
# UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

PROGRAMA DE MAESTRÍA Y DOCTORADO EN CIENCIAS MÉDICAS, ODONTOLÓGICAS Y DE LA SALUD

## Relación entre la baja absorción de los fotorreceptores y los cambios anatómicos foveales asociados a la edad

TESIS:

QUE PARA OPTAR POR EL GRADO DE
MAESTRA EN CIENCIAS

PRESENTA:

**JIMENA CASTORENA RODRÍGUEZ**

DIRECTOR DE LA TESIS:
MICHEL PAQUES (Hospitral 15-20, CIC. Paris, France)
YONATHAN OMAR GARFÍAS BECERRA (Hospital Conde de Valenciana, Ciudad de México)
GENARO VÁZQUEZ VICTORIO (LaNSBioDyT, UNAM, Ciudad de México)

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**Abstract:**

**Background**: Vision is affected by aging in many ways. Visual acuity is not pertinent to fully evaluate the effect of aging on visual function, since decline of visual function appear long before visual acuity is affected. Measuring other visual parameters such as contrast sensitivity (CS) is more pertinent. Using new procedures to evaluate CS, Angelo Arleo's team reported an age-related decline in photon capture efficiency of unclear origin. To elucidate the origin of this his, examining photoreceptors at high resolution is obviously of interest. A recent study (Soto, 2019) failed to identify a correlation between photoreceptor misalignment and photon absorption rate. Therefore, we investigated other possible causes such as age-related changes in the foveal structures using a recently developed imaging procedure.

**Purpose:** To correlate the presence of age-related subfoveal material with age-related decline of constrast sensitivity.

**Methods:** The Clinical Investigation Center of the Quinze-Vingts hospital recently developed a novel procedure for quantifying the accumulation of age-related deposits under the fovea from AOO images. The current study comprised 16 young adults (mean age 31.5 ± 7.11 years), and 21 older adults (mean age 74.23 ± 5.24 years). CS was measured by Angelo Arleo´s team. The adaptive optics ophthalmoscopy (AOO) and optical coherence tomography (OCT) were used to identify subretinal age-related abnormalities using a procedure called "Grazing light imaging" consisting of specifically extracting shadows due to backscattered light. The amount of foveal reflectance revealed in grazing light images was quantified and correlated to functional data.

**Results:** The correlation between contrast sensitivity and foveal hiperreflectance on grazing light imaging showed that contrast sensitivity is significantly inferior in the elderly group ($p<0.05$); and is inversely correlated with the degree of foveal abnormality.

**Conclusion:** Identifying the cause of the age-related decline in central visual function is still underway, but we may have identify a novel biomarkers of age-related visual loss.

**Aknowledgement:**

I would like to thank all the members of the Paris Group because they have contributed to my academic formation. Pr. Michel Paques who gave me the opportunity to be part of his team, Kate Grieve who kindly accepted me as a student, Daniela because she helped me everyday and made me feel comfortable, the orthoptists members of the Vision and Action in Aging Lab Sonia and Aude for their help with the tests; and Georgina Soto for sharing her results and experiences.

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My completion of this project could not have been accomplished without the support of my family so I thank my parents Jorge and Silvia and my little brother Santiago.

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**Introduction:**

Due to great advances in medicine, life expectancy has increased and so, elderly population. Age-related functional decline has become a trend topic to scientists, psychologists and researchers. Aging and the senses have been studied since the earliest 80´s with taste versus smell, and some other studies that aimed to clarify how some organs decline their function as we get older.
The eyes are not the exception. Vision is affected by aging (Owsley, 2011) but the origin of this decline is complex and not well understood. A lot of tests have been developed in order to evaluate vision. The most common way to do it is measuring visual acuity and there are different charts to this purpose, nevertheless it is not pertinent to fully evaluate the effect of aging on visual function with this method, since decline of visual function appear long before visual acuity is affected. Measuring other visual parameters such as color vision and contrast sensitivity is more pertinent.
Contrast sensitivity is the visual ability to distinguish an object from its background; it changes in situations of low light and it is not the same as visual acuity which is the ability to resolve detail that is usually expressed as the reciprocal of the minimum angular separation in minutes of two lines just resolvable as separate and that forms in the average human eye an angle of one minute. Visual acuity is measured on maximum contrast (black on white), while contrast sensitivity varies the contrast, and it defines the threshold between the visible and invisible.
In order to estimate a contrast threshold, the observer is tested over many trials, at various contrasts. Each trial is at some contrast and is scored right or wrong. The proportion of correct responses at each contrast is recorded. The observer's probability of correct response as a function of contrast is the psychometric function. There are several ways to measure contrast sensitivy: With printed charts and computer testing. The advantage of the second way is that allows use of efficient adaptive methods to accurately estimate threshold for a diverse range of stimuli and conditions. (Pelli, Bex, 2013)
It is important to measure contrast sensitivity correctly, because a low result could be a symptom of some diseases, but it can also be due to healthy aging.
The contrast sensitivity test has standardized results for some pathologies (Fig.1), however there

is no standard result derived from aging. In the charts below we can appreciate the standard result of two conditions: an ophtalmological and a neuronal.

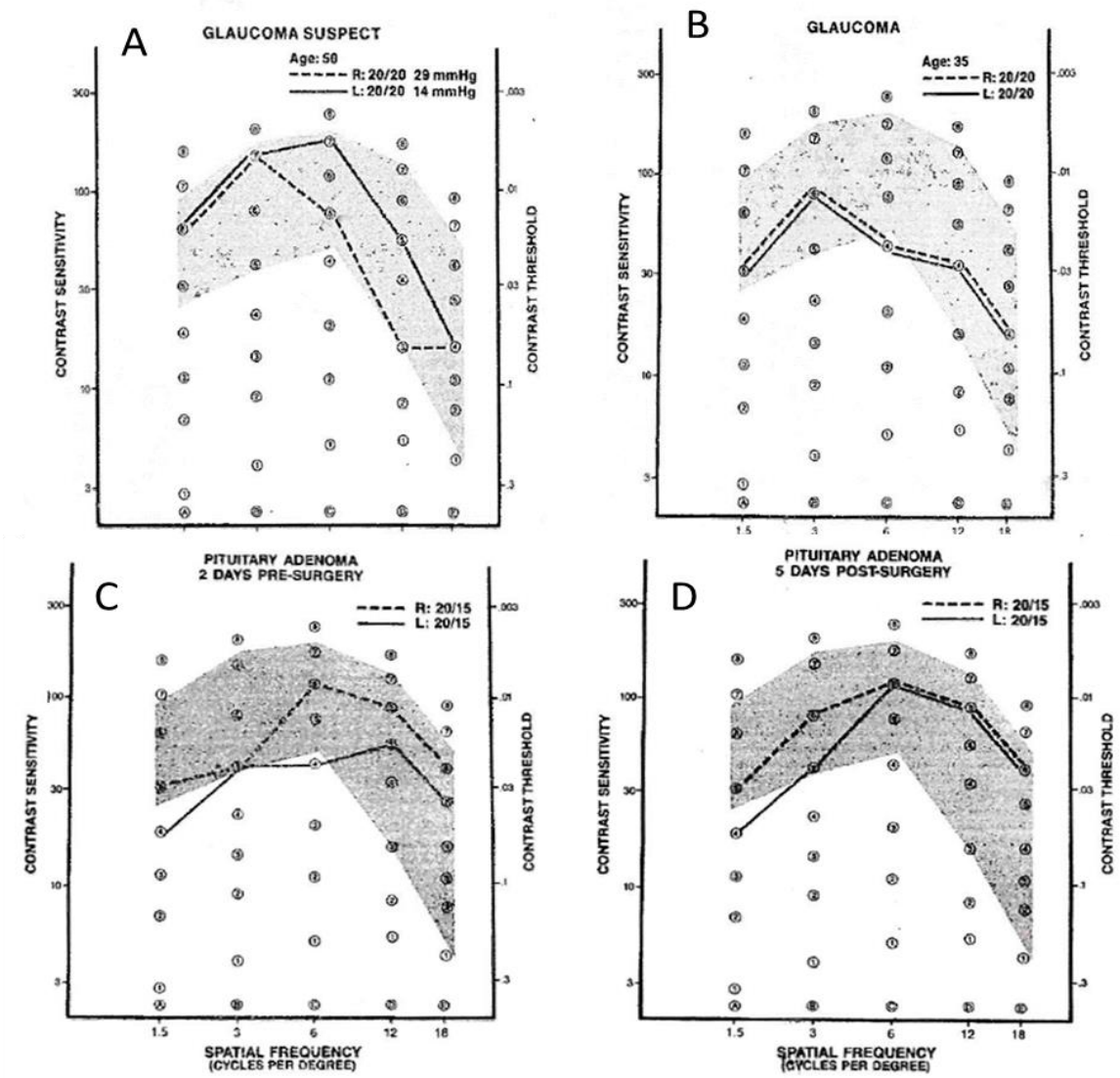



Fig.1 Shows the standard results of CS test in some different cases, the normal results are in grey A) Glaucoma suspect B) Glaucoma C) 2 Days Pre-surgery pituitary adenoma D) 5 Days post-surgery pituitary adenoma

Some authors have reported functional degradations in healthy aging like contrast sensitivity. Indeed, many studies report losses in contrast sensitivity in elderly, but the underlying causes are not well understood. Silvestre and colleagues developed a psychophysical noise paradigm that

quantifies the impact of various internal factors on contrast sensitivity, including internal noise sources at the photoreceptor and neural levels. They also showed in another study that elderly absorb considerably less photons (4X) than young adults (Silvestre et al., 2018). This lower absorption rate was attributed to a lower absorption efficiency of the cones.

To further progress in the understanding of age-related functional loss, the goal of the present study is to correlate the presence of age-related subfoveal material with photoreceptor function. With aging it is common that drusen appear. Drusen are yellow deposits under the retina that are made up of lipids, a fatty protein, among other substances. The drusen appear between the retinal pigment epithelium and the choroid layer that is the layer of blood vessels that supply nutrients to the macula. Having drusen increases a person's risk of developing age-related macular degeneration (AMD). Given that these kinds of deposits are one of the earliest histopathological and clinical signs observed in the early stages of AMD, to examine them really close seems to be necessary to better understand this disease.

One way to try to better understand healthy aging or retinal diseases, such as AMD, is throught direct visualization of individual cells which is now possible with tools for high resolution *in vivo* imaging of the retina such as Adaptive Optics Ophthalmoscopy (AOO) and Optical Coherence Tomography (OCT). And to complement imaging it is also possible to evaluate visual decline with tests of contrast sensitivity and microperimetry.

We hypothesized that the lower efficiency of the photoreceptors with aging could be due to a morphological change such as a misalignment of the photoreceptors occurring with aging. While conventional fundus cameras, SLOs, and OCT systems provide a macroscopic view of the living retina, they do not have the transverse resolution needed to reveal retinal features on the spatial scale of single cells while Adaptive Optics does. In 1989, Andreas Dreher and colleagues, working in Joseph Bille's laboratory in Heidelberg, described the first attempt to use a deformable mirror to improve retinal images in a scanning laser ophthalmoscope. The purpose of adaptive optics is to minimize the effects of wavefront distortions in order to improve the performance of optical systems. Its first use was at the field of atronomy. In these devices light goes onto the eye and reflected off of the retina, it creates a wavefront analogous to the red reflex (Paques et al., 2018). This wavefront of light passes through an array of lenslets; that focuses the light wave onto a charge-coupled device sensor, producing multiple focal points on this detector.

The pattern that these focal points make on the detector is then analyzed for uniformity by a computer algorithm previously designed.

To elucidate the origin of this his, examining photoreceptors at high resolution is obviously of interest. Hence, we used since 2018 AOO to try to understand the cause of age-related CS decline. A recent study by a M2 student of the BIP master (Georgina (Soto, 2019) failed to identify a correlation between photoreceptor misalignment and photon absorption rate. Therefore, we investigated other possible causes such as age-related changes in the foveal structures using a recently developed imaging procedure.

**Methods:**

Participants:

The current retrospective study comprised 37 patients of the Silver Sight cohort at the IDV, in Paris, France that includes young 16 (mean age 31.5 ± 7.11 years) and elderly 21 (mean age 74.23 ± 5.24 years). Participants were evaluated by the Orthoptists from Angelo Arleo´s team with tests that include memory, equilibrium, visual fields, color vision, visual acuity, contrast sensitivity just to mention some.

Then, *in vivo* images of the retina were acquired with optical coherence tomography (OCT) and RTX1 adaptive optics ophthalmoscopy (AOO). Finally the images were processed in some softwares developed to visualize drusen and correlated with the results of contrast sensitivity.

Participants were recruited from the Silversight cohort of the Vision Institute in Paris, France, and participated after signing an informed consent. Ethical Approval was obtained from the Comité de Protection des Personnes Ile de France V in agreement with the Declaration of Helsinski, and the clinical screening and image acquisition were done at the Hospital Quinze-Vingts, Paris. All participants of the Silversight cohort were screened and included in the current study if they had no known visual, audiovestibular, sensorimotor, neuropsychological or cognitive pathologies and if they had a good visual acuity (≥8/10 with their best correction in trial frames).

There were 46 participants who met the inclusion criteria, nevertheless only 37 patients were considered in the current study due to the quality of the images acquired.

At the end we included 10 participants of each cohort: 10 elderly (mean age 74.3 ± 5.24 years). and 10 young (mean age 27.8 ± 2.74 years).

Contrast Sensitivity.

Data were obtained from a previous study that was developed by Angelo Arleo´s team in 2019 (Soto 2019), where they were interested in the absorption rate of the photoreceptors.

Contrast Sensitivity (CS) is a basic visual function; it varies with spatial and temporal frequency and luminance intensity. It depends on optical aberration, internal noise and processing efficiency. Although there are many internal noise sources, most of them have negligible impact on CS in a given condition as only the greatest internal noise source considerably affects CS.

Using a 41x41cm screen and a projector (LCD Panasonic PT-EW730Z, Kadoma, Ozaka Japan) with a refresh rate of 60 Hz, a spatial resolution of 1280 X 800 pixels, and a luminance intensity up to 3700 cd/m2, they used a home made program to perform a motion discrimination task with a two -alternative forced - choice procedure (right or left movement). A chin rest was used to fix the head to ensure the same fixation point in the three different eccentricities for all participants that were evaluated. The signal was a sinusoidal pattern drifting at 2 Hz with a spatial frequency of 0.5 cycles per degree (cyc/deg). Low luminance intensity was obtained by using a neutral density filter with an optical density of 3.5 on the trial frames of the observer. In order to minimize spatial uncertainty a fixation point at the center was presented. Stimuli were presented for 250 ms plus an on and an off half-cosine ramp of 125 ms each, resulting in a total presentation duration of 500 ms.
They evaluated different eccentricities and different luminance conditions with and without external noise. Thresholds were measured with staircase procedure.(Silvestre et al., 2019).
The spatiotemporal frequency (0.5 cyc/deg and 2 Hz) was chosen based on a previous study (Silvestre et al., 2019) so that the motion sensitivity was expected to be limited by photon noise in the low luminance intensity condition, while the motion sensitivity was expected to be limited by late noise in the high luminance intensity condition. Based on the thresholds obtained, the calculation efficiency and equivalent input noise at high luminance and low luminance intensities were estimated.

Contrast sensitivity can be factorized into two components which are the observer's efficiency and equivalent input noise, by measuring the contrast threshold on a blank background, and additionally on a noisy background (Silvestre et al., 2019)

Image Acquisition

a) Optical coherence tomography (OCT).

It is a non-invasive imaging test which uses light waves to take cross-section pictures of the retina, with that data we map and measure thickness on it. To get high-quality optical images it can be focused in different layers and different points depending on the objective.
The images were taken with an SPECTRALIS™ that combines scanning laser fundus imaging with simultaneously acquired OCT. (Fig. 2 and 3)
During the image acquisition, participants were asked to fixate a point fixation. Optical coherence tomography was performed in all the participants and then correlated with the Rtx1 AOO images, most of the images were taken in the same session, and if not between them there is not more than 3 months difference.

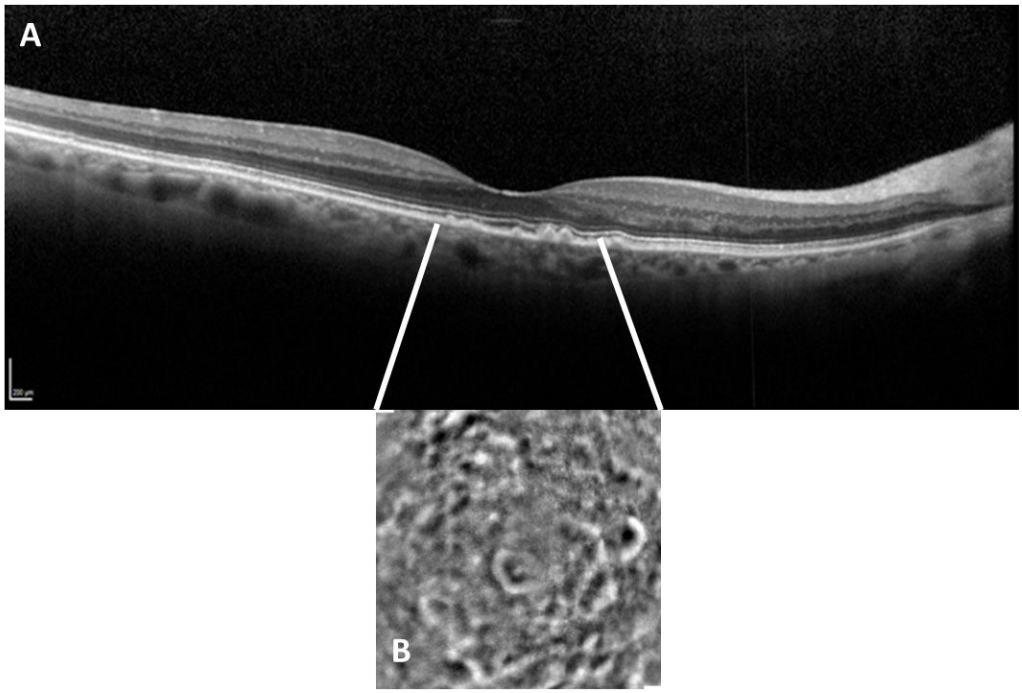



Fig 2. A) OCT was performed in all participants. B) AOO images were acquired in all participants, the most important point was the central because the fovea is located there.

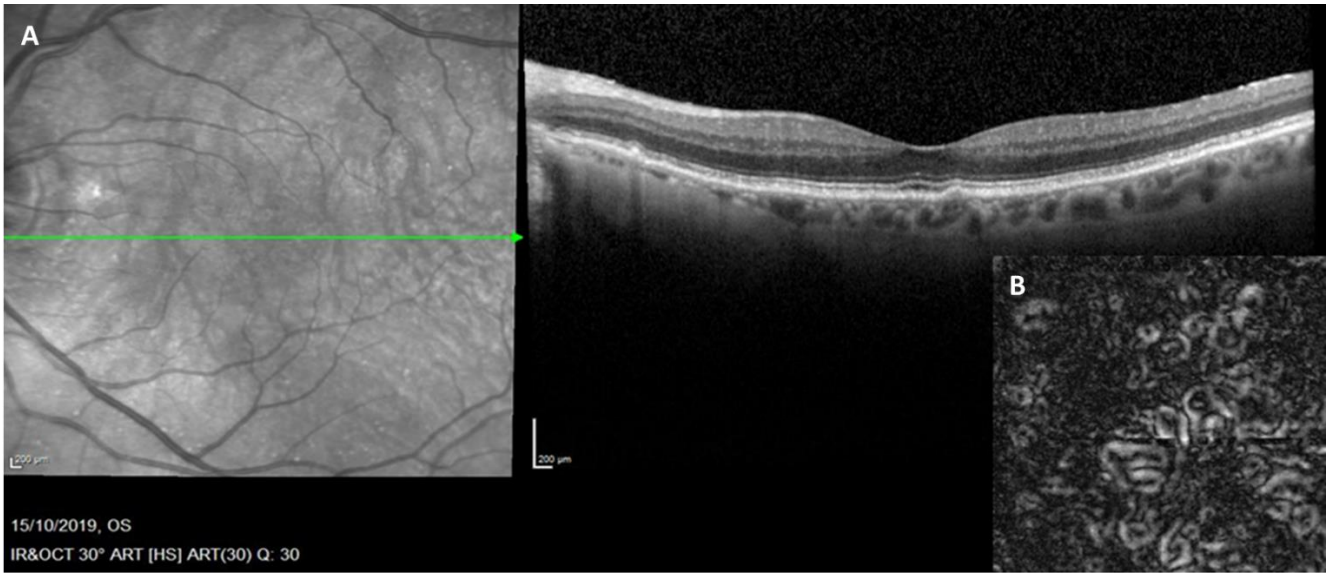



Fig. 3 A) OCT was performed in all participants, it was well focused on the fovea B) After calculating the standard deviation, images were analized to better determinate whether they could be included or not in the current study.

Adaptive optics ophtalmoscopy (AOO)

AOO images were acquired with an RTX1 camera (Orsay, France), that allows to examine the retina at a scale of individual cells (Fig.4). It uses LEDs to produce broader illumination of the retina and allows to record photoreceptors and to visualize the microscopic walls of retinal aterioles without the need of contrast agents.
This camera uses 3 primary components: a Shack-Hartmann wavefront sensor, a deformable mirror for correction of ocular aberrations, and a fundus camera.

RTX1 is an adaptive optical fundus camera using flashed, non-coherent flood illumination centered at 850 nm to record series of 40 images, each cover 4°x4°, Rtx1 has a pixel resolution of 1.6 μm and resolves 250 lines per millimeter. The system corrects imaging aberrations created by ocular refractive surfaces such as the cornea and the lens.

During the image acquisition, participants were asked to fixate a yellow fixation cross internal to the AOO fundus camera. The main idea was to obtain between 9 to 13 images overlapping the adjacent image to stitch them after and get a bigger frame. The most important part needed was the center of the macula, so in some cases, acquiring at least 5 central images was enough.
If necessary, patients were dilated. Then the images were processed with some different softwares to better visualize subfoveal material (described below).

After acquisition, the images were carefully analized to better determinate whether they could be included in the current study, or not. Some of the images had different artifacts, that means that the quality was not as good as expected. It could be due to lack of fixation of the participant, or because of some software overlapping issues that caused an extra signal that was interfering with the final big frame.

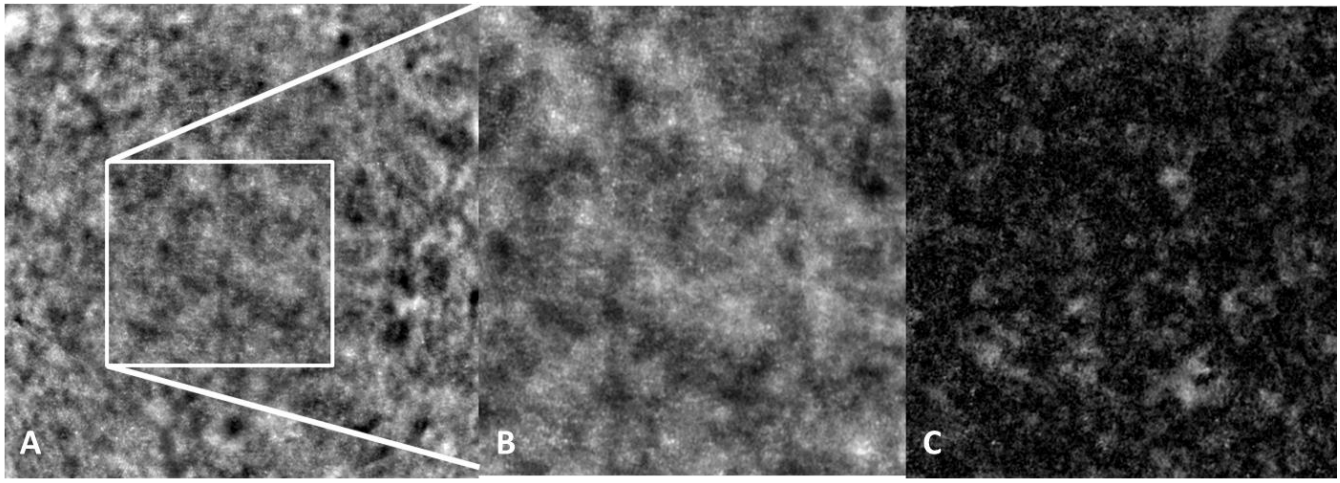



Fig. 4 A) Adaptive optics images were acquired in all patients B) With adaptive optics it is possible to record single cells C) After processing images with ImageJ, standard deviation was calculated to better visualize drusen if there is any.

## Image processing (AOO)

The optical principle of the software that was used is the grazing light (Fig 5). It is used to emphasize the materiality, structure and properties of surfaces. It extracts the small differences between two overlapping images, which is likely due to the amount of side-ilumination which is higher in the border of images as shown below.

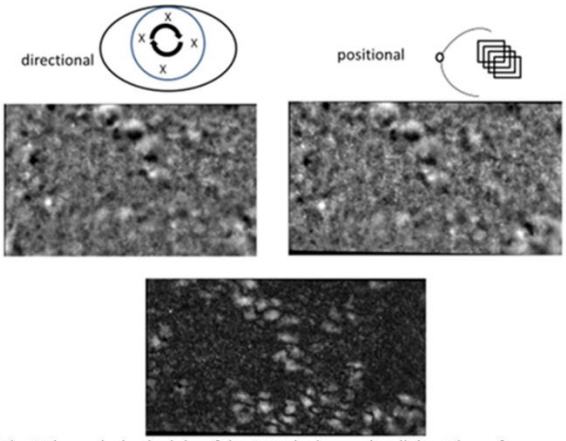



Fig 5 The optical principle of the AOO is the grazing light. The software extracts the small differences between two overlapping images, which is likely due to the amount of side-ilumination which is higher in the border of images.

After acquiring the retinal images, they were processed with two different softwares in order to visualize drusen and photoreceptors: ImageJ and MATLAB™ (The MathWorks, Inc., Natick, MA, USA).

Image J software can display, edit, analyze, process, save and print 8-bit, 16-bit and 32-bit images. With it we can work with an specific part of the fovea. It means that we could work with the 0,0 coordinate and the 8 images around it.
The purpose of this is to align each image aroud the central point and with the deviation standard function, extract some extra bright in order to emphasize the drusen, if there is any.

MATLAB™ Is a high-performance language for technical computing. It integrates computation, visualization, and programming where problems and solutions are expressed in mathematical notation. It allows to create quick programs and to create complete large and complex application programs.

For this research we used the computational code "The Drusen code" made in MATLAB™ by the group of Dr. Ethan Rossi in Pittsburgh University, USA. The main task of the algortihm is to extracts the small differences between two overlapping images, which is likely due to the amount of side-ilumination which is higher in the border of images. Collecting these intersections enables to construct a patchwork image which highlights the presence of drusen. The aim is to form a big picture to better visualize the fovea and quantify the size of drusen, if there is any.

The code can be used with a window that allows to make a quick use of all the functionalities implemented in the code. Once inside it, is only needed to select the images to analyze and drag into the boxes to get the resulting one already without the overlapping. Is important to notice that in all procces the path of the images and code should be the same, and should be known by the user.
In order to clarify the process, there are some steps described bellow.

1.- Collect 5 or 9 RTX images at locations:
5 central: 0,0; 2N,0; 2T,0; 0,-2; 0,2.
4 corners: 2N,2; 2N, -2; 2T,2; 2T, -2.

Sometimes it is not possible to get 9 images, in this case, the best option is to get, at least

the 5 central images. It is important to know the specific location of each image (Fig. 6)

2.- Open up Matlab. Run the “drusen code” and choose the 5 or 9 images collected.

3.- Matlab generates a big picture based on the smalls pictures that were taken with the Rtx 1 camera.

The aim of the code was to develope a new method to visualize drusen and their progression using AOO, taking advantage of the fact that the contrast of drusen varies with gaze position. Using math, they probed that combining images from different gaze position, drusen could be visualized with high resolution and contrast. The images were acquired at 4°×4° fovea-centered and then its internal fixation target was moved to obtain 4–8 additional, overlapping images, with gaze displaced by +/- 2° vertically and horizontally (or both). Custom software registered images and calculated the standard deviation (SD) of each pixel across areas of overlap. (Rossi et al., 2019)

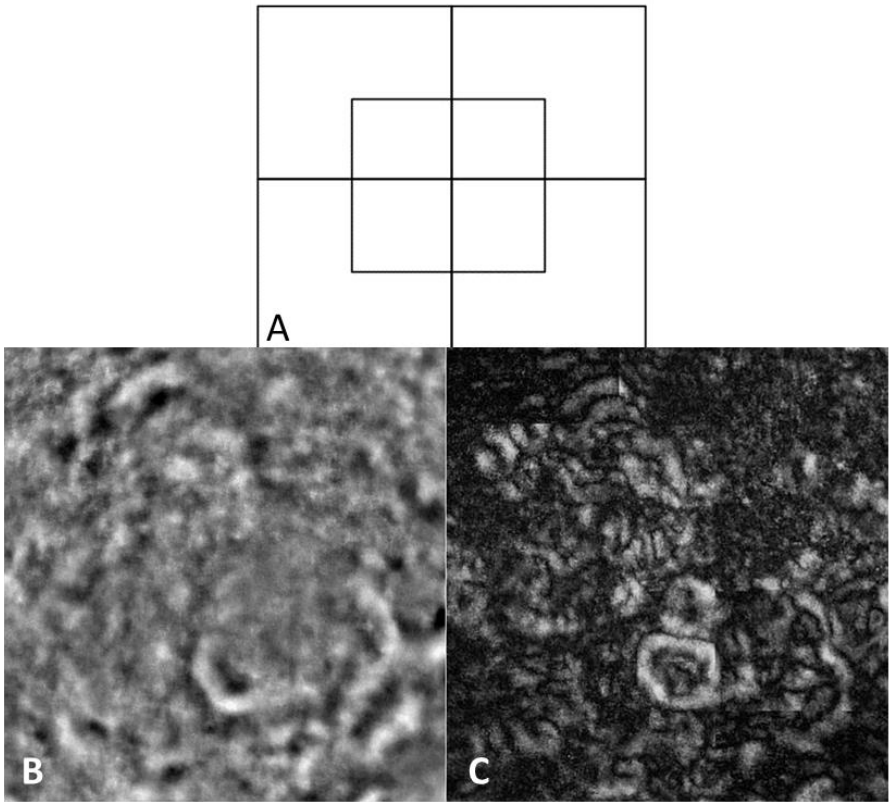



Fig. 6 A) To construct the final big frame, it is necessary to get 9 images, 5 central and 4 corners. B) After the softwares proccess the images to stitch all the little pictures C) With ImageJ it is possible to calculate the standard deviation of each pixel across áreas or overlap

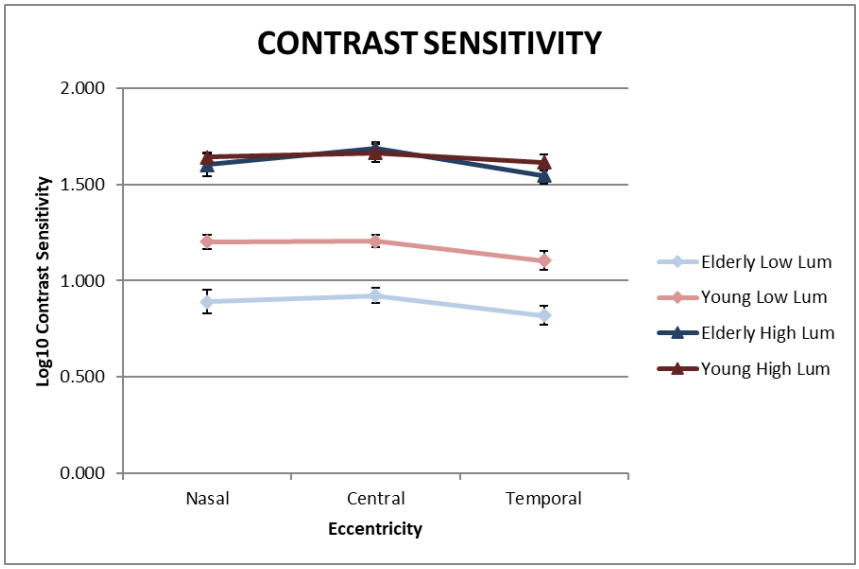
All the images were processed with some different sofwares, compared and then analized to select the ones without artifacts. An image was considered good if it was not blurred, because it could be a sign of movement during the acquisition of the image; if it had not black boarders due to lack of information; if it had not vessels because it indicates that the eye was not well fixated and if the final frame was continuous.
An image was considered arficated if it was blurred, with black boarders, with vessels or discontinuous.

**Results:**

Contrast sensitivity.

Elderly were less sensitive than young adults at the low luminance intensity, but not at high luminance intensity. These results agree with previous studies. Contrast sensitivity showed a significant effect of age, a significant effect of eccentricity and a significant effect of luminance intensity (Soto, 2019).

A significant interaction between age and luminance was found, which can be explained by the greater age related sensitivity loss at low luminance intensity. No significant interaction between age and eccentricity was observed.



Despite 46 participants could have been included in the current study, not all of them had high quality images acquired. The foveal images of 37 patients were analized. Out of 66 images that were acquired, 33 were considered of sufficient quality to be included in the current study.

**AOOimages**

Although the softwares were designed to process the images acquired with the Rtx1AOO, not all of them were well processed. The main limitation was that some of the final big frames had some artifacts that did not allow to visualize the fovea, it could be due to a bad quality of the picture, or movement of the patient. As a result in the final frame we had lost of information of the foveolar area.

The most common artifact is represented by whitish aspect of all four borders of a 4° image (Fig 7a) This appears as a image of "white cross" on the 8°x8° image. Since the fovea is located at the corners of four adjacent images (that is, at the middle of the white cross), these whitish border accumulate white pixels from the four contiguous corners hence the fovea itself appears artifactually white. To avoid this, one must take care that there is no image of such white cross, as shown below. We identified the source of this type of artifacts, which is due to a slightly increased brightness of the border of AOO which depends on fixation stability; this brightness cumulates at corners and hence in the reconstructed fovea which is the sum of four corners. This prevented us to obtain more high quality images. To circumvent this, we developed an alterative procedure that significantly reduced this artifact but requires to reacquire images; it cannot be done retrospectively.

Another artifact is due to misalignment of two images. Hence, any difference will generate white pixels on the std image. This is easily identified if there is a vessel, which will generate a white line on the final image (Fig. 7b).

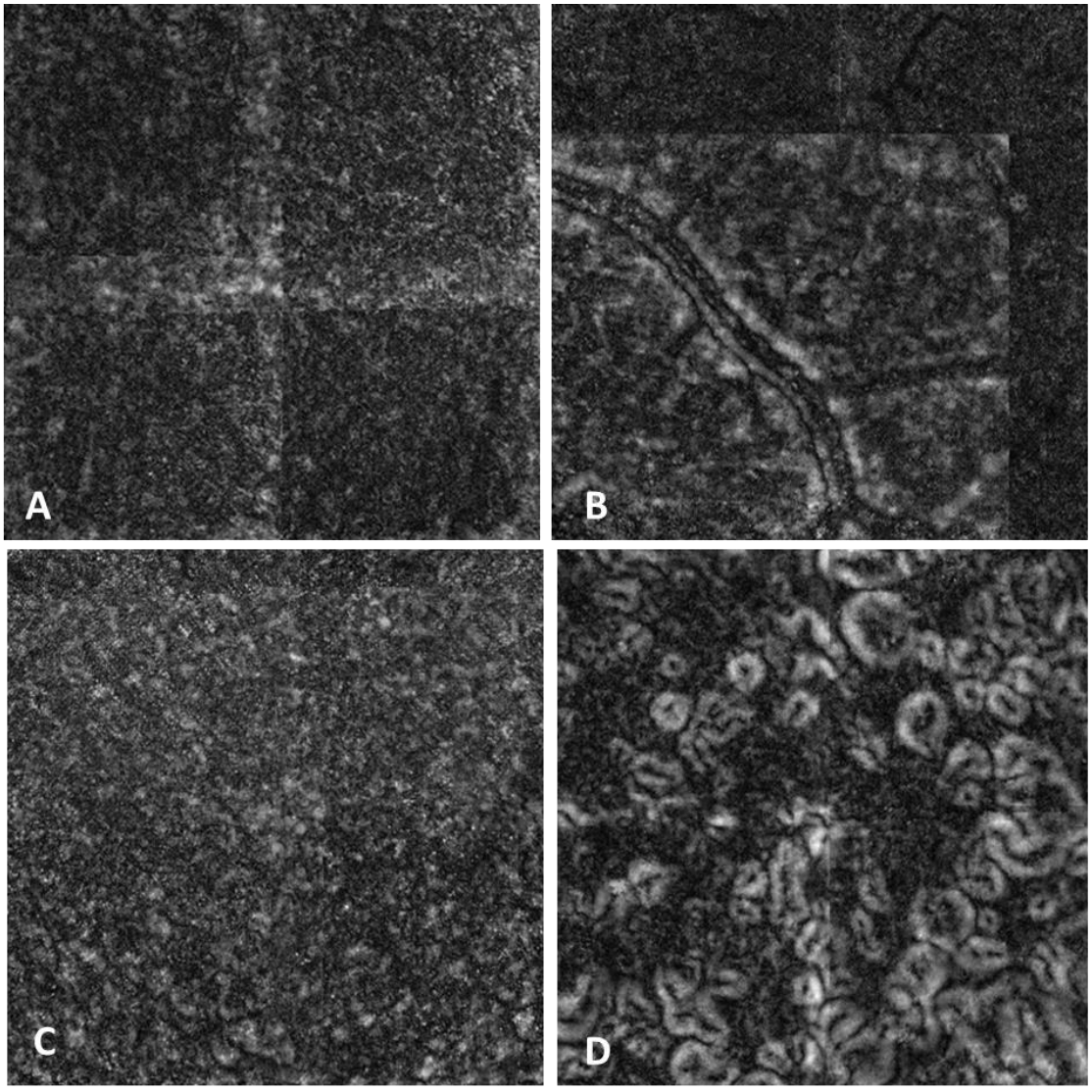



Fig 7 A) The most common artifact is represented by whitish aspect of all four borders of a 4° image. B) Misalignment of two images, it is evidente because of the presence of vessels C) In controls, there were not gaze-varying structures revealed. D) In patients, drusen as small as 30 μm were detected with high contrast.

In patients, drusen as small as 30 μm were detected with high contrast (see image). The smallest appeared as uniformly bright. Larger ones had bright margins with a dark center and the largest sometimes had additional bright structures within the dark center. Individual drusen were clearly delineated within densely packed drusen (Fig. 7d) (Rossi et al., 2019).

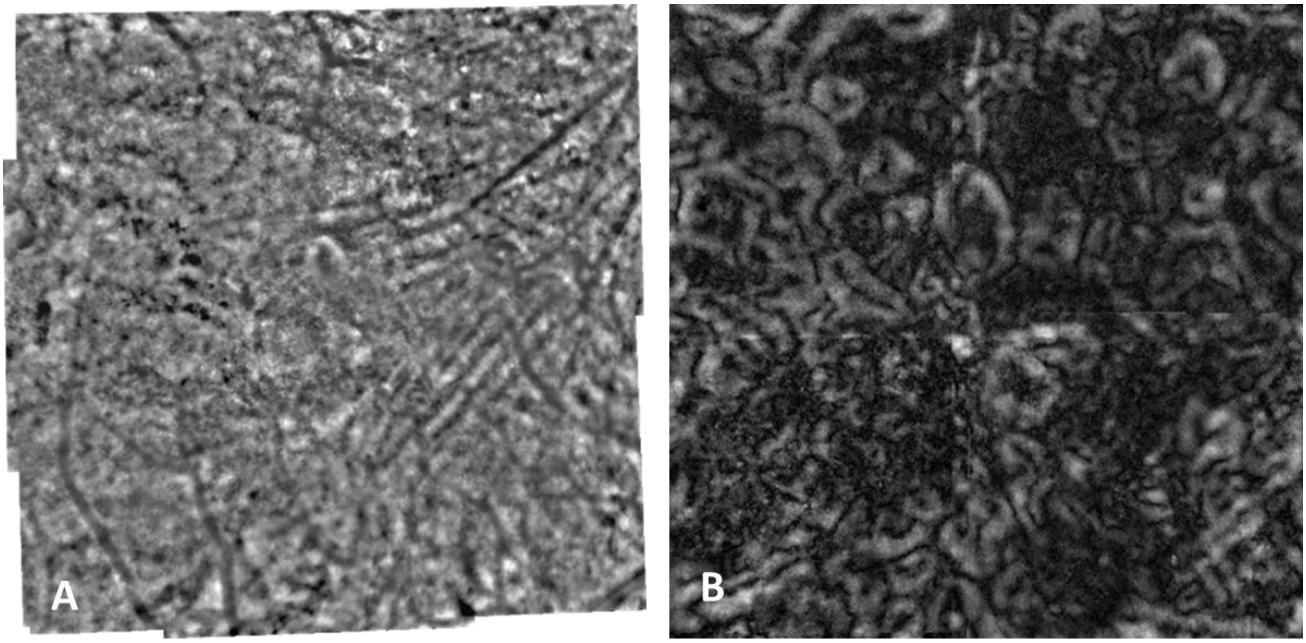
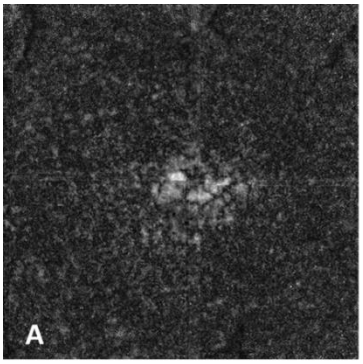


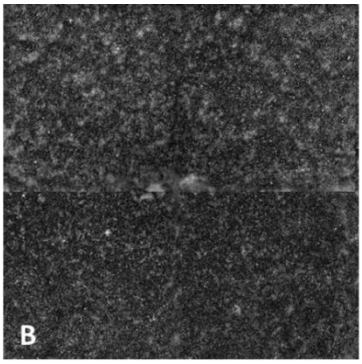

Fig. 8 After acquiring the 9 central images they are processed in order to construct a big last frame as a "puzzle". All of them were processed more tha one software and then compared :A) Montage was done with AO Detect B) Montage was done with the Drusen code in Matlab

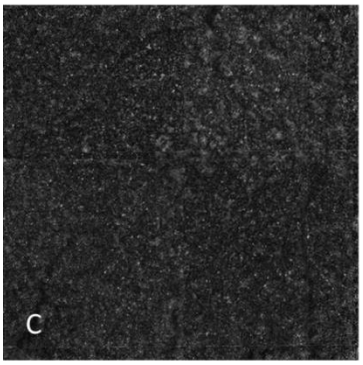
After carefully selecting the non artifacted images, they were clasified in two different groups:

- Normal fovea (Young and elderly) n=8
- Abnormal fovea (Young and elderly) n=8

The abnormal fovea looks like a whitish spot in the center of the image as shown below, where we can notice the presence of "abnormality" in young (Fig. 9) and elderly participants (Fig. 10)

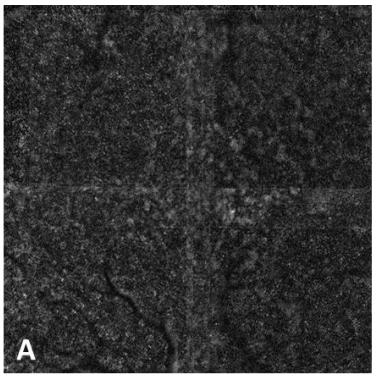


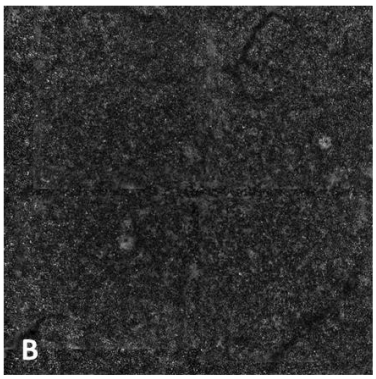



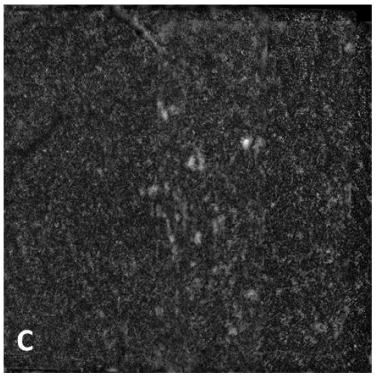




| D | Log CS Low luminance | Log CS High luminance |
|---|---|---|
| A | 1.132 | 1.377 |
| B | 1.248 | 1.697 |
| C | 1.190 | 1.765 |

Fig. 9 A) 22 year old participant B) 30 year old paricipant C) 31 year old participant D) In the table are shown the results of Contrast Sensitivity (CS)




| | Log CS Low luminance | Log CS High luminance |
|---|---|---|
| A | 1.190 | 1.736 |
| B | 0.793 | 1.387 |
| C | 0.715 | 1.833 |

Fig. 10 A) 61 year old participant B) 74 year old paricipant C) 69 year old participant D) In the table are shown the results of Contrast Sensitivity (CS)

| Subject | Eye | Age | Log CS - Low luminance |
|---|---|---|---|
| **YOUNG** | | | |
| COS | RE | 23 | 1.03485006 |
| LIT | RE | 29 | 1.12206455 |
| LEF | RE | 22 | 1.13175831 |
| HAG | LE | 31 | 1.18990465 |
| PAE | RE | 23 | 1.23197641 |
| BEM | RE | 30 | 1.23835778 |
| SID | LE | 30 | 1.24805149 |
| AGC | LE | 39 | 1.35464652 |
| | | | |
| **ELDERLY** | | | |
| BEC | LE | 69 | 0.71504617 |
| DEC | RE | 68 | 0.84102872 |
| PEA | RE | 72 | 0.85071929 |
| ALG | RE | 75 | 0.88310017 |
| ATM | RE | 70 | 0.92824683 |
| ATA | RE | 70 | 1.1447535 |
| ALA | RE | 70 | 1.16083003 |
| GAG | RE | 61 | 1.18990196 |

**Table 1. Results of contrast sensitivity in subjects with an artifact-free gasing light imaging. The difference in contrast sensitivity is significantly inferior in the elderly group ($p<0.05$).**

Due to the difficulty to quantify the foveal reflectance, we choose to evaluate qualitatively the grazing light images. In the following figure we ordered all images by decreasing contrast sensitivity; top left is the case with the highest contrast sensitivity, and bottom right the case with the lowest contrast sensitivity. Although there seem to be a few outliers, there is a general increase of foveal reflectance from top to bottom, suggesting that contrast sensitivity is inversely correlated with the degree of foveal abnormality.

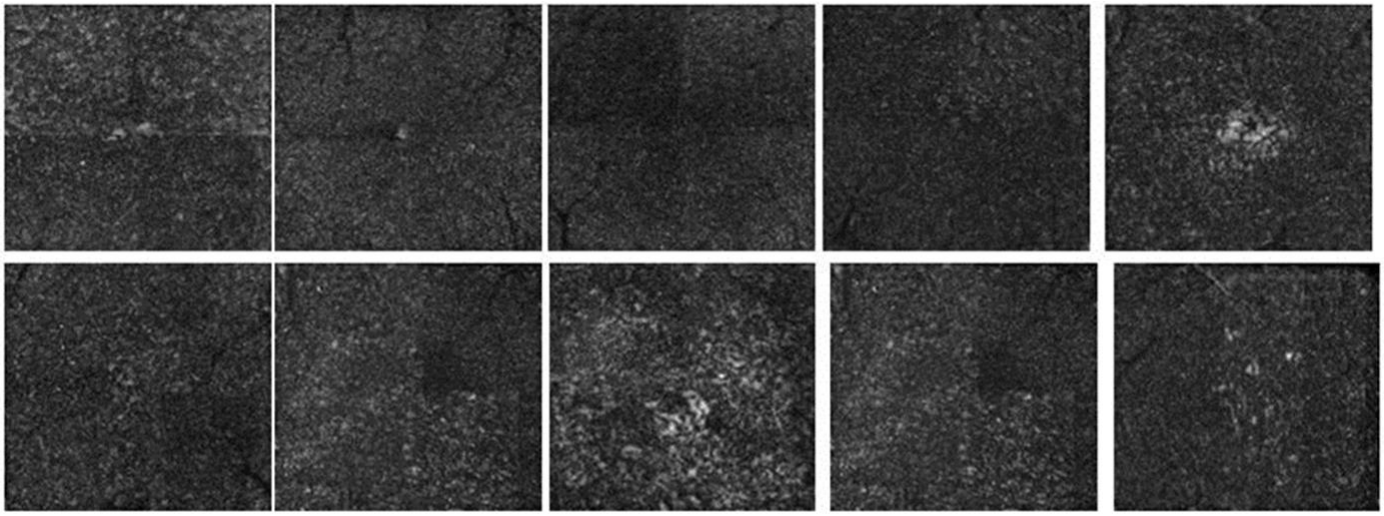


Fig. 11 Recapitulation of grazing light images of 10 subjects (after additional control of artifacts and check of the side of the eye). Images were ordered by decreasing contrast sensitivity;top left is the case with the highest contrast sensitivity, and bottom right the case with the lowest contrast sensitivity. The corresponding images are from the following patients (in that order): SID, BEM, PAE, HAG, GAG, ALA, LEF, COS, ATM, ALG, DEC, BEC

## Discussion

The goal of the present study was to correlate the presence of changes in light reflectance of the fovea assessed by grazing ligh timaging with photoreceptor function assessed by contrast sensitivity. We have been developing recently an image processing protocol that may reveal very fine changes in the foveal structures that may be present in the absence of detectable abnormality by OCT, yet of unknown origin at present. In order to determine if such changes were correlated to visual function, we investigated if contrast sensitivity was correlated to such changes evidenced by our “grazing light” protocol. We found that within a group of 16 subjects, there was indeed a trend toward a more pathological fóvea, although the correlation appears rather weak.

Limits of the study are primarily the small number of subjects. Another limitation was the frequency of artifacts, the cause of which are still under investigation. Finally, we could not establish a quantitative appreciation of the foveal abnormality. Nevertheless, the techniques developed here appears promising to identify the cause of the age-decline in visual function given the technique be refined and more subjects be examined.

## Conclusion

Identifying the cause of the age-related decline in central visual function is still underway, but we may have identify a novel biomarkers of age-related visual loss. Although they may be multiple combining factors and actors, the interation of photoreceptors plays probably a significant role. Due to the fact that most cases of central visual loss do show some degree of morphological alteration, it is legitímate to pursue the goal of identifying pathological images related to the loss of CS in elderly patients. The next step will be to refine our current protocol for grazing light imaging together with high-resolution imaging.

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