	228	104	40		120	94	59	233	120	94
	134	66	3	245	134	66		132	101	62	216	132	101
	128	64	0	251	127	63		132	101	60	230	132	101
Average	119	53	1	241	119	53		132	101	62	231	132	101

Table 4.1: RGB averages values of MMSI pigment checker.

					(6	6) Ve	rdigris						
		VIS			FC				VIS			$\mathbf{FC}$	
	R	G	В	R	G	В		R	G	В	R	G	В
Lincod	1	125	180	198	0	126	Walnut	127	233	252	233	128	233
Linseed	2	129	177	206	2	129	wannut	124	244	254	224	123	245
	1	119	171	211	1	120		117	237	253	232	117	237
	1	140	190	216	1	140		96	214	245	221	96	214
Average	1	128	180	208	1	129		116	232	251	227	116	232
(7) Malachite													
		VIS			FC	-			VIS			FC	
	R	G	В	R	G	В		R	G	В	R	G	В
Lingood	0	100	89	133	1	99	Walnut	0	92	97	193	0	92
Linseed	1	80	81	153	2	80	wannut	0	97	95	195	0	97
	1	79	78	142	0	79		0	109	109	202	0	109
	2	108	104	155	2	108		1	94	101	222	1	94
Average	1	92	88	146	1	91		1	98	100	203	1	98
		1	1		(8)	Burn	t Sienna			1	1	1	
		VIS			FC				VIS			$\mathbf{FC}$	
	R	G	В	R	G	В		R	G	В	R	G	В
Lingood	61	44	62	253	65	47	Walnut	43	26	35	201	43	25
Linseed	82	51	28	254	100	66	wannut	41	25	37	222	41	25
	70	50	42	252	79	55		37	22	34	188	37	22
	91	54	40	254	95	57		38	20	26	171	37	19
Average	76	50	43	253	85	56		40	23	33	195	39	23

exception because the color that provide the mix of the pigment with linseed oil resulted into a dark color, but for walnut oil keep its characteristic orange color [38]. So, the CIE-L\*a\*b\* values summarized that will be useful for an analysis are presented in the table 4.3; those are the values of the table 4.2 transformed in the other color model, also it contains a little comparison of VIS and FC colors for each pigment.

Finally, this pigment checker could be always amplified according with the analysis requirements such as new pigments and preparations, taken into account details as binders or varnishes. It is clear that a bigger database will provide wide possibilities of identification, but, in this case this pigment checker is focused into find the 8 pigments with which it is build; if it is the case that the pigment is not one of the database so there can be applied another analysis technique as the presented by Bonneau et. al. [39] such as SEM-EDS, Raman spectroscopy and FTIR and also the wide list of procedures that Cosentino relates in his web page in the "art examination" section (https://chsopensource.org) . That is another focus of the system: be a first stage of analysis (quickly, easy and cheap) before the application of another type of analysis more sophisticated.

Pigment	Code	$\mathbf{v}$	VIS (1	.)	H	FC (2	)	1	2
-	-	R	G	В	R	G	В	-	-
White Lead	1a	253	255	255	252	253	254		
Azurite	2a	3	32	141	254	3	31		
Read Lead	3b	252	139	32	172	251	139		
Vermilion	4a	117	28	50	199	117	29		
Yellow Ochre	5a	119	53	1	241	119	53		
Verdigris	6a	1	128	180	208	1	129		
Malachite	7a	1	92	88	146	1	91		
Burnt Sienna	8a	76	50	43	253	85	56		

Table 4.2: MMSI Pigment Checker RGB values.

Table 4.3: MMSI Pigment Checker CIE-L\*a\*b\* values.

Pigment	Code	VIS				$\mathbf{FC}$	
-	-	L*	$a^*$	b*	L*	$a^*$	b*
White Lead	1a	99,8539	-0,6518	-0,2413	99,2613	-0,1478	-0,6029
Azurite	2a	$19,\!9453$	$36,\!8239$	-61,2899	53,2056	79,9603	57.4327
Read Lead	3b	68.9427	36.5188	68.9785	91.5181	-43.8486	46.0180
Vermilion	4a	26.2818	39.8198	9.2267	57.0299	26.1626	57.3309
Yellow Ochre	5a	30.5693	26.2031	41.1305	63.4678	42.8294	55.8369
Verdigris	6a	50.3817	-10.2240	-35.5710	45.6569	74.6159	-11.2086
Malachite	7a	34.7324	-24.1014	-4.3962	31.5506	57.5380	-9.7373
Burnt Sienna	8a	23.5453	10.7588	9.2096	59.5850	62.3653	51.2131



Figure 4.4: Paintings from San Francisco´s Church for each sample analysed with the MMSI.

## 4.3 Pigment Identification in Cultural Heritage

For this application of the MMSI three samples were chosen from different artistic paintings that nowadays are in the San Francisco's Church in Quito-Ecuador from the  $17^{th}$ age (Fig. 4.4). The samples were taken by trained personal of the INPC, attached in acrylics with the same process as the acrylics of the pigment checker and submitted to the MMSI in order to generate the FC photos. The RGB sampling, as mentioned in the previous section, is realized taken 4 points of the pigment's image in RGB format and calculated its average. Since it is visible three different layers of paint those were used for the analysis. The method results semi-quantitative for the fact that the operator choose in which part is feasible take a RGB sampling because, in certain occasions, the only area for sampling is a little trace of a color involved commonly by certain amount of white lead or another mixture pigment. Also, there will be compared the VIS and FC information in order to rule out other possible pigments. Finally, the standard  $\Delta E$  is at maximum of 15 in order to accept that two colors are similar; this value is consider high in the comparison of colors but in this case it is necessary have a big tolerance because the pigments to be compared are pure versus preparations of pure pigments made a lot of years ago by ancient artist.

### Test Sample N°1

This sample correspond to the painting "San Francisco de Asis" by Francisco Zurbarán and it was retrieved from the representation of the sky (Fig. 4.4). Since the human sight tells that this color is blue just it is necessary to confirm or not if this pigment correspond to Azurite (2a) that is the blue pigment present in the database of the MMSI.



Figure 4.5: VIS, IR and FC images of the sample N°1.

In this case the only blue trace is at the top of the sample and this will be the area of study. In the figure 4.5 is presented the VIS, IR and FC photographs and the table 4.4 are the sampling in RGB for the posterior transformation to the CIE-L\*a\*b\* format. The table 4.5 represents the calculation of the Color Difference using the equation 2.4.1 of the VIS and FC data; from this it is possible to see that the pigment does not correspond to Azurite due to the high VIS Color Difference value ( $\Delta E_v$ =48.79), however for the FC Color difference value ( $\Delta E_f$ =13.59) it is evident that these two pigments have similar behavior under FC. So, using the figure 1.3, roughly, it is possible to say that could be Ultramarine or Egyptian Blue discarding the rest of blue pigments. A final result could be provided amplifying the database of the pigment checker.

Test Sample N°1									
		VIS		$\mathbf{FC}$					
	R	G	В	R	G	В			
	35	38	72	234	36	39			
Sampling	38	57	96	235	42	62			
	36	41	71	235	28	43			
	34	38	69	236	32	37			
Average	36	43	77	235	37	45			
Tranf.	L*	a*	b*	L*	a*	b*			
	18.53	7.93	-21.98	50.89	71.40	47.12			

Table 4.4: RGB sampling and CIE-L\*a\*b\* transformation of Test Sample N°1.

Table 4.5:	$\Delta E$	calculat	ion of	sample	N	Ί.

	V	IS					
	Sample N°1	Azurite	$\Delta$				
L*	18.53	19.94	-1.41				
a*	7.93	36.82	-28.88				
b*	-21.98	-61.28	39.30				
		$\Delta E_v$	48.79				
False Color							
	False	Color					
	<b>False</b> Sample N°1	Color Azurite	Δ				
	False Sample N°1 50.89	Color Azurite 53.20	Δ -2.31				
L* a*	False           Sample N°1           50.89           71.40	Color           Azurite           53.20           79.96	Δ -2.31 -8.55				
L* a* b*	False           Sample N°1           50.89           71.40           47.12	Color           Azurite           53.20           79.96           57.43	Δ -2.31 -8.55 -10.30				

#### Test Sample N°2

In this sample is possible to identify at least two different pigments: one orange pigment at the top and a green pigment at the bottom. This sample was retrieved from the "Sancha Reina D´ Napoles" (Fig. 4.4), exactly at the representation of a gem in the crown. In this case the base pigment to compare the first layer is the Red Lead since is the orange color in the pigment checker; for the green layer it is compared with Malachite that is the dark green of the MMSI system. In the figure 4.6 is presented the FC transformation with the two images that compose this.

### **Orange Layer**

The table 4.6 lists the RGB samples taken from the orange layer with the posterior CIE-L\*a\*b\* transformation. The Color Difference calculation of the sample compared with Red Lead is in the table 4.7. In this case, the results of  $\Delta E$  for VIS and FC are above 40, this means that that this pigment does not correspond to Red Lead in first instance; also is not possible to discern what red pigment could be using the Cosentino pigment checker, this is common between these pigments because all the reds have a similar behavior under IRFC [4]. In this case is necessary apply a stronger analysis method to determine a result.

### Green Layer

For this big green layer it is also possible to see two greens with a slight difference. In this case the samples are taken from the lowest layer in order to compare. In the table 4.8 are presented the RGB samples taken from the green layer with the subsequent CIE-L\*a\*b\* transformation. The  $\Delta E$  calculation is carried out in table 4.9 and is possible to see that  $\Delta E_v$  this pigment could be confused with Malachite, however looking the  $\Delta E_f$ it does not fulfill the requirement to be less than the standard value (15) but is not too



Figure 4.6: VIS, IR and FC images of the sample N°2.

Test Sample N°2 (orange layer)									
	VIS			FC					
	R	G	В	R	G	В			
	171	109	41	215	180	116			
Sampling	157	100	52	195	158	103			
	155	90	54	219	153	95			
	167	108	59	215	174	118			
Average	163	102	51	211	166	108			
Tranf.	L*	a*	b*	L*	a*	b*			
	48.91	19.8	38.27	71.01	9.4	36.22			

Table 4.6: RGB sampling and CIE-L\*a\*b\* transformation of Test Sample N°2 (orange layer).

Table 4.7:  $\Delta E$  calculation of sample N°2 (orange layer).

	I	/IS	
	Sample N°2	Red Lead	$\Delta$
L*	48.91	68.94	-20.03
a*	19.8	36.51	-16.71
b*	38.27	68.97	-30.7
		$\Delta E_v$	40.28
	False	e Color	
	Sample N°2	Red Lead	$\Delta$
L*	71.01	91.51	-20.5
a*	9.4	-43.84	53.24
b*	36.22	46.01	-9.79
		$\Delta E_f$	57.88

far. This can say that the pigment are a mixture of some green pigments that can contain Malachite; this is also evident looking the green areas of the sample: it contains little parts of the characteristic FC transformation of Malachite. Once more, a larger pigment checker can provide a tighter result.

### Test Sample N°3

The painting that provide the third test sample is "La Pereza" and comes from the series of the 10 Commandments attributed to Miguel de Santiago (Fig. 4.4) and comes from the representation of a calf from the character on the left. In this sample the area to study is a tinny particle in order to test the grain size able to analyze with the MMSI. In the figure 4.7 is the VIS, IR and FC photographs of this test.

The table 4.10 relates the RGB sampling and the CIE-L\*a\*b\* transformation; in the same way, the table 4.11 shows the calculation of the  $\Delta E$  values:  $\Delta E_v=31.62$  and

Test Sample N°2 (green layer)						
	VIS			FC		
	R	G	В	R	G	В
	52	94	95	170	58	98
Sampling	44	77	80	173	38	68
	49	83	86	186	55	89
	46	80	80	161	43	83
Average	48	83	85	172	48	83
Tranf.	L*	a*	b*	L*	$a^*$	b*
	32.85	-12.03	-5.15	40.42	52.34	8.98

Table 4.8: RGB sampling and CIE-L\*a\*b\* transformation of Test Sample N°2 (green layer).

Table 4.9:  $\Delta E$  calculation of sample N°2 (green layer)

VIS						
	Sample N°2	Malachite	$\Delta$			
L*	32.85	34.73	-1.88			
a*	-12.03	-24.1	12.07			
b*	-5.15	-4.39	-0.76			
		$\Delta E_v$	12.23			
	False Color					
	Sample N°2	Malachite	$\Delta$			
L*	40.42	31.55	-8.87			
a*	52.34	57.53	5.19			
b*	8.98	-9.73	-18.71			
		$\Delta E_f$	21.34			



Figure 4.7: VIS, IR and FC images of the sample N°3.

 $\Delta E_f = 24.96$  say that this red dot in the sample is not Vermilion or that this particle result too small for a proper analysis due to the invasive white pigment surrounding the particle.

Test Sample N°3						
	VIS			FC		
	R	G	В	R	G	В
	127	94	87	230	128	94
Sampling	127	90	87	229	127	91
	127	93	89	229	128	93
	131	98	93	230	131	99
Average	129	94	89	229	128	94
Tranf.	L*	$a^*$	b*	L*	a*	b*
	43.23	13.11	9.29	64.08	35.8	35.41

Table 4.10: RGB sampling and CIE-L\*a\*b\* transformation of Test Sample N°3.

Table 4.11:  $\Delta E$  calculation of Sample N°3.

VIS					
	Sample N°3	Vermilion	Δ		
L*	43.23	26.28	16.95		
a*	13.11	39.81	-26.7		
b*	9.29	9.22	0.07		
		$\Delta E_v$	31.62		
False Color					
	Sample N°3	Vermilion	Δ		
L*	Sample N°3 64.08	Vermilion 57.02	Δ -7.06		
L* a*	Sample N°3 64.08 35.8	Vermilion 57.02 26.16	Δ -7.06 -9.64		
$ \begin{array}{c} L^*\\ a^*\\ b^* \end{array} $	Sample N°3 64.08 35.8 35.41	Vermilion 57.02 26.16 57.33	$     \Delta      -7.06      -9.64      21.92 $		

## 4.4 MMSI System Capacity

As is known, the resolution in a microscope depends directly on the wavelength in which the sample is irradiated, so using the formula (4.4.1) provided by Davinson [13] is possible to calculate the value of resolution for visible and infrared light, where  $\lambda$  is the wavelength of the source of light and NA are the Objective Numerical Apertures. The values of NA for 10x and 20x of the objective were retrieved from the web page of Olympus Corp of technical details (www.olympus-lifescience.com). In the table 4.12 is presented the resolution calculations, those values say that, theoretically, with both objectives is possible to differentiate traces of 1.56 $\mu$ m and 0.94 $\mu$ m respectively.

$$R = \frac{\lambda}{2 * NA} \tag{4.4.1}$$

Objective	NA	<b>VIS</b> (550 <i>nm</i> )	<b>IR</b> (940 <i>nm</i> )
10x	0.30	0.91	1.56
20x	0.50	0.55	0.94

Table 4.12: MMSI resolution calculation  $(\mu m)$ .

Now, in a more experimental way, with the tree previous tests is possible establish a minimum detectable particle size of the MMSI system. In the figure 4.8 is presented the measurement of the smallest particles of the test samples that were analyzed. It is important to mention that this was realized by a maximum magnification of 200x due to it was carried out with the 20x objective and the 10x ocular. The first sample was used to study a particle the has a extension of  $3\mu m$  of diameter; in this sample the list of possible blue pigments was shortened until two, this was thanks to the amount of pixels to take RGB values. For the orange layer  $(4\mu m)$  in the second sample was obtained enough information to discard completely the possibility to have Read Lead there. Finally, the last sample analysis give information for just suspect that the pigment is different from Vermilion, first it could be due to the mixture with the invasive white pigment that produce a change in the color; in this case the particle could be too small for the method  $(1\mu m)$ . So, the limit of detection of the MMSI is for particles with diameter above  $3\mu m$ ; this is in function in tree main aspects: detector resolution, type of microscope and amount of database information in the pigment checker.



Figure 4.8: Pigment traces measurements for each sample in order to establish a resolution limit in the MMSI.

### 4.5 Discussion

The constructed system has as a first weak flank the number of base pigments in the pigment checker; this is evident when the comparison of test samples was just with one base pigment. Also, it can be seen in the results provided by the calculation of the Color Difference, which just allows recognizing or not certain pigment present database. Also, in other kinds of analytical methods, it is important an extensive database, for example, in the FTIR spectroscopy, the peaks of spectra are compared with certain database previously constructed, it is possible because the spectra work as a "fingerprint" for all type of sample [40]. Nowadays, the amount of FTIR spectra for pigment analysis make this technique extremely robust since it is possible to find huge databases on the web ([23, 41, 42]), making more bearable the identification of a sample. In that way, the MMSI will increase its power of identification by the construction of a big database.

Now, for the case of the red and yellow pigments is important to take into account that these colors commonly has a similar behavior under IRFC [4] and with the MMSI will be a hard work identify these pigments; in this case, the MMSI, at this point, could not be enough for this purposes. The similar behavior under IRFC means, directly, a similar infrared reflected information in the spectral band at, for example, 940*nm*, which is the band that the MMSI works. In this case, the application of another kind of FC transformation can be a help to differentiate some pigments; for example, according to Andersson [43] UVFC helps to differentiate color as Yellow Ochre, Naples Yellow, Lead White, Brown Yellow better than IRFC. This is common in the characterization of pigments where it is better to use one or another technique; as an example, it is more suitable for FTIR spectroscopy differentiates siennas and ochres, and by using SEM-EDS, it is better to identify umbers and green earths [44]. However, in the MMSI is possible to apply the two transformations in just one device.

About the structure of the system, it is really optimal at this point since the components are easy to use and attach to the microscope. Also, the application of the MMSI in a sample does not exceed the 3 or 4 minutes between the photo-taking and the transformation; this allows the applicability of a large number of samples in a short time thanks to the simplicity of the system set up and software. However, through the process could occur the movement of the sample at the moment to change the sources producing misalignment of the VIS and IR photographs as it is evident in the FC image of the sample N°1 (fig. 4.5), even though this does not affect the FC transformation nor the RGB sampling it provokes the meticulous alignment of photographs, and this requires extra time. In order to optimize this part can be necessary to use just the VIS support to implement IR LEDs at the same time with the VIS Light Emitting Diodes (LEDs) but using the same circuit of the MMSI stage III. In this field, it is important to mention that the rest of the spectra, ultraviolet for example, also can be part of this system, as mentioned before, by just implementing in the same VIS support some UV sources in order to generate other kinds of False Color types (UVFC).

The theoretical resolution in microscopy is expressed commonly with a value that says the minimum size between two particles in order to be differentiated [13]. For the MMSI, this resolution always will be ruled by the resolution provided by the IR light, since it has a relatively big wavelength, it produces a diminish in the resolution of the image (bigger R) [45], for example, at 20x it is  $R = 0.94 \mu m$ . But experimentally, the resolution of the system is limited by two aspects: the type of modified microscope, due to that the structure does not allow an optimal sample illumination for 40x and 100x objectives, and the detector due to it is not the best camera in the market; it lands on a resolution for the MMSI of  $3\mu m$  with a total magnification of 200x. Now, according to Higgit et al. [46] the application of SEM-BSE is feasible to the analysis of pigment stratigraphic samples using magnifications from 126x and above with resolutions of 1nm. This positioning the MMSI as a little competitive method against giants as the Scanning Electron Microscopy, at least in the pigment identification field in terms of cost, time, and formal operation experience. It is important to mention that the total resolution and the magnification of the MMSI can be improved by upgrading some components anytime.

As was mentioned at the very beginning of this chapter, choosing a  $\Delta E$  is essential in order to establish a limit for the acceptance or rejection of two colors as equals [28] but, according to Mokrzycki [47], fix a  $\Delta E$  has its dependence in several aspects such as if the analyzed colors are darker or brighter that, in such case, is possible to put a low or high  $\Delta E$  value respectively. Also, Mokrzycki mention some calculations of  $\Delta E$  for two colors in RGB: (255,25,137) and (255,25,131) giving  $\Delta E = 3.40$  [47]; it shows a "big" difference of colors even though the RGB values are very similar. Now, taking into account that the analyzed samples in the MMSI show mixes of pigments that could interfere with the color in comparison with the base pigments that are pure, it is feasible to choose a high value of  $\Delta E = 15$  in order to compare two colors. Furthermore, it is important to comment that the number of pigments in the database could contribute to the reduction of the Color Difference limit value because if there are some mixes, preparations, and pigments to compare, it is necessary to be more strict in the comparison of colors. Finally, it is remarkable that in the literature does not exist a method that, as the MMSI system, presents results in the field of FC analysis at micro-scale for pigments of the  $17^{th}$  century in order to make a critical comparison of colors, which makes this work an important contribution as a simple method to identify pigments in paintings of this epoch.

# Chapter 5

# **Conclusions and Recommendations**

## 5.1 Conclusions

This experimental work allows to conclude that:

- The MMSI system is an easy-to-build system with a low cost of construction that uses simple components and software and does not need specific training before the application.
- It is feasible to say that the production of FC photographs at micro-scale is possible under many conditions of light using some components; however, in order to make a deep analysis of pigments, it is important to ensure a good quality of image, the correct light irradiation into the sample and the adequate operating conditions.
- The FC transformation for the MMSI is not complex since it does not need a white balance (for the utilization of the same light in the darkness); just the change of color channels using PS is essential.
- The set of base pigments of the MMSI helps to identify or reject pigments retrieved from cultural heritage; withal, this checker would fall short in front of more test samples with different mixtures, preparations, and pigments.
- The capacity of the MMSI of magnification and detection of particles are attached in the 20x and  $3\mu m$  respectively, due to the type of microscope and the resolution of the detector.
- The test samples submitted into the MMSI allow to identify the scope of the system in terms of grain or particle size; also, this brought out the lack of pigments in the database with different mixtures.
- The utilization of just IRFC is a binding for the MMSI that, in the case of red pigments, does not allow the identification or the discard of some of those.

## 5.2 Recommendations

- The first recommendation for future applications is to adapt the system specifically into an ILM in order to break the limitation of the 20x magnification and the  $3\mu m$ minimum particle size.
- The detector can be changed at any time, so if there is the possibility to improve this component, it will boost the system and also break the size particle limitation.
- The amplification of the Pigments Checker must be carried out by implementing new pigments and also the same pigments with different preparations and amount of mixtures, for example, a pigment with 50%, 40% or 30% of Azurite and 50%, 60% or 70% of Smalt respectively.
- A special recommendation is the construction of a full spectra probe following the model of the VIS source but using IR and UV LEDs in separate circuits in just one 3D piece that surrounds the sample.
- The system could also be applied in other fields of study, such as the identification of fungi, bacteria, and other kinds of samples.
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# Attachments

#### **A.** MSI components.

InGaAs camera BOBCAT-320-Star



GigaPan EpicPro



Nikkor 200mm 1:4



Nikkor 20mm 1:3.5









 ${\bf B.}$  Optimal location of the GP .

Examples of MSI configuration

Component	Quantity	Unitary Price (USD)	Total
3D impression VIS support	1	5	5
3D impression Cam support	1	17	17
Chip Leds GMKL	4	1	4
IR Clusters BL016-15-28	3	8.33	24.99
WebCam	1	36	36
Goose-necks	3	2	6
MultiDimmer	1	20.22	20.22
(materials and construction)	L	09.00	J9.JJ
Wood Frame	1	10	10
Convering	$3.27m^{2}$	$8/1.8m^2$	14.50
		TOTAL:	156.82

Table 5.1: Attachment K: Cost of the components of the MMSI system.

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		2. Click the	7 eyedropperica	on. 3. Choose a grey point in the
4. Click the save icon.	5. Choose JPEG type file and click "save".			image and click one more time in the eyedropper icon.
Integrate for all and all all all all all all all all all al	the second seco	Property or an interval of an interval of a first of the second of		
		6. Open f	the IR image	7. Click the eyedropper icon,
		as the say (step 1)	me of the VIS	choose the same point that was chosen in the VIS image and click
Result of the step 7				one more time the eyechopper.
Compared to the mean of interview When it is partial at the second seco		Diverse for an and any of		

### ${\bf c.}$ White Balance using UFRaw.

10/02/2020 11:48

Fecha de modifica... Tipo

Archivo NEF

Tamaño

41.878 KB

8. Click the save icon. 9. Choose JPEG type file and click "save".

Q D D Q Q Q Q

2 Cpciones 2 200 2500 4

### **D.** Microscope Olympus BH2.



Microscope Olympus BH2 and objectives.

#### E. Data Sheet IR clusters.

## Kingbright



APPROVED : J. Lu Downloaded from Arrow.com.

CHECKED :Allen Liu

DRAWN:D.L.HUANG

## Kingbright

Symbol	Parameter	Device	Min.	Тур.	Max.	Units	Test Condition
١v	Luminous Intensity		180	320	-	mW/sr	IF=60mA
201/2	Viewing Angle		-	40	-	Deg.	-
VF	Forward Voltage		-	6	8	v	IF=60mA
<b>λ</b> peak	Peak Wavelength	BL0106-15-28	-	940	-	nm	IF=60mA
∆ <b>λ</b> 1/2	Spectral Line Half-width		-	50	-	nm	IF=60mA
İr	Reverse Current		-	-	30	uA	V R = 5V
Parameter			Ту	pe			Units
Parameter			Ту	pe			Units
Parameter ower dissipa	ition		5-	40			Units mW
Parameter ower dissipa C Forward C	ution Current		5- 5-	7 <b>pe</b> 40 90			Units mW mA
Parameter ower dissipa C Forward C everse Volta	ition Current ge		5 5 9 -40 T	7 <b>pe</b> 40 90 5 5 *70			Units mW mA V °C
Parameter ower dissipa IC Forward C leverse Volta operating Tem torage Temp	ition Current ge iperature erature		<b>Ty</b> 5- 5- 5- 5- 5- 5- 5- 5- 5- 5- 5- 5- 5-	7 <b>pe</b> 40 50 5 +70 5 +85			Units mW mA V °C °C
Parameter ower dissipa C Forward C Reverse Volta Operating Temp torage Temp	tion Current ge perature erature 1.0 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0		<b>Ty</b> 5- 5- 5- 5- 5- 5- 5- 5- 5- 5- 5- 5- 5-	40       30       5       5       5       5       5	TA = 25°C		Units mW MA V °C °C

APPROVED : J. Lu
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CHECKED :Allen Liu

DRAWN:D.L.HUANG



### ${\bf F.}$ Specifications of the WebCam support.

#### **G.** Data Sheet Chip LEDs.



EE. UU. IntermatixFino y suave, uniforme en tamaño, mejor rendimiento en lúmenes, mejor uniformidad de emisión de luz.



**H.** Specifications of the VIS Support.



I. Black Box Structure.



J. Acrylics Test.

Transparent, White and Pink acrylics



FC transformation images with transparen acrylic.



FC transformation images with white acrylic.



FC transformation images with pink acrylic.

