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# **Biomedical Instrumentation to Analyze Pupillary Responses in White-Chromatic Stimulation and Its Influence on Diagnosis and Surgical Evaluation**

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Additional information is available at the end of the chapter

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## **1. Introduction**

The pupil is an important element for image perception helping in the focus of parallel rays inside of the eye on the macula and because of its autonomic innervations had been studied in many scientific areas [1-11]. The pupil diameter has been measured with different methods and luminance conditions. Characteristics of pupil diameter like peak velocity contraction, maximum contraction time, amplitude and more [9] have been studied under white light conditions [12] and darkness. Authors have mentioned latency time does not change with age from 14 to 45 years old [3].

Experiments with flicker chromatic lights of equal and different luminance levels have been done; specifically using blue, green and red stimulus [13-16]. Kimura E. and Young R. studied pupil responses to 6 wavelengths [17]. Rodríguez D. and Suaste E. analyzed pupil responses from 400 to 600 nm [18]. Video techniques or image based methods called Video-oculography (VOG), have been employed in the study of pupillary responses [3,8,10,12,14,18,19] with capture ranges of 20, 25 and 102.4 Hz [3,10,12] and pupilometers [17]. High speed video-oculography (HSV) [5, 20] is a technique used in eye tracking systems [21], the study of mice speed movements [22], nistagmus analysis [23], and for pupil dynamic analysis with white light. Another application of VOG had been the objective perimetry as a new proposed method that does not require the opinion of the subject in study using pupillary response.

On the other hand, the World Health Organization describes that 314 millions of people have a visual incapacity, 45 million are blind and 90% live in developing countries, being cataract and refractive errors the principal causes of treatable blindness. During the searching by to resolve

cataract blindness, Scientifics have been developed techniques of cataract extraction and many kinds of Intraocular Lens (IOL) with the purpose of giving transparency and flexibility as a natural lens for the eye. IOL could be only for a vision (monofocal), two visions (bifocal) or multiple visions (multifocal). IOL are a method of correcting cataract and refractive errors (from moderate to high) [24]. Speaking of refractive errors and their correction, IOL represent the next step in the refractive surgery. Authors have explained the importance of considering pupil size when an IOL will be or have been implanted [25-27]. Others authors have spoken about visual effects and complications as results from IOL implantation [28-30].

The purpose of this chapter is to show the biomedical instrumentation based on two VOG techniques used to study pupillary responses as well as to describe a useful methodology in order to determine pupillary dynamic evoked by chromatic stimuli plus a white stimulus, using High Speed Video-oculography (HSV) and image eye processing. The mentioned evaluation is applied to know pupillary responses in healthy subjects and in subjects with an implant of IOL (multifocal) which are called pseudophakics.

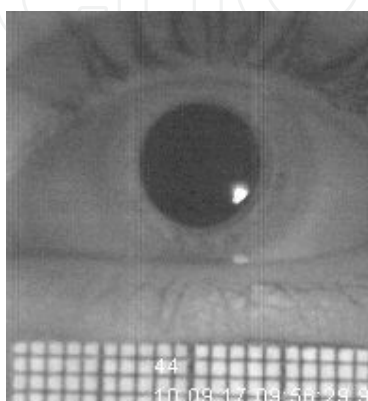
## 2. Instrumentation and methodology

### 2.1. Introduction

In the late 40's, researchers used cameras to record eye movements of pilots [31]. But until the 70's the eye monitoring based on video began. Eye monitoring systems have been classified in two categories: invasive and active against non-invasive and passive. Passive eye monitoring is popular by being non-intrusive [19]. Techniques as electrooculography, electromyography, infrared-oculography and VOG, have been used to monitoring and tracking eye lid, pupillary responses and gaze direction [32]. The VOG is classified as passive eye monitoring because it is non-intrusive. In the next paragraphs is going to be described VOG employed in some preliminary experiments.

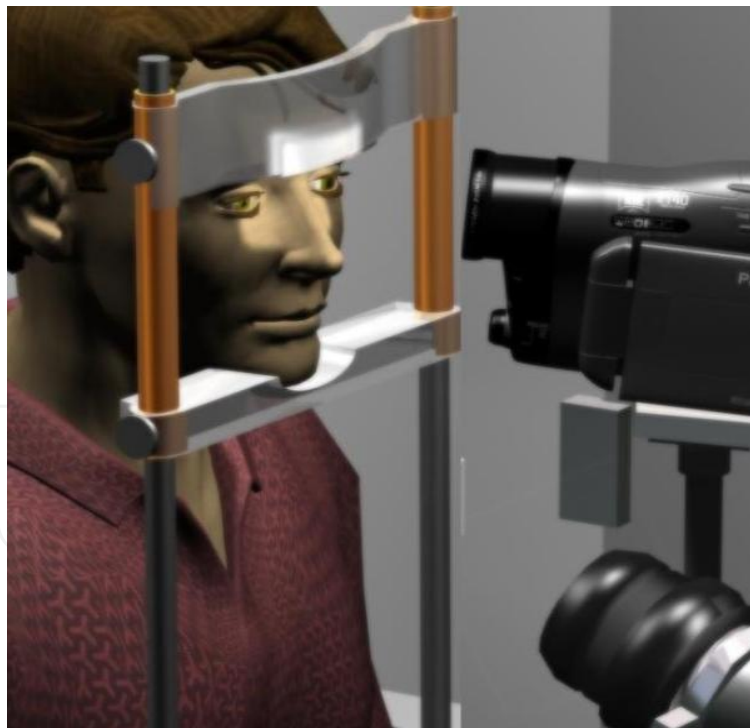
### 2.2. Video-oculography

A reference scale is collocated on the lower eyelid of the left eye to convert pixels to millimeters. It is shown in Figure 1.

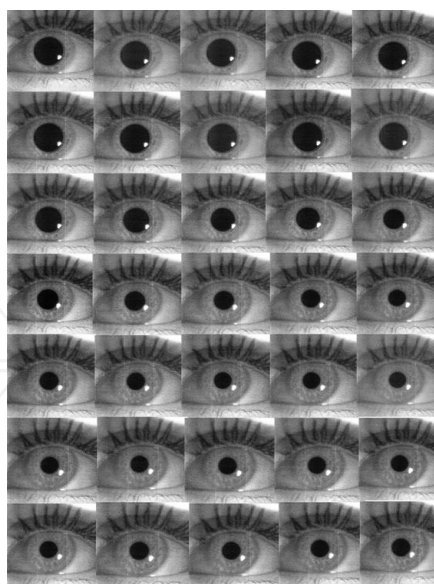


**Figure 1.** Reference in millimeters scale. Pupillary area in this picture is of 34.686 mm<sup>2</sup>.

The chromatic stimulus is projected on right eye while left eye is being recorded. Eyes can be stimulated with 4 wavelengths inside of the visible spectrum plus a white stimulus. The duration of the stimulus is 6 seconds; 155 fps (frame) are captured per second approximately, which means that pupil measurements were taken each 7ms (time between photos). Eye images are recorded inside of a dark room with infrared light (IRL  $\lambda=900$  nm) which is placed at 10 cm from the left eye temporal side. HSV starts with darkness adaptation of 10 min and then the first stimulus was projected. The stimuli sequence is red, blue, green, yellow and white. Five minutes of darkness adaptation between stimuli are necessary for pupil expansion. The HSV is performed from 7:00 to 10:00 hours. IRL is deactivated if there is not recording. In order to avoid gaze deviation, the subject is being sat on a height adjustable seat and his head is placed on chinrest. Camera and IRL are collocated on an elevation table. See Figure 2. All this allowed for specific adaptations, for subject comfort during the HSV. In Figure 3 is showed a series of pupillary pictures from high speed chromatic video-oculography.



**Figure 2.** The VOG image represents medical center where HSV was performed. Chromatic stimulus was projected to right eye while left eye was filmed and the video was recorded in a computer.



**Figure 3.** It is illustrating a series of pupillary pictures obtained from high speed chromatic video-oculography

### 2.1.1 Cameras

The next paragraphs are going to describe the cameras used to evaluate pupillary responses in the applications forward in the text, in two categories of VOG. The main difference between them is the number of frames per second. Commercial video cameras (NTSC format) with CCD and CMOS sensors give generally 50 or 60 fps. A little bit number gives 120 fps [33].

#### 2.1.1.1. SONY camera

A CCD SONY video color camera, DCR-SR42 model is employed in conventional VOG, with 500 lines of horizontal resolution and peak velocity of capture of 30 fps. This camera has a night shot, option which let film using IR illumination. See Figure 4.

#### 2.1.1.2. DALSA HIGH-SPEED video-camera

A DALSA monochromatic camera, CA-D6-0256W-ECEW model, has a shooting range up to 929 fps and 260x260 pixels of resolution; it gives possibility of taking approximately a frame each second. Pictures could be kept in BMP, JPG, etc formats. See Figure 4.

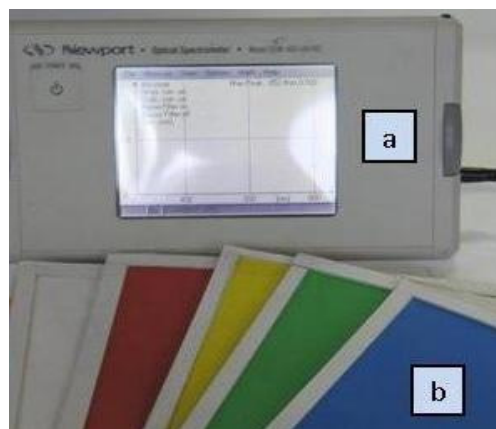


**Figure 4.** SONY camera (left, own elaboration) and DALSA camera (right, Dalsa camera user's manual).

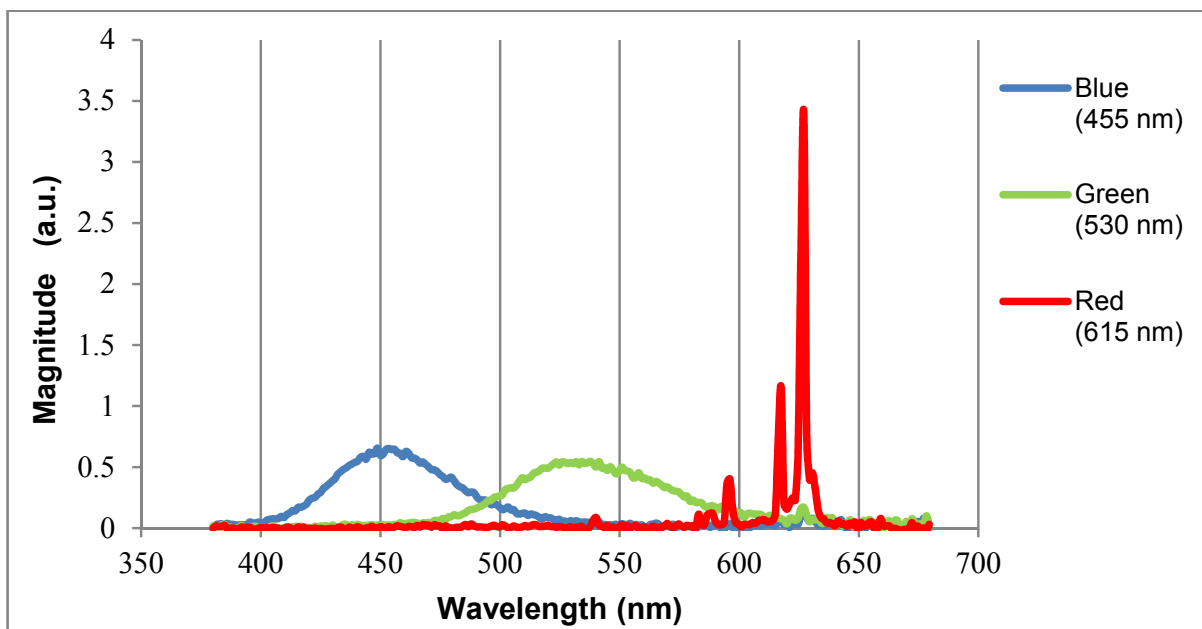
### 2.1.2. Stimuli

These stimuli included the two chromatic-opponent-cone axes, a red–green (L–M) axis and a blue–yellow [ $S - (L + M)$ ] axis. Four chromatic stimuli plus white are used in these experiments but they could be changed according to the purpose in other studies. Each one is characterized by measuring its reflectance spectrum; it will be helpful when pupillary responses will be analyzed. Previous studies can be considered in this aspect [13-18].

The luminance level is measured with a Light Photometer IL 400 A. The photometer sensor is collocated in front of the stimulus where the eye will be, in order to know luminance in that precisely place. Luminance levels are regulated adjusting the input voltage to the light source to each stimulus. See Figure 5. The reflectance spectrum was measured with a spectrometer OSM-400-UV/VIS in order to know the wavelength components in each stimulus [34]. Thirty-six reflectance spectra were taken from each stimulus, they were averaged and plotted. Stimuli were indicated according their principal component. See Figure 6.



**Figure 5.** a) Spectrometer OSM-400-UV/VIS and b) Stimuli.



**Figure 6.** Examples of reflectance spectra.



2.2. Objective perimetry

The perimetry is another test where is possible to use VOG. Perimetry is a helpfulness test, in diagnosing ocular diseases as glaucoma or blindness areas, analyzing the visual field. Perimetry has been classified as: Static and Kinetic. Static, keeps an immobile stimulus in a visual field position, and increases progressively its luminance until the subject not be able to detect it. Kinetic, moves the stimulus along visual field. Here is described the objective perimetry as an application of VOG. The design is based on the Goldmann perimeter dimensions [35]. The light source is a stroboscopic lamp and the targets are based on fiber optic with a diameter of 1 mm, controlled by a computer system. The projection of dims targets is based on fiber optics which permits to perform test in colors, in all the areas of the visual field. A VOG system is used for monitoring and recording the pupil constriction. This system consists of an SONY camera and a PC equipped. After pupillary images are processed and analyzed. It is possible to use together at Evoked Visual Potentials (EVP), electro-oculography and electro-retinography with the aim of to observe cerebral activity when the pupil is stimulated and know alterations in the visual pathway [36]. An example of it, it is showed in Figure 7.

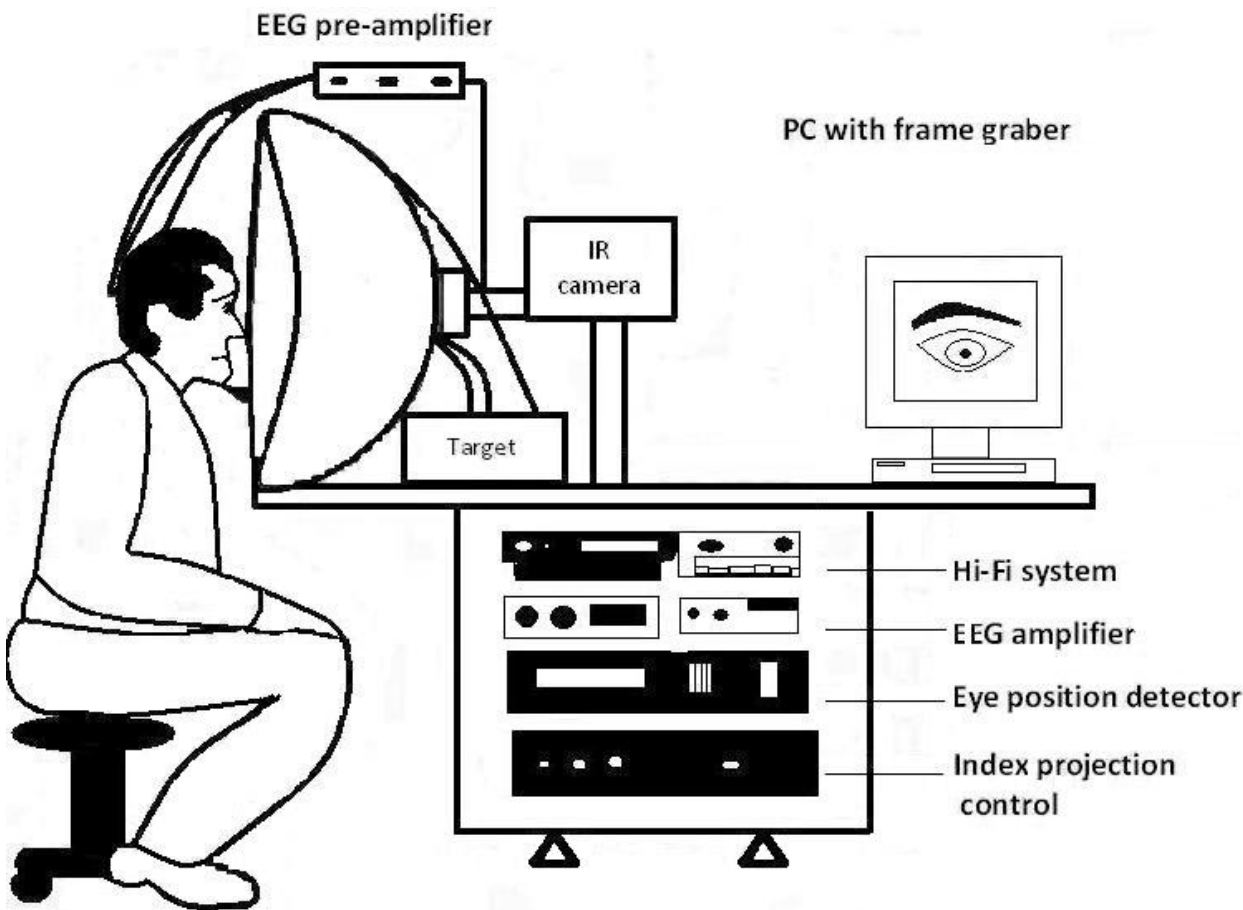


Figure 7. It shows objective perimetry and EVP.

## 2.3. Image processing and statistical analysis

This procedure can be done in any programming language of your choice (MATLAB, C Language, etc). The aim of this stage is to identify pupillary areas in each picture. A graph is illustrating the points on the line selected to observe the pupil dynamic is shown in Figure 8. Variations in pupillary areas of the left eye while the stimulus is projected on the right eye are shown in Figure 8a. Pupil parameters are determined from HSV as follows: Area at the beginning (BA), area at latency time (LA), latency time (LT was taken as Barbur J *et al* described the point at which the trace first departed from the baseline), maximum contraction area (MCA shows time at which pupil area comes to its maximum contraction area), time of maximum contraction (TMC refers to period of time measured from the stimulus beginning at the moment when pupil comes to its peak velocity value) and pupil area from 0 to 5 seconds. The first derivate was calculated from the typical graph in order to obtain values as Peak velocity contraction (PVC presents value of maximum velocity during the pupil responses in each stimulus) and time of peak velocity contraction (TPVC). An example of the last two values is presented in Figure 8b.

Images are processed in a Vision Builder AI 3.5 using graphical programming. First a calibration was performed in order to convert pixels to millimeters. Filters were used to highlight the area of interest in the image sequence. Intensity variations were decreased, noise was eliminated and outlines were identified. Steps of image processing are described in Figure 9.

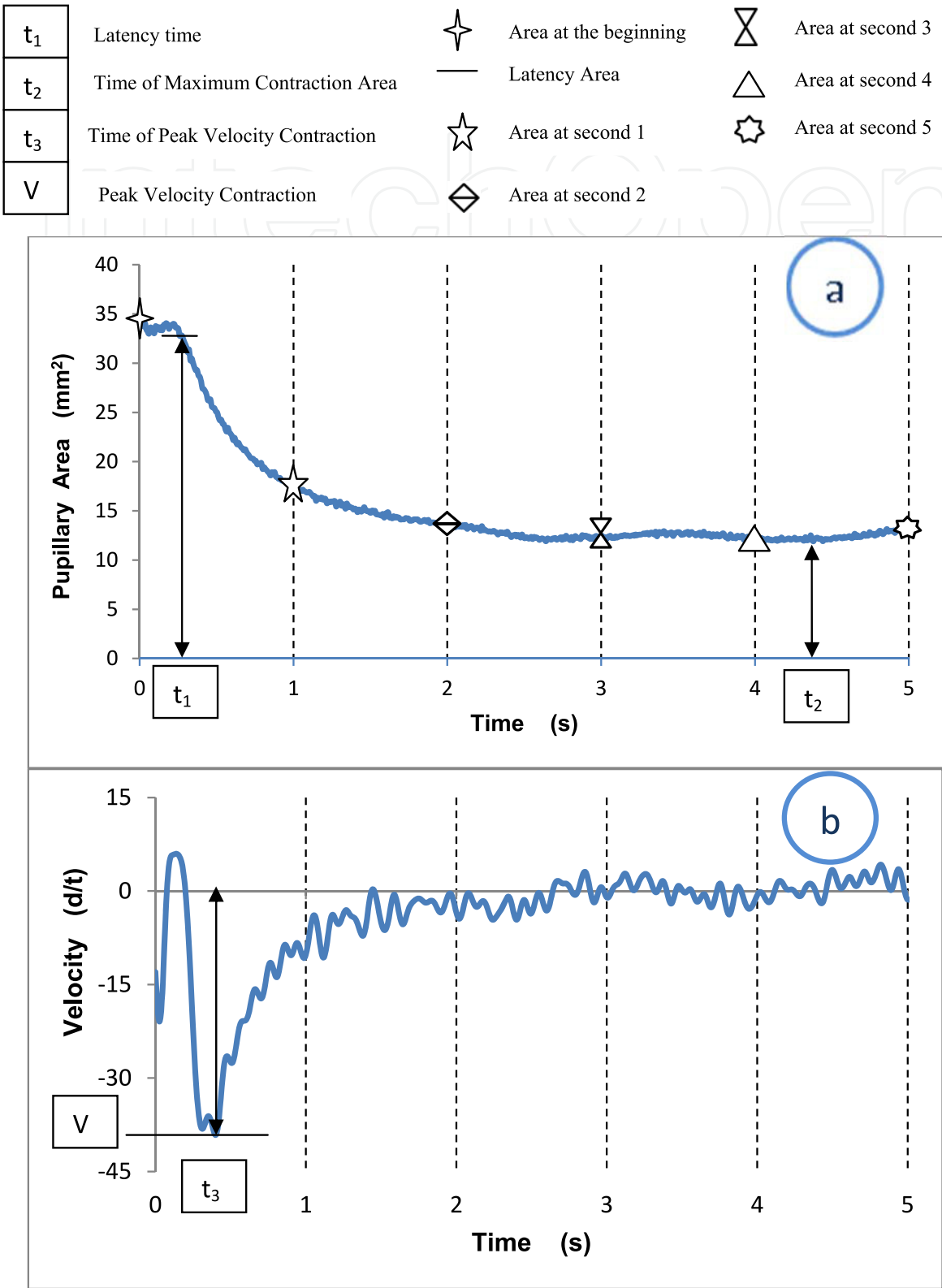
In this context, dates obtained from images can be analyzed for are used statistical analysis. The statistical analysis can be done in any software of your preference depending on the purpose of each experiment. Below, Mean Difference (F test) and Analysis Of Variance (ANOVA) are used to get the necessary comparisons among groups; and, in addition the measures known as average and standard deviation. These statistical analyses are applied in the next section in this chapter.

## 3. Applications of pupillary evaluations and results

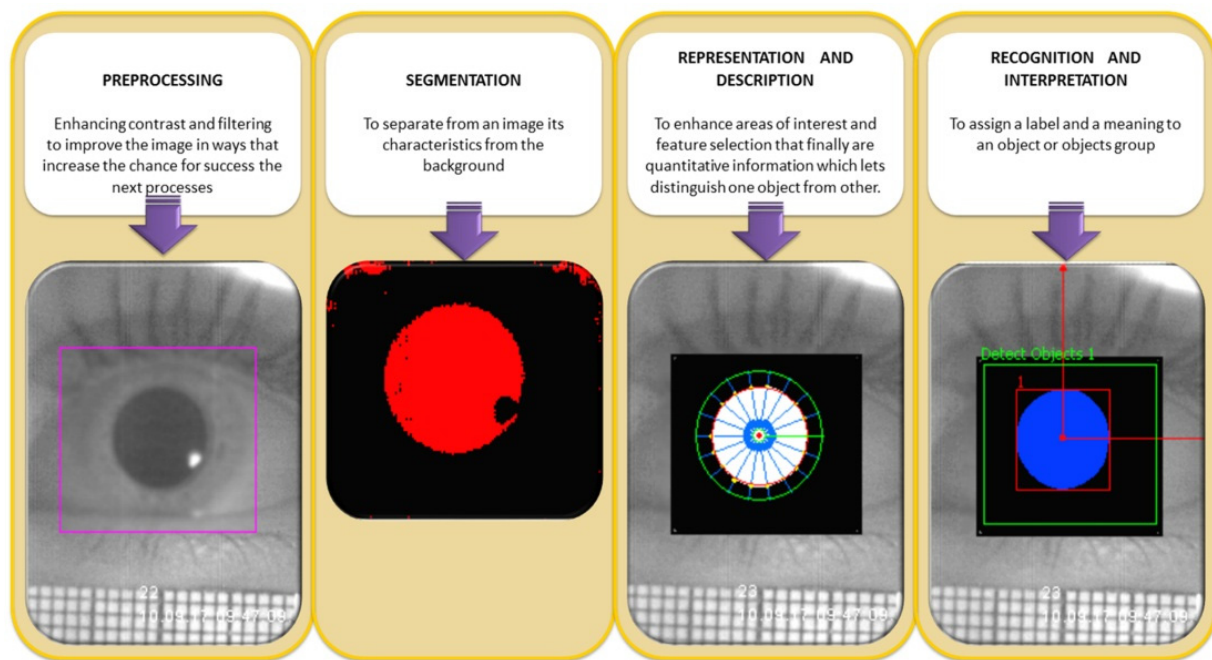
### 3.1. Introduction

In this section there are described many applications of Conventional and High-speed VOG, in diagnosis and postsurgical eye applications. These studies were approved by Bioethical Committee for Research in Humans from Center of Research and Advanced Studies (CINVESTAV). Subjects were informed about the project and they signed in their agreement as it is established in the Helsinki Declaration. For that, it is necessary to apply a preview subject's evaluation with the purpose of having a homogenous sample. Tests are applied to volunteers in order to select the sample group: visual acuity, static retinoscopy, D15, and ophthalmoscopy. D15 test was applied to all subjects in order to exclude subjects with color perception problems. Static retinoscopy was done with a Welch Allyn 18240 NY USA under scotopic conditions. Ophthalmoscopy direct was performed with a Welch Allyn ophthalmoscope 11735 NY USA model, without mydriatic application under scotopic conditions. In the next experimental applications HSV was done as it was described before. Subjects with IOL, were facilitated by **Ophthalmologic Hospital Nuestra Señora de la Luz**, Mexico City.





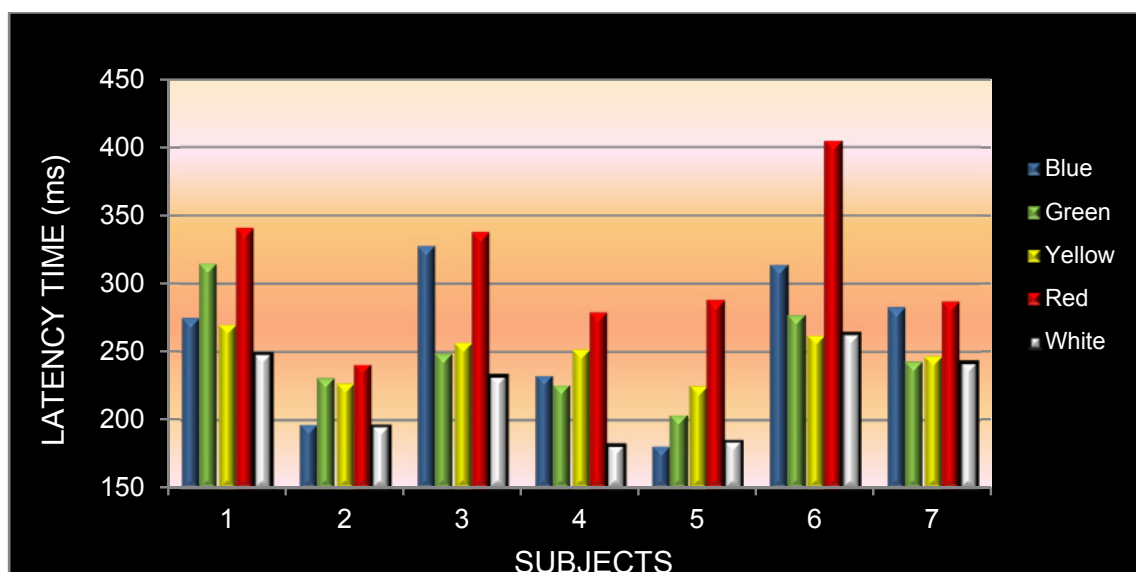
**Figure 8.** a) A typical pupillary response and b) It is first derivate. Where d=mm<sup>2</sup> and t = ms.



**Figure 9.** Steps of Image Processing are described in the top image.

### 3.2. HSV to measure pupillary latency evoked by isoluminant chromatic stimuli

One important characteristic of Pupillary response is latency (LT), which is defined as the time since the stimulus starts until the pupillary begins its contraction. The aim of this study was to determinate LT in response to 4 chromatic stimuli, they were Blue (455 nm), Green (530 nm), Yellow (570 nm), Red (615 nm) plus white employing HSV and image processing in 7 healthy subjects. Latency measures corresponding to each subject are presented in Figure 10.



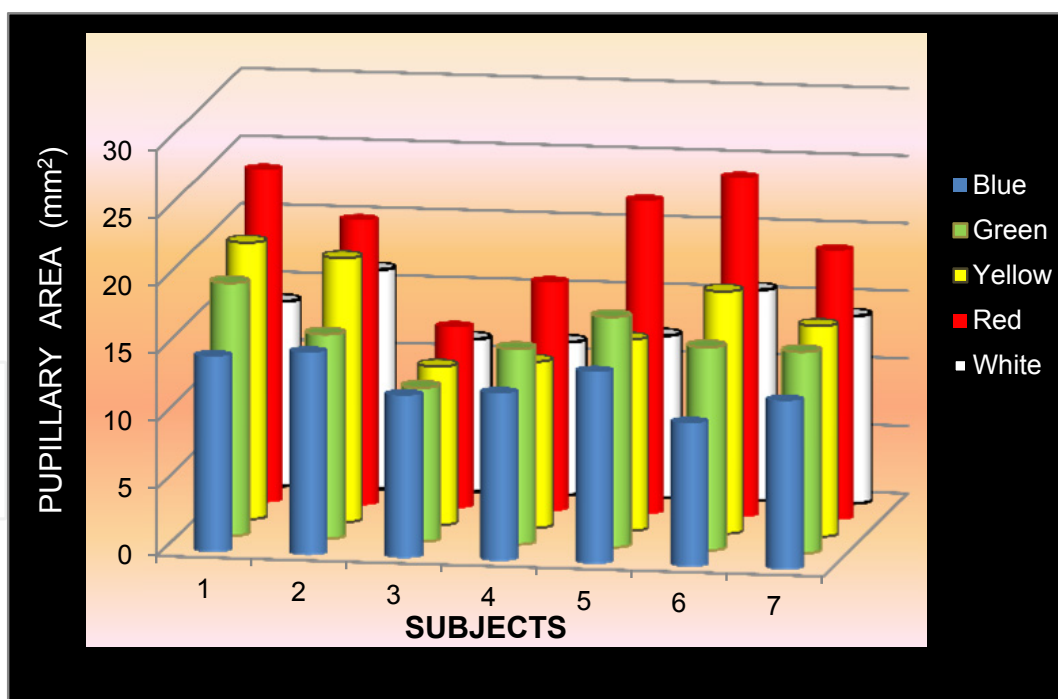
**Figure 10.** Six of seven subjects had the longest times in red. Five of seven subjects showed the shortest times in white. Mean latency in Red stimulus shows larger latency than blue, green, yellow and white.

Statistical analysis: ANOVA test was done among stimuli, considering  $\alpha=0.05$ , ANOVA  $p=0.0075$  which means that at the least one latency time mean is statistically different to the others. And Means comparisons with difference were: red VS blue  $p=0.026$ ; red VS green  $p=0.0099$ ; red VS yellow  $p=0.0091$ , red VS white  $p=4.04 \text{ E-}4$ .

Considering the written above, Bos J. E. describes latency can be a reliable parameter to detect nervous dysfunctions, while the difference between the beginning moment and the maximum velocity can be used to determine muscle diseases [6].

### 3.3. Analysis of pupillary areas at second 3 in response to isoluminant chromatic stimuli with HS-VOG

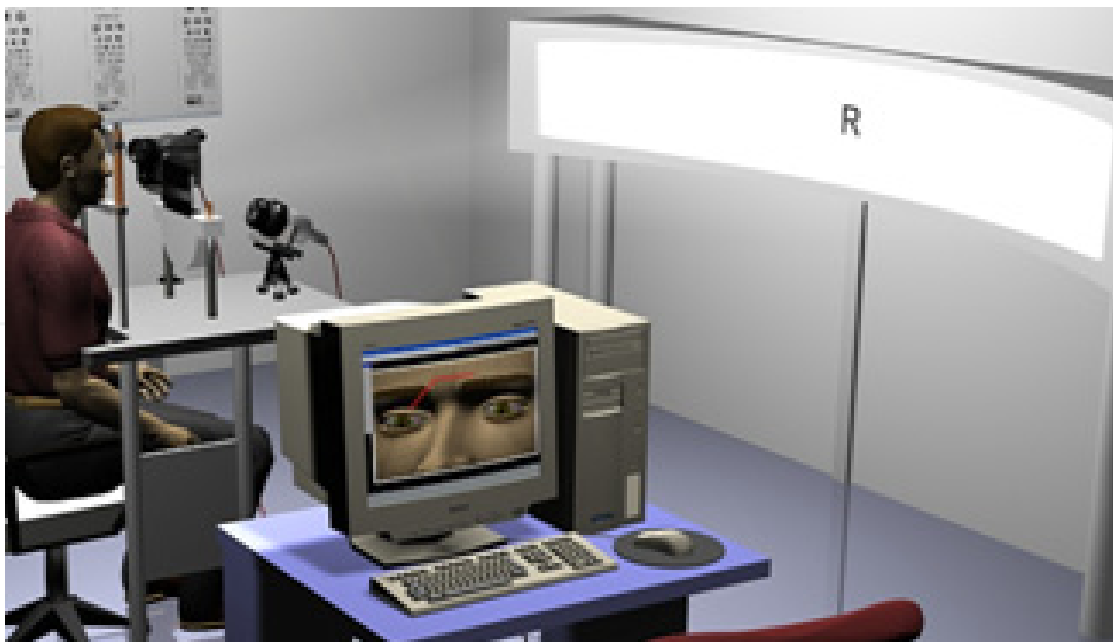
Pupillary responses from 7 healthy subjects were obtained. Images were processed and it is chosen the pupillary area presented in the third second (since it is considered steady state) of each stimulus to 7 subjects. Areas were plotted in Figure 11; each stimulus is called by its main wavelength component that has been obtained from Reflectance Spectrum. They were Blue (455 nm), Green (530 nm), Yellow (570 nm), Red (615 nm) and lastly white. A luminance level for comfortable reading [37] was chosen,  $29 \text{ cd/m}^2$ .



**Figure 11.** It describes areas corresponding to steady state presented in each color by each subject. As it is possible to observe, red responses are the biggest and the smallest without consider white stimulus are in blue. Observing all stimuli, in white 4 of 7 subjects presented the smallest areas in stable state (comparing all).

### 3.4. Conventional video-oculography to evaluate dynamic visual acuity to subjects with intraocular lens implant as a treatment for high refractive errors

Dynamic visual acuity (DVA) is defined as the eye capacity to distinguish details in relative movement conditions between object and subject. DVA have been less studied than Static Visual Acuity (SVA) [38]. In 1985 “The Committee on Vision of the National Research Council” suggested DVA evaluation for car drivers, pilots and sport people [39]. A high refractive error (HRE) is a condition in which parallel rays of light do not focus on retina resulting as blurred vision. Also, glasses in this days; LASER surgery and IOL are used as treatment of HRE. DVA has been studied in its relation with saccadic movements [40]; with an objective method using pupillary responses [41] patented on 2009 [42] and in different luminance levels comparing subjects with monofocal IOL versus multifocal IOL [43]. In this experiment is described a VOG application to evaluate DVA in subjects with IOL implant as treatment of HRE. DVA is done before and after IOL implant. Subject is positioned to 3.10 m in front an optotype used as stimulus (optotype were adjusted considering Lovie-Baleya card) on a chinrest in order to avoid head movements. Optotype are showed until the maximum measured is obtained from DVA, in an angle of  $20^\circ$  on a curve white screen using a projector in a galvanometer controller by a function generator. It starts with the biggest optotype projection to 0.1 Hz, increasing frequency in steps of 0.1 Hz, until the letter becomes blurred. After, it is showed the second optotype, and the procedure is repeated until the smallest letter that the subject can see. The luminance level on the projection screen is 0.3 cd/m<sup>2</sup> and on the optotype is 23.6 cd/m<sup>2</sup>. Figure 12 shows VOG in DVA. While optotype are projected, VOG is done. When the test finishes, VOG is analyzed in order to know the frequency in which subjects could not see the optotype.



**Figure 12.** Video-Oculography used in DVA. It describes the subject position, Video camera matched to a PC to record the video, optotype and screen of projection.

It is explained to the subject that a letter is going to be projected in front of him/her, which will be in movement from left to right and right to left increasing velocity. The subject in study should indicate when he/she could not be able to see the projected optotype, pushing a button, keeping his head static.

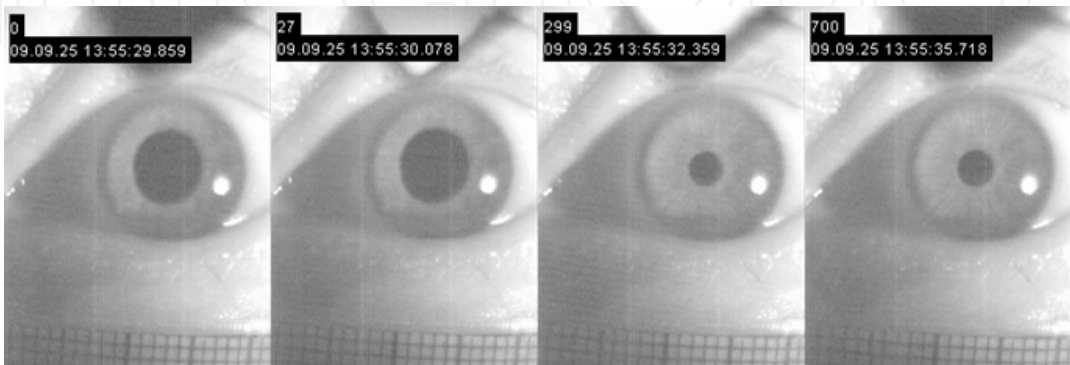
Frequency values in Hz from DVA of two voluntaries, PRE (before) and POST (after) implant are showed in Table 1.

LETTER	PRE		POST	
	S1	S2	S1	S2
N	0.9	0.9	1.2	1
R	0.9	0.8	0.9	1
D	1	0.8	0.9	0.9
C	-	-	0.8	0.9
O	-	-	0.8	0.8
K	-	-	0.8	-

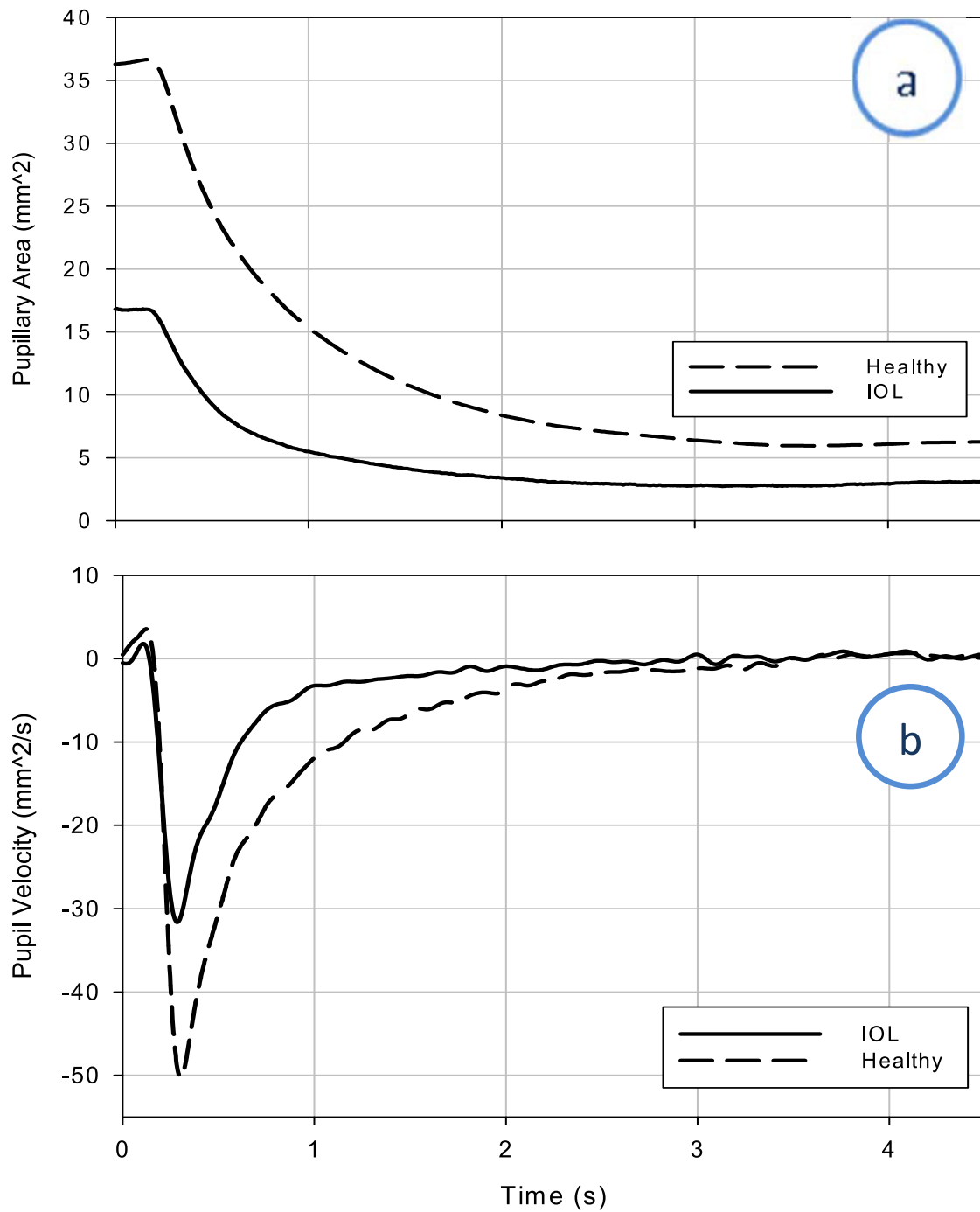
**Table 1.** Frequency values (Hz) of DVA. An increment of frequencies is observed in subjects 1 and 2 (S1 and S2 respectively).

**3.5. High-speed VOG applied in a Comparative study between IOL and health eyes**

The pupil size has been studied in healthy people [18] and in people with IOL [24, 44] implant; finding that it increases the camp depth and improves near and middle vision, after cataract extraction surgery. The next VOG application has the aim to compare pupillary parameters as: LT, PVC, TPVC and to know if they are related with age or the luminance levels. Two groups are compared: Young-healthy subjects (YS) and IOL Older-subjects (IOL-OS). The luminance (white stimuli) used was 171.31 cd/m<sup>2</sup> for YS and 55.16 cd/m<sup>2</sup> for OS. Images were captured with HSV methodology as it had been described above. Once eye images were captured, they were processed with a graphic program (Vision Builder AI V3.5), from which the pupil areas were obtained about be statistically compared between groups. See Figure 13 and Figure 14.



**Figure 13.** A sequence of images obtained by the high speed camera DALSA, left to right: beginning area, latency area, maximum contraction area and steady state area.



**Figure 14.** a) Curves of pupillary responses from IOL and healthy eyes respectively, b) Pupillary velocity. These curves correspond at average for each one of the groups.

Measures obtained from groups are described in the Tables 2 and Table 3. Those are LT, PVC, TPVC and the age and area (at 3<sup>rd</sup> second) of each subject are added. The statistical analysis was done in sigma plot V10, with the aim of knowing if they present a significant relation with one or some of them.



Eye	LT (ms)	PVC (mm <sup>2</sup> /s)	TPVC (ms)	Age	Area (mm <sup>2</sup> )
1	125,38	-27,15	267,47	75	9,22
2	183,89	-40,69	242,39	66	19,81
3	200,60	-20,06	300,90	58	11,47
4	167,17	-44,56	267,47	74	22,95
5	167,17	-25,67	284,19	62	9,05
6	125,38	-31,52	334,34	65	13,44
x	161,60	-31,61	282,79	67	14
S	30,67	9,36	31,90	6,68	5,78

**Table 2.** It shows the pupil characteristics evaluated in this HSV application for IOL-ES eyes. Where x is the average and s is the standard deviation corresponding at each characteristic of the group.

Eye	LT (ms)	PVC(mm <sup>2</sup> /s)	TPVC (ms)	Age	Area (mm <sup>2</sup> )
1	209,2	-45,80	298,11	19	37,66
2	251,04	-63,45	321,645	21	34,4
3	188,28	-48,74	303,34	22	33,54
4	203,97	-39,41	316,415	22	32,75
5	177,82	-57,21	295,495	22	29,01
6	214,43	-48,28	303,34	24	27,85
x	207,46	-50,48	306,39	21,67	32,54
s	25,34	8,56	10,38	163	3,61

**Table 3.** It shows the pupil characteristics evaluated in this HSV application for YS. Where x is the average and s the standard deviation corresponding at each characteristic of the group.

All averages were smaller in the IOL group. From *t* paired test  $\alpha=0.05\%$ . The latency (contraction time)  $p=0.02122$ , the PVC  $p=0.01838$  and TPVC  $p=0.3763$ . Although the luminance levels used and the age between groups were different, it does not exist a significant statistically difference in latency and maximum contraction velocity (Figure 14) between groups. At the same time, the results indicated that age and IOL do not affect statistically on them, however, the TPVC, is far from it [45].

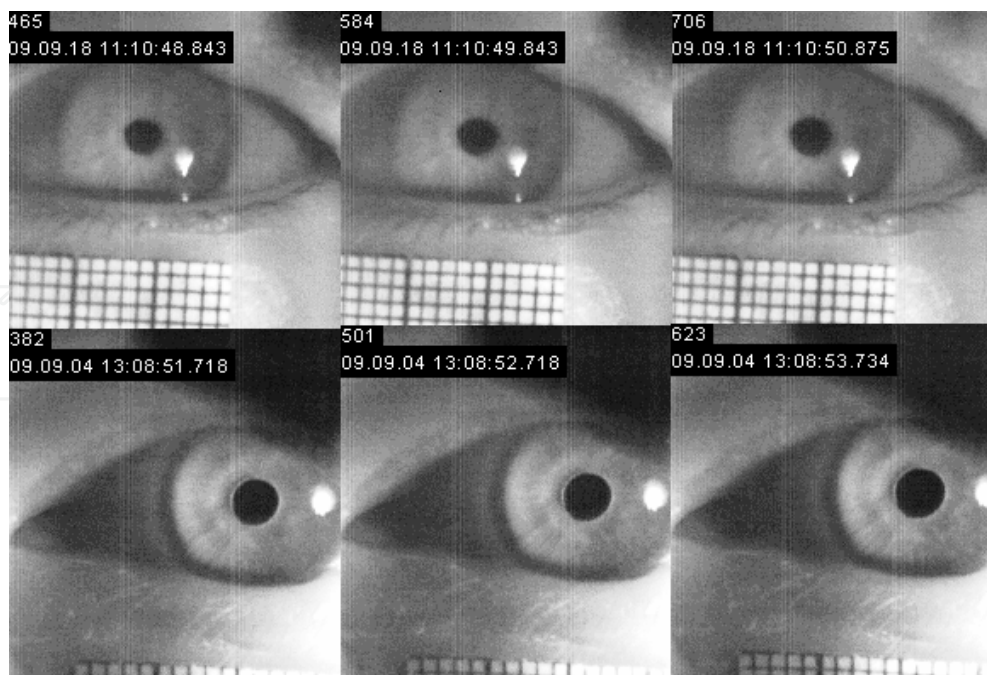
This experiment describes that it is possible to establish differences and similarities between healthy subjects and subjects with an ocular and/or systemic diseases.

### 3.6. ANOVA to analyze pupillary variations in pseudophakic eyes, in response to chromatic stimuli during steady state

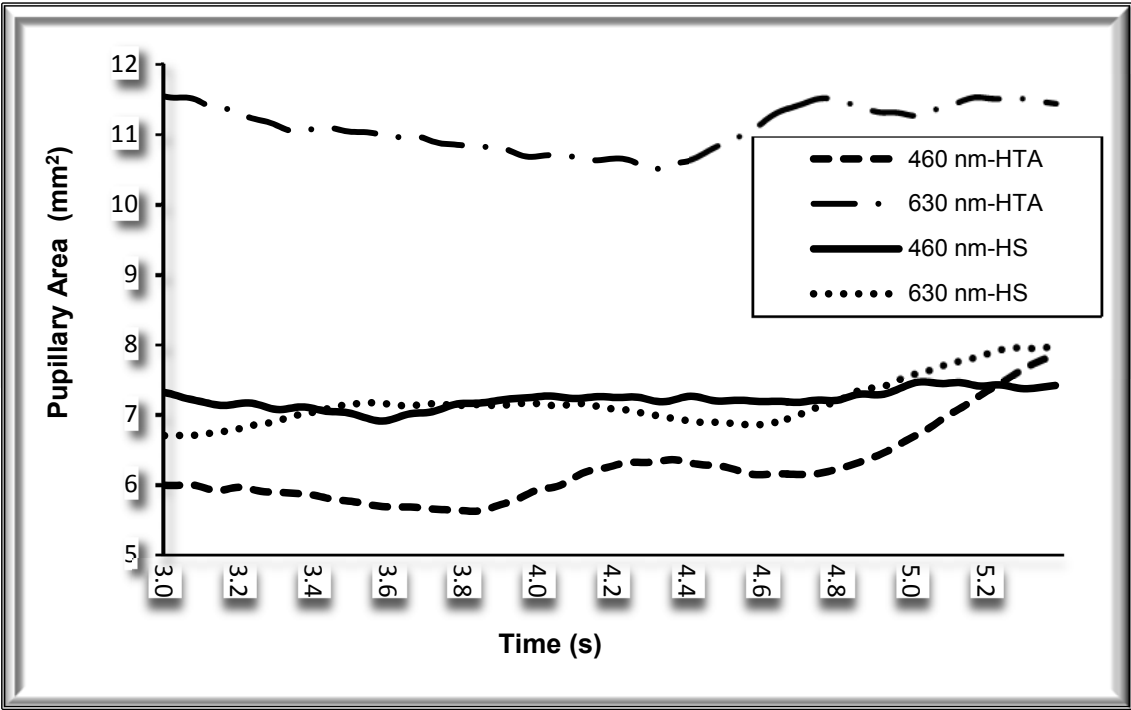
Authors have studied visual performance under mesopic and photopic conditions [46], and have defined which IOL provides better contrast sensitivity in diabetic subjects [47-49]. On the other hand, studies of pupillary responses have been done in health and diabetic subjects [18,50]. Also ANOVA test is a helpful tool in optometric studies [51-52].

In this study, all subjects have multifocal IOL implant (AcrySof ReSTOR), normal chromatic vision and are older than 50 years old. The pseudophakic eyes have been classified in two groups considering their health systemic characteristics: in hypertension group (HTA) and healthy subjects (HS). Each group is formed by 4 subjects. The statistical test used was ANOVA in order to analyze oscillations of pupillary areas, obtained from pseudophakic eyes employing HSV during steady state (from second 3 to second 5.4). A total of 2288 eye images were analyzed in Vision Builder, 286 from each subject. Pupillary pictures of a Healthy subject and a HTA subject are in Figure 15. Averages obtained by group are showed in Figure 16.

ANOVA test was done from 2288 measures of areas. Results from average, standard deviation and ANOVA test (p values) are described in Tables 4, 5, 6 and 7. Average areas obtained per group to each stimulus are described in Table 4.



**Figure 15.** At the top, pictures of a HS subject. At the bottom pictures of a HTA subject. Pupillary responses, second 3, 4 and 5 (from left to right). A millimeter scale is observed used to convert pixels to mm during image procession process.



**Figure 16.** It shows average pupillary areas by group, during steady state (from second 3 to second 5.4) in eyes with MIOL implant.

	460 nm	630 nm
HTA	6.22	11.08
HS	7.21	7.17

**Table 4.** It describes averages areas per group in mm².

	460 nm	630 nm
HTA	0.54	0.32
HS	0.13	0.33

**Table 5.** It shows standards deviations per group.

	HTA	HS
460 nm VS 630 nm	1.84 E-18	2.98 E-51

**Table 6.** It describes P values of ANOVA with  $\alpha=0.05\%$ , in comparison between wavelengths.

	460 nm	630 nm
HTA VS HS	4.5 E-101	0.43

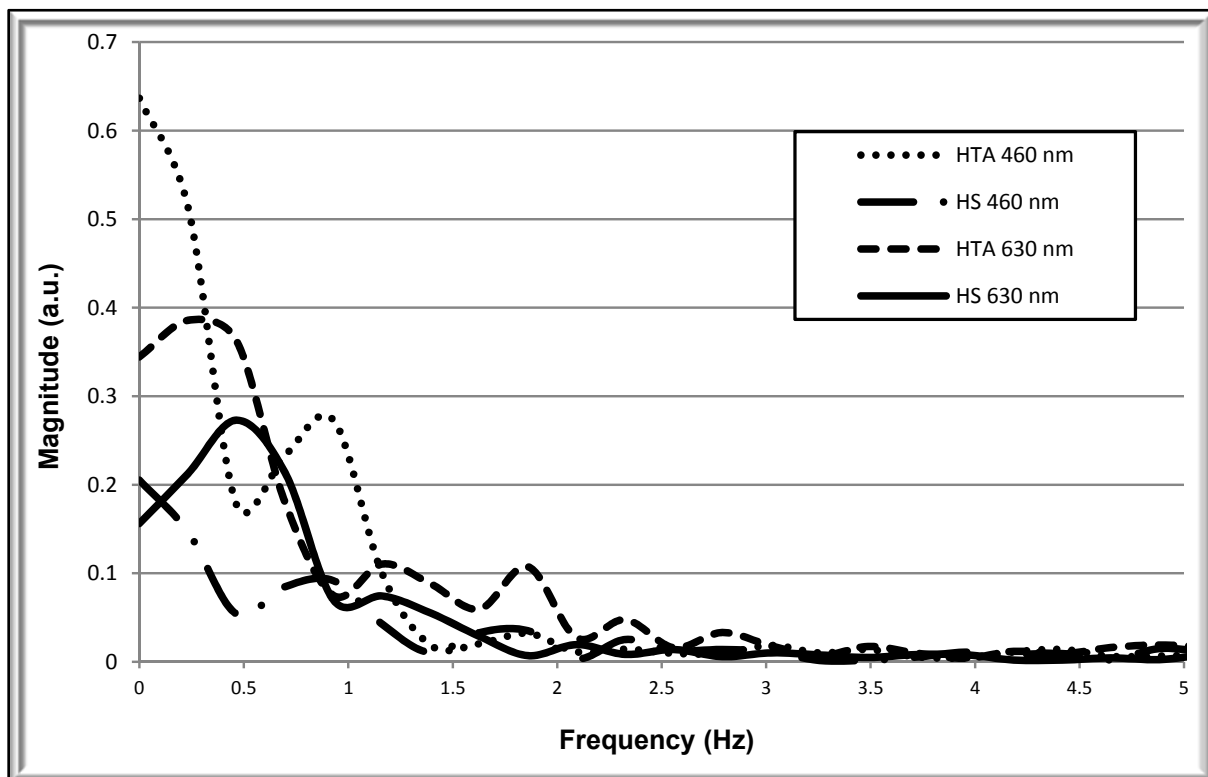
**Table 7.** It shows P values of ANOVA with  $\alpha=0.05\%$ , in comparison between health state.

Based on the results, HTA presents more variation in 460 nm than 630 nm, in pupillary areas during stable state, although pupillary areas of HTA in 630 nm are the biggest; against case in HS. Also, the comparative study inside of 460 nm suggests more variation from HTA subjects in contrast to HS. At the same time, was obtained the sum of energy corresponding to all components of frequency components of Fourier, from pupillary responses, to each group. Values of energy are described in Table 8. Frequency Fourier components are described in Figure 15.

	460 nm	630 nm
HTA	2.147	1.964
HS	0.834	1.196

**Table 8.** Frequency components of Fourier (total sum).

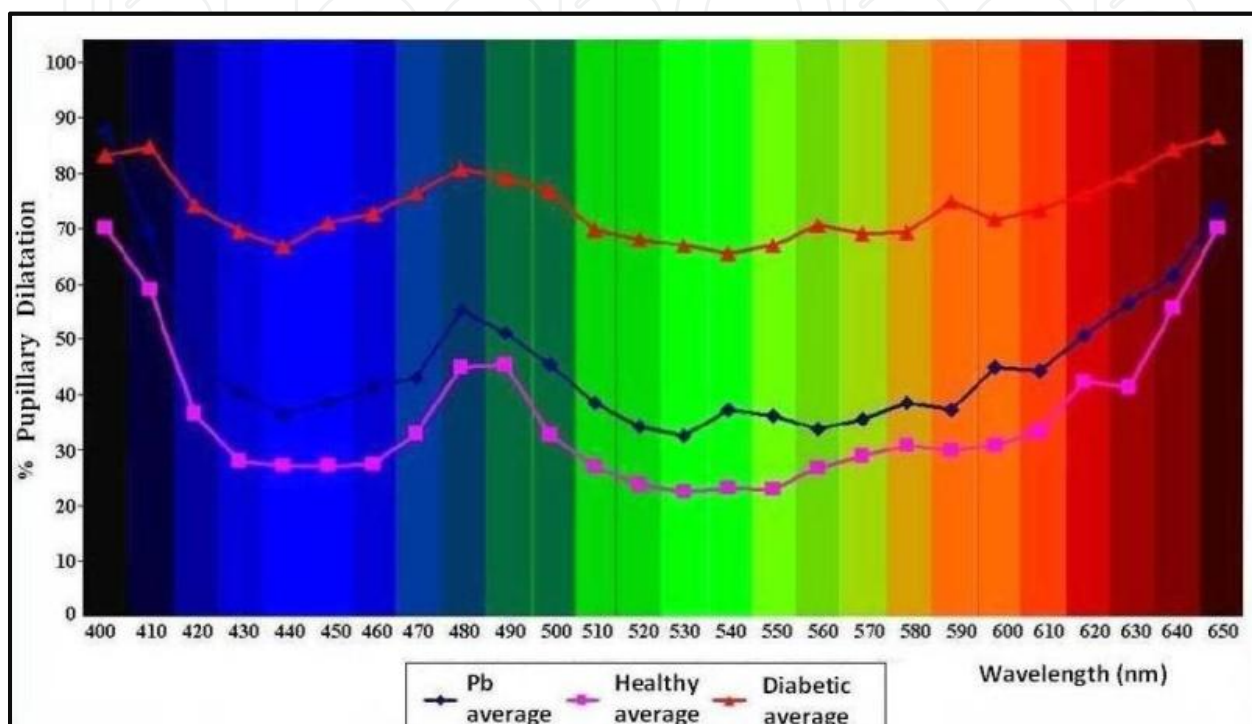
Results obtained from frequency components of Fourier, are in agreement with the results obtained in ANOVA test. Fourier components were obtained from dates that are showed in Figure 17. Observing Figure 16 and Figure 17; Figure 16, shows, bigger average areas in HTA subjects to 630 nm than the other three groups. Figure 17, shows frequency components of major magnitude in 460 nm by HTA subjects; this is related with the results of Table 5 and Table 8 where bigger values are observed in HTA subjects to 460 nm. HTA group has more variation in oscillations (Table 5) and energy content in 460 nm (Table 8).



**Figure 17.** Fourier frequency components by group (averages from Figure 16).

### 3.7. Conventional VOG to observe pupillary responses in sickness and healthy people

Another application of conventional VOG, inside of our laboratory is to evaluate pupillary dynamic in the visual spectrum in subjects with diabetes mellitus type 2, poisoning on lead Pb and healthy people. Results of it evaluation are described in Figure 18. It was patented on 2009 [53].



**Figure 18.** Pupillary dilatation in three different types of subjects.

## 4. Conclusions

As described above, it is possible to observe that the biomedical instrumentation proposed in this chapter has an important contribution in diagnosis of systemic diseases as well as in therapeutically and surgical applications. Also, the methodology proposed is a powerful tool, employing VOG techniques in pupillary evaluations and its application; in order to know both eye physiologic conditions and systemic health situation. Using this methodology, with high speed video-oculography as a tool to film a prolonged chromatic stimuli and white; it is possible to determinate details in the pupillary responses and its derivatives.

The HSV with chromatic stimuli can help in diagnosis, treatment and care of subjects with systemic diseases, also with/without IOL implant. Besides this, HSV let to know changes in pupillary responses until each millisecons. At the same time; allows knowing differences among pupillary responses to chromatic stimuli and white stimulus by eye image processing, which can be standardized considering the parameters exposed in Figure 8, in

healthy subjects and later compared in subjects with some systemic diseases or surgical ocular procedure. Added to these, applications of ANOVA test and Frequency components of Fourier let to analyze pupillary responses in a more complete form.

Therefore VOG techniques plus the methodology proposed plus statistical and Frequency Fourier analysis let to know differences between healthy and with IOL people, as was observed in the above examples. It was in the subjects that were tested with different colors and luminance levels.

While, conventional VOG lets to know frequency changes in DVA. In this aspect, it is necessary to do more measures in healthy and sick subjects. An application of VOG in perimetry is going to be explained in a posterior written because of it let to observe brain activity through pupillary reflex in an objective form.

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