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Capture and analysis of autofluorescence in biological tissues for identification and demarcation of lesions

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Resumen

Los cromóforos son sustancias características que se encuentran en determinados tipos de células. Estas sustancias hacen que las células respondan de una manera particular frente a la incidencia de una luz específica. En concreto los cromóforos son los responsables de la autofluorescencia de determinadas células. En este trabajo se muestra cómo el uso de técnicas de iluminación y filtrado de la imagen aprovechan estas propiedades para llegar a distinguir, incluso en tejidos formados por las mismas células, desórdenes no perceptibles en condiciones de iluminación estàndar. En el trabajo se expone cómo estas técnicas podrían llegar a mejorar determinados procesos quirúrgicos permitiendo al cirujano disponer de una información más completa y detallada durante la propia intervención.

Palabras clave: *Cromóforos, autofluorescencia, fuentes de luz, tejidos.*

Abstract

In certain cell types substances called Chromophores are found. These substances are responsible of a particular way to the incidence of a specific light. Specifically, chromophores are responsible for the autofluorescence of certain cells. This work shows how the use of lighting techniques and image filtering take advantage of these properties in order to distinguish, even in tissues formed by the same cells, disturbances that are not perceptible under standard lighting conditions.In the paper, we explain how these techniques could improve certain surgical procedures, allowing the surgeon to have more complete and detailed information during the intervention itself.

Keywords: *Chromophores, autofluorescence, lighting techniques, tissues.*

1. Introduction

Once an anomaly appears in a human body, its early detection is a key element to improve the efficiency of the treatment and the evolution of the disease. In the case of oncology pathologies, part of the treatment is the resection and extirpation of the tumoral mass of cells. Therefore, the improvement itself of the process of the resection of the pathologic mass decreases the probability of the disease to reappear. In many cases, the harmful mass happens to be in sensitive tissue, near vital organs or even into the brain or the uterus. Therefore, the accurate demarcation of the mass to be extirpated is a key point to avoid leaving traces of the it, which will eventually become a new complication; and preserve the parts of the tissue which are healthful.

This paper analyses the state of the tools and techniques already developed to improve the recognition of tumoral masses; and proposes a new procedure to improve the direct visual discrimination. This proposal has been validated with good results at a lab level.

2. Analisys of the state of art

Absorption, fluorescence, and scattering are the three physical phenomenon that take place with electromagnetic radiation interacts with a material. The analysis of the three of them reveals information about the nature of the material.

The phenomena of absorption and fluorescence depend on the chemical composition of the particles. The components of the tissues responsible for the appearance of the aforementioned phenomena are called CHROMOPHORES. In particular, the chromophores that trigger fluorescence are known as FLUOROPHORES.

On the other hand, the dispersion phenomenon depends on the shape and size of the particle on which it is incident, that is, on the texture of the fabric. This phenomenon appears at the point at which the refractive index of the medium changes.

The above phenomena are usually recorded with the help of spectroscopes and multi and hyper-spectral cameras.

When an oncological lesion appears in a medium, two processes are triggered: angiogenesis and hyper-metabolism. As a result of them, there is an increase in abnormal blood supply in the area. (1)

The absorption differences characteristic of a medium (in this case blood) have been used in (2) to create an oxygenation map with the help of hyper-spectral data. The oxygenation map of the medium has been used in a uterine transplant to monitor and detect possible ischemic damage.

The characteristic absorption spectrum of the blood has been used to increase the visualization of veins (3) and increase the visualization of inflammatory tissues (4) among others.

In all of them, hyper-spectral cameras have been used, selecting the bands containing the information of interest and carrying out a data processing based on statistics (5).

The fluorescence causes a reflection of light of wavelength greater than that of incidence. Therefore, to study this phenomenon are usually used illuminants included in the UV range.

Thus, and with the help of digital cameras and filters or instruments marketed as VELScope, it is possible to detect the presence of fluorophores in concentrations different from the usual ones and that can be indicators of tumor presence.

The most abundant fluorophores in the cellular matrix are collagen and elastin. The main fluorophores present in the cell metabolism process are NADH, FAD and lipo-pigments.

In (6) the influence and why each of the endogenous fluorophores are useful for the identification and diagnosis of each of the treated pathologies is explained in detail.

For example, if a beam whose wavelength is comprised between 300-400 nm is influenced by collagen, the energy that bounces off it will be characterized by a wavelength between 400 and 600 nm. In this way, it will be possible to distinguish, for example, epithelial tissue from the connective (1).

Various artificial fluorophores and contrasts have been synthesized that can be injected into the patient and that adhere to a specific type of cells or molecules, thus allowing the identification of these when excited by a specific energy. The most common are the 5-ALA and the INDOCIANINE GREEN.

When no contrast is introduced to increase the visualization of the lesions, we will speak of auto-fluorescence. Numerous otorhinolaryngological studies focus on detecting cancer using auto-fluorescent imaging systems (7) (8) (9).

In dermatological studies (10) it is explained how the decrease in fluorescence in melanomas results from increased collagen and hyperpigmentation of the tissue under an excitation light of 335nm.

The auto fluorescence of the bladder under an excitation of 308nm has been studied in (11).

This paper also details the use of an emission fluorescence intensity ratio at 358 and 455 nm under an excitation light of 337 nm. In (12) , (13) and (14) we find more applications of the technique in the field of urology.

The use of non-external markers in the operating room as a method of detecting necrotic areas, besides having clinical utility, allows a better interaction, communication and training to the team as a whole.

3. Materials and Methods

A Paulmann lamb made of 15 leds of blue color with paramenters 230V-1W is used as source of illumination. This type of light is quite monochromatic (blue range of the electromagnetic spectrum) and unexpensive.

The device employed to capture the images has two sensors: RGB with 12 Mpix and monochromatic with 20 Mpix. The second one allows the capture of more light in the scene (similar to a photomultiplier).

Moreover, it is possible to control the exposition time of the sensor and provides a file with metadata (EXIF) of any of the images taken. In this files it is specified the the value of the aperture, the focal of the image, the time of the capture, time of exposition, etc. It corresponds with the model of the sensor integrated in the smarthophone IPHONE X.

A revious research[i] had already employed different cameras integrated in smartphones to effectively capture areas of skin with large resolution (5 mm). This capture provided a mapping of the chromophores in the area of the study.

Table 1 shows the characteristics of the sensors, as given by the metadata file.

Table 1-Metadata of the images

F-number or aperture	f/1.8
ISO sensitivity	ISO 20
Focal distance	4 mm
Focal distance for 35mm	28mm
Flash	Activated
High DPI	72.00
Withg DPI	72.00
File siez	798 KB
Depth	8
Color space	ICC Profile
Compressed bits per pixel	0
Contrast	regular
Exposition time	1/329 s
White balance	Automatic
Metric mode	Pattern
Sharpness	Regular
Resolution	12.2 MPix
Aspect ratiton	4/3
Color model	RGB

In terms of filters, the following were employed in the capture of the images:

- Schott VG9* (green). It is a bandpass filter centered around 526nm in the range [450-633]nm
- Schott REG610* (red). It is a highpass filter. It blocks any wavelength below 610 nm.

4. Results

1st TRIAL

In the first lab trials, several natural teeth were pictured. In Figure 1 it is visible the more yellow tone some zones, due to the wear of the enamel. As a consequence, the dentine layers happened to be at more superficial strata and therefore visible at a simple glance.

Fig 1. Lab teeth with white light and no filters. Worn out enamel zones where dentine is visible are encircled.

If dentine did not have the property of fluorescence, once illuminated with a blue light, and even applying an special filter, it would be seen with less intensity than the rest of the enamel, just because of the light reflection. White color reflects all visible light, whereas a yellowish color would reflect less amount of it.

In figure 2 it is observed that applying a blue light without any filter does not make any difference: The parts of the teeth with different wear of the enamel could not be differentiated.

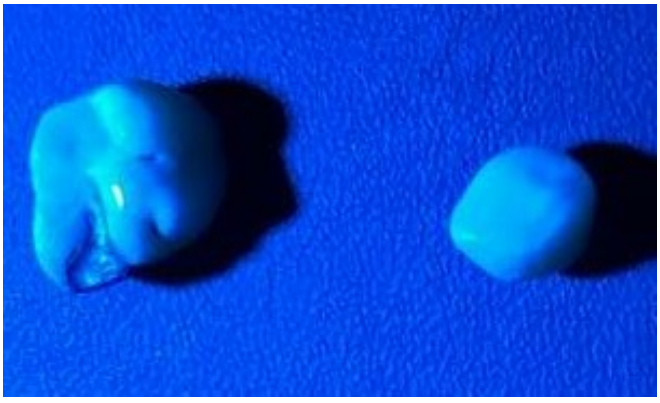

Fig 2. Lab teeth with a blue light without filter

However, if filter RG610 is applied, the zones with greater wear and therefore with more visible dentine, appeared in a more white tone. This is due to the fact that dentine shows fluorescence and emission of energy in the range of the red of the electromagnetic spectrum.

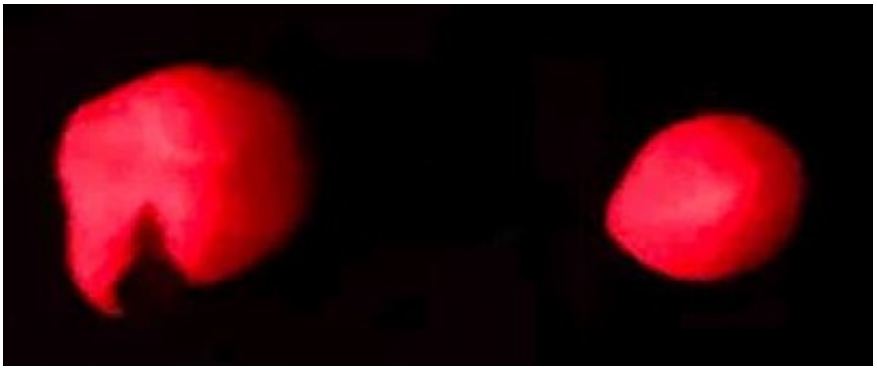

Fig 3. Lab teeth with a blue light and filter RG610

Moreover, if filter RG610 is placed just in front of the objective of the camera without contact, leaving a tiny distance so that a small quantity of blue light could pass through, dentine zones are further enhanced.

Fig 4. Lab teeth with a blue light and filter RG610 located at a small distance to the sensor

2nd TRIAL

In the second trial, a complete oral structure is pictured to identify the natural teeth (with dentine) from the artificial prosthesis (without dentine). All incisive and canine are prosthesis, whereas molar and premolar teeth are natural.

If the oral cavity is illuminated with the visible light without any filter, is virtually impossible to differentiate natural teeth from prosthesis. The same happens if a blue light without any filter is applied (see Fig4.)

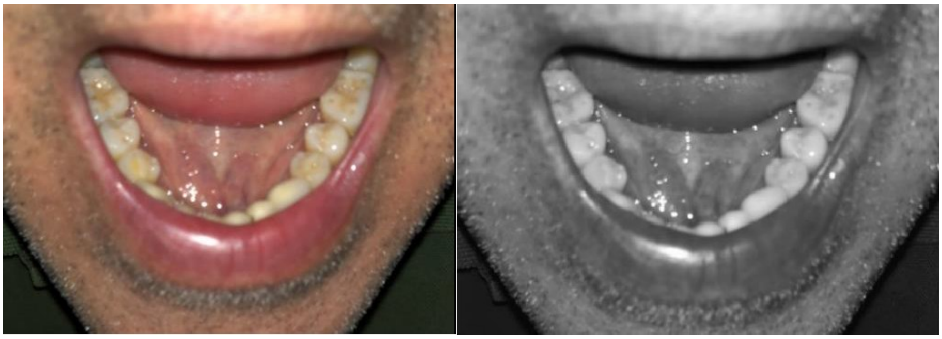

Fig 5. Oral cavity illuminated with visible light

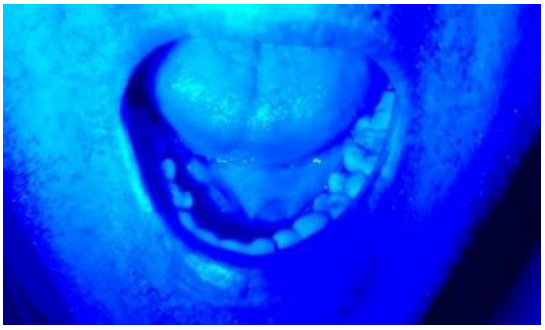

Fig 6. Oral cavity illuminated with blue light

Even in the rage blue-green-yellow-orange of the electromagnetic spectrum no difference could be allocated (seen Fig 7.). The filter employed Schott VG9 [459-633]nm centered in 526 nm.

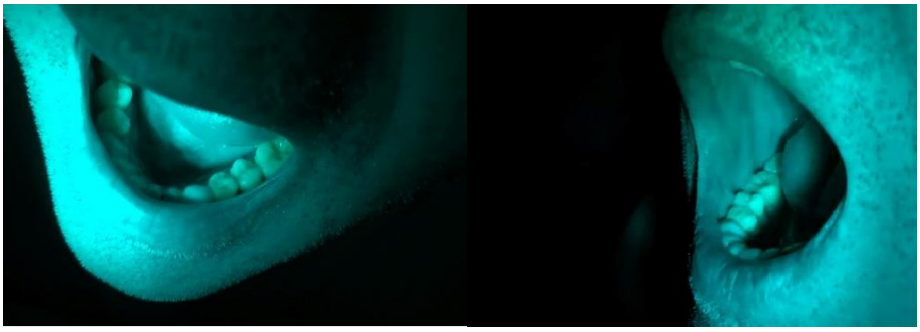

Fig 7. Oral cavity capture in the range of blue-green-yellow-orange spectrum. Filter Sc.hott VG9

However, if the cavity is illuminated with a blue light and the highpass filter RG610 is employed, the difference between the natural teeth and the prosthesis is clearly revealed

(see Fig8.). For a better identification of the dentine, it is possible to edit the last image to highlight the natural parts.

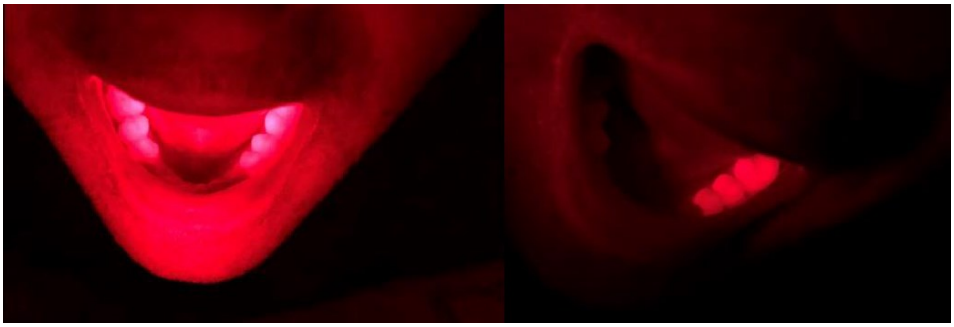

Fig 8. Oral cavity capture illuminated with blue light. Filter RG610

5. Conclusions

It is possible capture a digital image of a given biological area, select the convenient light to shot the picture and process this picture with the convenient filter to seizure the natural fluorescence of a particular substance and allocated it. Therefore, it is possible to use the self-fluorescence of healthy and injured tissue to improve the detection and delimitation of tumoral masses in human beings.

The emission of light in a characteristic way by endogenous fluorophores will give information on the presence and delimitation of the edges of diseased tissues.

This will serve as the basis for the development of a non-invasive and low-cost tool that serves to discriminate pathological tissues of beningno tissue with the combination of lighting and filtering of information captured by a conventional digital camera.

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