Protocol cover sheet

Wearable Dark-adaptometer in Normal Adult Healthy Volunteers

NCT number NCT02674425

Version 2.0 27/01/2017

WONDER STUDY
Evaluation of a novel wearable light-emitting system for measuring dark-adaptation thresholds in normal adult healthy volunteers

STUDY PROTOCOL

Chief Investigator: Rachel Williams
Principal Investigator: Alessandro Giuliano
Co-Investigator: Simon P. Harding
Collaborator: Noelia V. Pitrelli

Clinical Eye Research Centre, St Paul's Eye Unit, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP, UK
University of Liverpool, Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, William Duncan Building, 6 West Derby Street, Liverpool, L7 8TX

Contents

1. Project summary

2. Background

3. Aims

4. Methods
4.1. Study design
4.2. Setting
4.3. Investigational system
4.4. Participants
4.5. Inclusion criteria
4.6. Exclusion criteria
4.7. Procedures
4.8. Detailed description of investigations
4.9. Study schedule
4.10. Recruitment
4.11. Primary outcomes
4.12. Secondary outcomes
4.13. Data collection/Project management
4.14. Data analysis
4.15. Adverse event management
4.16. Study personnel

5. References

6. Dissemination

1. Project Summary

Aim:

- Assess the dark-adaptometry testing performance of a novel wearable light-emitting system by generating full dark-adaptation threshold functions in normal adult healthy volunteers

The study intends to answer the following research questions:

- Is it possible to reproduce state-of-the-art threshold measurements as good or better than those produced by commercially-available dark-adaptometers and reported in the literature?
- Do the threshold measurements in the elderly compare with literature data adjusted to exclude aged crystalline lens and pupillary miosis contributions?
- Are test results from the wearable light-emitting system repeatable?
- If the results are not repeatable, can any assumption be made about reducing their variability through software and/or hardware optimisation of the novel light-emitting system?
- Is the novel light-emitting system comfortable and easy to use by normal healthy adults despite age differences?

Study design:

- Single-centre, observational healthy case-only study
- 20 healthy volunteers

Study investigations:

- General wellbeing
- Retinal structure, visual and behavioural test series
    - Refractive error
    - Distance Visual Acuity (DVA)
    - SKILL card
    - Contrast sensitivity
    - Pupillometry
    - Confrontational visual fields
    - Direct ophthalmoscopy
    - Psychomotor capability
- System performance
    - Dark-adaptometry testing
- System usability
    - Ad hoc pen-and-paper questionnaire

Results of the study will be publicised by papers submitted to international peer-reviewed journals and proceedings of international conferences.

2. Background

Human sensitivity to visible light is altered by the retina based on the surrounding illumination and varies with the state of adaptation of the observer. The eye becomes accustomed to a light brighter than the surrounding illumination it is exposed to through a process called light adaptation [1-2]. As the surrounding illumination decreases, the eye becomes accustomed to the lower light level through a process called dark adaptation [2-3]. Between the two extremes there is an infinite number of adaptation or threshold states [4].

The threshold luminance refers to the smallest difference between two light levels that can be distinguished by a subject. The threshold luminance is inversely proportional to the eyes' sensitivity: as the sensitivity of the retina increases the threshold decreases (i.e., a lower

luminance evokes a response); vice versa, as sensitivity decreases a brighter light is required to evoke a visual response in a subject and the threshold is raised. Thus, large changes in the ambient light level are required for the light-adapted eye to perceive any change; in contrast, the dark-adapted eye is extremely sensitive to light perceiving very small variations in the luminance [5-6].

In 1865, Aubert [7] first described the dark-adaptation process as an observable increase in sensitivity of the visual system to light when the ambient light level decreases. It is a common experience initially to find it difficult to see anything when entering a dimly light room from the sunny outdoors conditions. Indeed, the visual function restores gradually [2, 8] and the human eye safely adapts to extremes of bright and dark lighting conditions spanning a range of about $1x10^{10}$:1 [9].

Dark adaptation primarily depends upon four processes: changes in pupil size, changes in the neural network, photochemical changes of the visual pigments, and changes in the calcium and sodium channels present in the photoreceptors [4, 8]. Certain eye diseases, inherited abnormalities, metabolic disorders and nutritional deficiencies may be identified by changes in dark-adaptation thresholds as determined by investigation of the dark-adaptation curve (i.e., repeated measurements of light threshold as a function of time). Dark adaptometers are instruments used to evaluate dark adaptation and are important in the diagnosis, prognosis and monitoring of identified symptoms including night blindness, retinal dystrophies, and hypovitaminosis A.

Measurement of dark adaptation typically involves the presentation of a light stimulus, which may comprise an ascending or descending threshold [10] (the staircase threshold method providing more accurate measurements). Tested subjects are asked to indicate when the stimulus appears or alternatively disappears [1, 11]. The time taken for the stimulus to become visible to the observer [12-13] is recorded. During a dark-adaptometry test the stimulus is always presented against a completely dark background field with the subject in complete darkness or blackout. At the start of the test the threshold luminance is initially high and decreases as one dark adapts.

The dark-adaptation curve is obtained by plotting a graph of each measured light threshold against the time [1, 14]. Each plotted threshold represents the time it takes the subject to perceive the light stimulus. A typical dark-adaptation curve is presented in Figure 1.

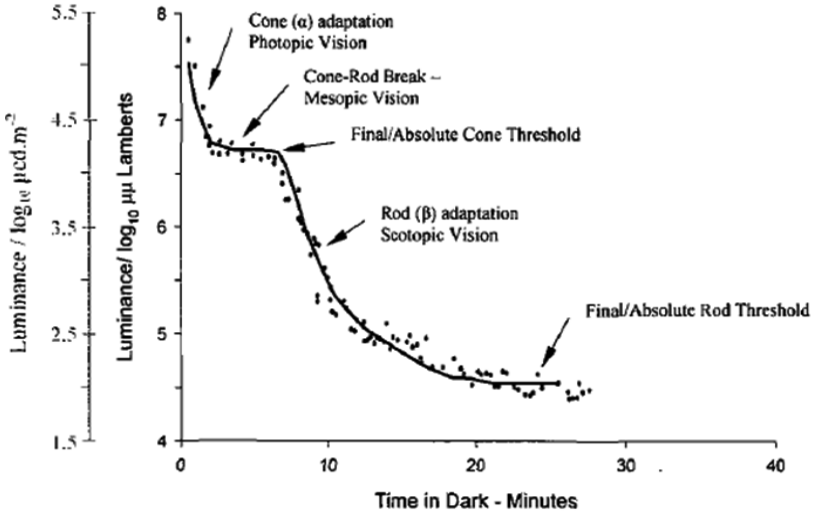

Figure 1. Normal dark-adaptation curve obtained using the Goldmann-Weekers adaptometer as a function of time. The discrete points are the recorded thresholds, whereas the best fit line identifies the dark-adaptation curve. The pre-adaptation light duration was 2 minutes at 1500 mL. (Figure reprinted from [15])

The typical range from highest threshold luminance to absolute rod threshold covers about 5 logarithm units of luminance [16]. The first part of the curve is the photopic phase, which identifies the cone adaptation. The last part of the curve is the scotopic phase, which identifies the rod adaptation [11]. The interval between the two phases is commonly referred to as the rod-cone break. It can be seen in Figure 1 that, as the luminance is reduced, there is an increase in light sensitivity, which is initially very rapid before levelling off after 5 to 10 minutes at the final or absolute cone threshold [5]. At this point, the foveal cone cells are fully dark adapted (i.e., they have reached their maximum sensitivity [17]); in contrast, the rod cells are saturated and inactive [l]. With time (the luminance of the stimulus is reduced) a second slower fall in the threshold luminance is observed as the peripheral rod cells become activated (rhodopsin is regenerated). Rod adaptation is slower than the cone adaptation [18] because of the regeneration half-life of the visual pigments. However, unlike cone cells, light of very low intensity is capable of stimulating the rod cells, when given sufficient time to accumulate rhodopsin [2]. After approximately 30-40 minutes in total darkness, the final rod threshold is achieved. Dark adaptation is now complete and no further increase in sensitivity of the rods photoreceptors is possible.

The time required to reach maximum sensitivity is dependent on the amount of pigment bleached by the previous light exposure [19] and neural adaptation. The wavelength of the incident light has also a strong influence on the dark-adaptation curve, given that cone mediated vision is responsible for colour vision whereas rod vision is colourless.

Both cone and rod dark-adaptation curves consist of an initial rapid nonlinear increase in sensitivity (i.e., the S1 component of recovery) followed by a second longer section (i.e., the S2 component of recovery) of near linear slope and a third section (i.e., the S3 component of recovery) where the curve asymptotically reaches the final cone and rod threshold, respectively [18]. The first section of the cone phase curve may be explained by the rapid increase in pupillary diameter [8, 20]. The near linear middle sections are determined by the regeneration of bleached visual pigments in the outer segment discs of the cone and rods [21]. The difference in the decay curve of both cone and rod mediated vision may be explained by different regeneration half-lives of the rod ($t_{1/2}$ = 5 minutes) and cone ($t_{1/2}$ = 90 seconds) cells thus cone pigments are regenerated at a faster rate than rhodopsin.

Despite the use of more objective modern instrumentation [22-23], the subjective determination of human dark adaptation has provided important information on the structure and functioning of the retina and continues to be a useful investigative tool.

Most dark adaptometers are based on the Hecht-Schlaer adaptometer developed in the 1938 [19], which is considered the first reliable instrument allowing for an accurate investigation of the various aspects of dark adaptation after previous attempts carried out by use of photometer devices [8, 15, 24]. Variation of the light stimulus was obtained by Hecht and Schlaer upon the insertion of several neutral density filters in front of a tungsten light source. The test field subtended 3° and was presented 7° nasally to the fixation light. The fixation intensity was adjustable and was maintained so as to be just visible by the subject throughout the test. Dark adaptation data obtained from this device under controlled conditions were accurate, reliable and reproducible. Many subsequent instruments are essentially modifications and improvements on this device [25-28]. A notable device was constructed by Wald [29] in 1941 and used to survey vitamin A deficiency in Newfoundland, but with limited success. A novel landmark, low cost instrument was developed by Henson and Allen [30] in 1977 consisting of a single stimulus of a green (565 nm) solid-state light-emitting diodes (LEDs) as opposed to tungsten filaments used in earlier devices. The stimulus intensity control setting was via a manual position switch that corresponded to 12 discrete luminance light levels that were approximately half (or a quarter) the previous presentation luminance. The fixation distance was 33 cm. Electronic circuitry controlled the LED light output to a quoted accuracy of 0.1%, which could be maintained over ten years of continuous use. A potential deficiency with this

instrument was that the test was self-administered/controlled leading to possible significant bias in the test results. In addition, it lacked a chin rest, thus the distance between the stimulus and the subject appears not to have been fixed; consequently, there is an uncertainty in the exact stimulus size and eccentricity to the fovea.

Particularly important is the Goldmann-Weekers adaptometer (Haag-Streit AG) [31]: an expensive, highly sophisticated and versatile instrument that has attained the status of the bench mark instrument by which other instruments past and present are compared and evaluated [32-37]. The instrument is still in use today but not commercially available any longer. It comprises an integrating sphere required for light adaptation and a chin rest that fixes the viewing distance. With the subject's head resting on the chin rest a single circular 11° target stimulus is located centrally and directly in front of the subject's eyes. A red fixation light is traditionally presented at 11°-15° to the fovea [6]. Newer models of Goldmann-Weekers adaptometer unit are available with a movable fixation light thus enabling examination of the full retina. The recording of the threshold luminance values is made by the operator activating a lever on a recording arm connected to an indicating "pricker" that perforates a logarithmic paper chart, which is mounted on a recording drum rotating once per hour.

Time is plotted on the abscissa in minutes while the luminance is plotted on the ordinate. Changes in stimulus luminance of both the target and fixation luminance are manually achieved. Up to 100 thresholds measurements are made until the threshold stabilises, typically after 30-40 minutes; the test is then terminated. The paper chart is removed and the best fit curve subjectively drawn by the operator through the series of perforations thus yielding the adaptation curve. A skilled and experienced operator must carry out the test in the dark along with the subject under examination throughout the test. A full description of the testing methodology is described by Russell et al. [10].

Field analysers may also be used to obtain dark adaptation measurements [38]. For example, a modified Friedmann field analyser described by Bedwell [39] has been used extensively to measure dark adaptation [40]. Neugebauer and Vemon [41] developed a dark adaptometer based on the Lister perimeter. This instrument was successfully used by Rayner and Tyrrell [40] to investigate nyctalopia in cystic fibrosis patients. The disadvantage of using field screeners and perimeters is that the instrumentation is not specifically designed for dark adaptation measurements; consequently they are of limited use compared to dedicated instrumentation such as the Goldmann-Weekers device.

However, there are significant disadvantages and limitations inherent in currently used dark adaptometers including:

1) the use of old and out dated technology;
2) most adaptometers are elaborate, large, heavy, non-portable and expensive;
3) test procedures require full attention of highly trained operators in a clinical environment;
4) electrical supply is provided by mains, thus making them immobile and unsuitable for field investigations;
5) the operator must carry out the test in total darkness with the subject under test;
6) test data acquisition is manually obtained by the operator;
7) data analysis is complex requiring additional operator time after the test has terminated;
8) frequently, only one area of the retina may be tested at any one time;
9) there is a test learning curve leading to the possibility of biased results.

The need for an entirely dark room is also a physical limitation and an economic burden for the use of most of the modern portable-adaptometer instruments such as the scotopic sensitivity tester-1 (SST-1) (LKC Technologies) and the AdaptDx (MacuLogix). The SST-1 instrument entered the market as an efficient device for quickly identifying night-blindness in patients with retinal degeneration. However, several drawbacks have been identified when testing with the SST-1 including: the narrow range of measurable intensities (3.0 logarithmic units); the too dim luminance at its maximum intensity in order to evaluate subjects who had severe scotopic

sensitivity losses (floor effect); the too bright luminance at its minimum intensity in order to evaluate thresholds in subjects with good scotopic sensitivity (ceiling effect); the non-adequate initial adapting light for photopigment bleaching. In addition to standard dark-adaptometry testing, the AdaptDx is claimed to allow for early AMD diagnosis but this use is to date restricted to research purposes only.

For the reason listed above, conventional dark-adaptometers are unsuitable as a mass screening tool; consequently, dark adaptation is not routinely used as a clinical technique because of the practical problems it presents [33, 39-42].
In order to overcome the associated problems with current instrumentation described above, the use of a novel, wearable and semi-automated dark-adaptometer is proposed. This is implemented as a pair of head-mounted googles and a handheld joystick, and it is easy to use without the need of any skilled operator. The novel dark-adaptometer is very versatile, uses low-cost solid-state electronics, and features low-power consumption, which allows for battery-powered operation. For a more comfortable user experience, the system is light and characterised by durable enclosures that totally black-out the inside of the goggles, hence avoid the need of a dark room to carry out dark-adaptometry testing.
The portability, ease of use, and relatively low cost of this system aims to spread the practice of dark-adaptometry testing and let it be adopted also by high-street optometrists. This will allow diagnosing a number of retinal pathologies more quickly and more reliably eventually bypassing the use of other visual electrophysiology equipment such as the ERG system: a more expensive instrument characterised by a larger form factor and a range of features that may be surplus to optometrists' requirements. Receiving a quick diagnosis may lead to starting a treatment sooner; faced with an ageing population, this represents a major asset to the Health Community and the NHS.
The wearable light-emitting system as described can represent a viable diagnostic tool for measuring dark-adaptation thresholds with proven good reproducibility and precision of the test data when compared to conventional instrumentation. Since the intensity, duration, colour, area and location of the light used for the fixation, bleach, and target stimuli all have an influence on the measured thresholds [8, 19, 44], the modified 3-down-1-up staircase procedure carried out in [43] will be adopted here for comparison purposes with data results reported in the literature.

3. Aim

The aim of this study is to assess dark-adaptometry testing performance of a novel wearable light-emitting system by generating full dark adaptation threshold functions in normal adult healthy volunteers.

The study intends to answer the following research questions:

I. Is it possible to reproduce state-of-the-art threshold measurements as good or better than those produced by commercially-available dark-adaptometers and reported in the literature?
II. Do the threshold measurements in the elderly compare with literature data adjusted to exclude aged crystalline lens and pupillary miosis contributions?
III. Are test results from the novel light-emitting system repeatable?
IV. If the results are not repeatable, can any assumption be made about reducing their variability through software and/or hardware optimisation of the novel light-emitting system?

V. Is the novel light-emitting system comfortable and easy to use by normal healthy adults despite age differences?

4. Method

4.1. Study design

Single-centre, observational healthy case-only study.

4.2. Setting

The research will take place at the Clinical Eye Research Centre (CERC) of St. Paul's Eye Unit at the Royal Liverpool University Hospital, Liverpool (UK).

4.3. Investigational system

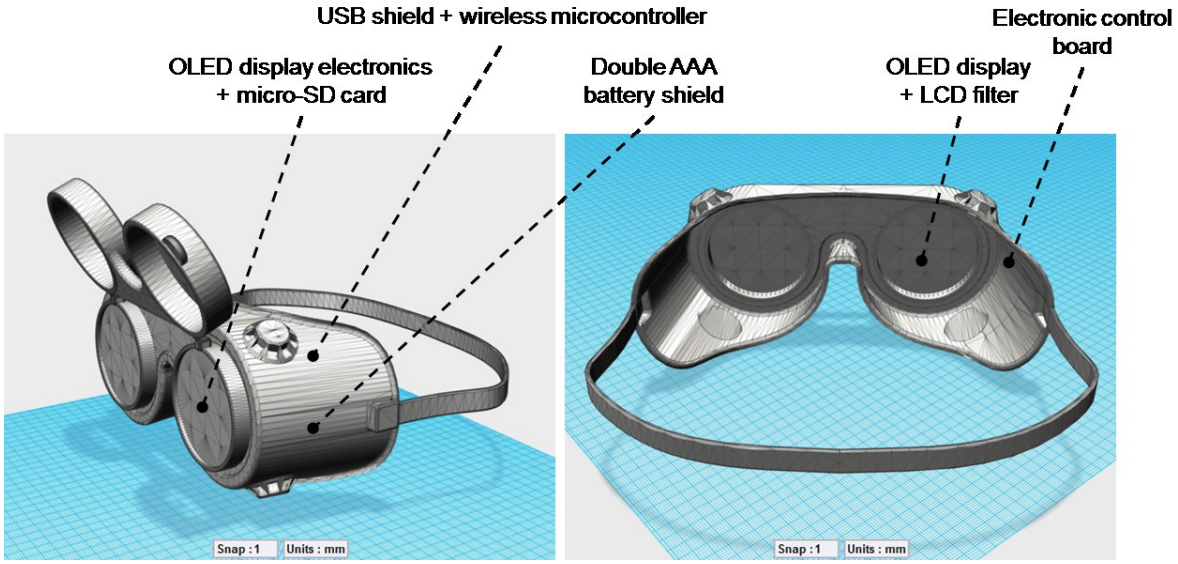

Figure 3. 3-D model of the head-mounted goggles with parts

The novel dark-adaptometer is designed as a wearable and semi-automated system consisting of a pair of head-mounted goggles (Figure 3) and a handheld joystick. Within each side of the goggles are included: an organic light emitting diode (OLED) display, which delivers the light stimuli; and a LCD filter sitting in front of the display to precisely tune the light stimuli within a range between 10^{-6} to 10 cd/m^2. A handheld joystick completes the system allowing the subject under test to wireless transmit its response to the visible light stimuli through the press of a button. The response is cached and interpreted by the computing unit inside the goggles, which automatically set light intensity and time interval of the successive stimuli based on a modified 3-down-1-up staircase procedure described as in [43] and schematically depicted in Figure 4.

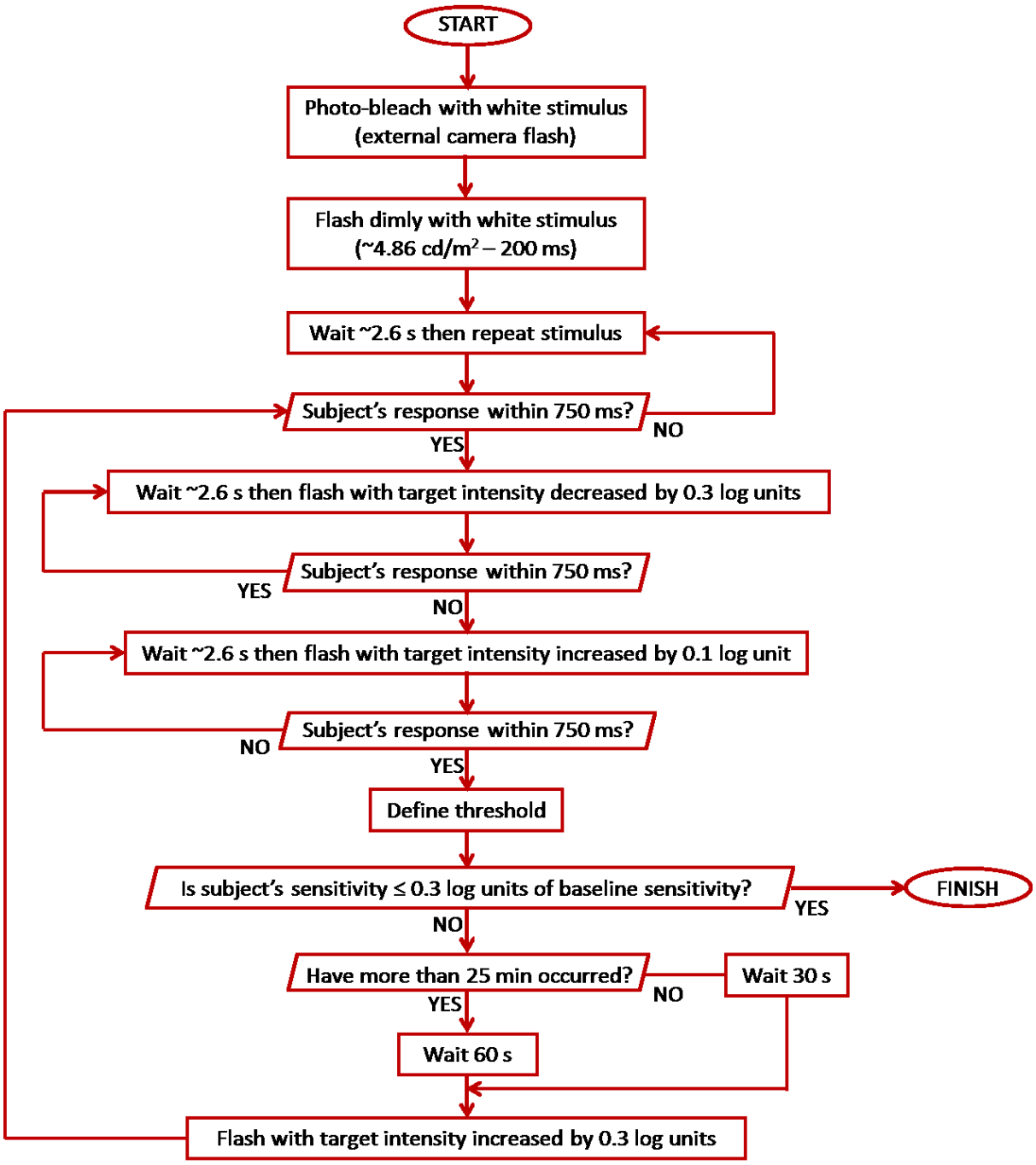

Figure 4. Flow-chart of the implemented dark-adaptometry testing procedure

Wearable goggles and joystick are given to participants in their switched-on status and hold by the subject under test respectively on the forehead, with an elastic strap, and on the dominant hand. In order to reset the photoreceptors of the retina, thus guarantee a similar non-dark-adapted background common to all participants, a photo-bleaching flash is presented through an external camera and channelled to the study eye at the start of the test. Immediately after the onset of the photo-bleaching flash, the goggles are pulled down over the eyes by the user that will now be exposed to white light stimuli of 200 milliseconds duration. Light stimuli are automatically emitted for the threshold measurements and will stop when the absolute threshold is measured, which in normal healthy adults typically occurs after 25-35 minutes from start.

Such a system implementation aims to be easy to use and operate avoiding the use of any skilled operator. In addition, the system requires minimal user training. Data are digitally stored

as a .csv file into a preselected memory location of a USB-connected PC and real-time plotted through an ad hoc graphical user interface. The novel light-emitting system is very versatile and uses low-power and low-cost solid-state electronics. Power is independently supplied via USB to each side of the goggles, thus enabling dark-adaptometry testing independently on each eye, and by mean of a single AAA rechargeable battery to the handheld joystick.

4.4. Participants

Healthy adult volunteers with no prior or current eye problems and good general health will be recruited from University of Liverpool students and staff, Royal Liverpool University Hospital students, and people accompanying patients attending St. Paul's Eye Unit.

Two age-groups of subjects will be enrolled:
Group 1: 10 people aged 18 to 40
Group 2: 10 people aged 50 to 70

4.5. Inclusion criteria

Group 1 & 2
Healthy adult volunteers with no prior/current eye problems or family history of genetic eye diseases and good general health.

1. Refractive error: $\leq \pm 5$ dioptres spherical (myopia/hyperopia) equivalent; ≤ 3 dioptres cylinder (astigmatism) equivalent.

4.6. Exclusion criteria

Group 1 & 2
1. Subjects not in age range and/or unable to fully understand the informed consent and/or unable to comply with study procedures.
2. Self-reported history of depression, lack of sleep, psychiatric disorders, or neurological diseases such as Alzheimer's and Parkinson's disease.
3. Self-reported history of diabetes, epilepsia, stroke, or multiple sclerosis.
4. Self-reported hypovitaminosis A, alcoholism, liver or intestinal disease, malabsorption, protein calorie malnutrition, or sickle cell anaemia.
5. Use of psychoactive drugs (including lithium salts for mood stabilisation).
6. Use of dietary intake of ascorbic acid, vitamin A, B, E or other antioxidant supplements in the last two weeks.
7. Recurrent practice of activities that expose the retina to ultra-violet radiation such as sailing, fishing, sunbathing or tanning saloons.

4.7. Procedures

Subjects included in the study will undergo the following investigations:
1. Detailed personal medical history (age of participant, ethnicity, smoking, diet, etc.) and family history of eye diseases (15 minutes)
2. Retinal structure, visual and behavioural test series (45 minutes)

- Refractive error
- Distance Visual Acuity (DVA)
- SKILL card
- Contrast sensitivity
- Pupillometry
- Confrontational visual fields
- Direct ophthalmoscopy
- Psychomotor capability

3. System performance
   - Dark-adaptometry testing (45 minutes)
4. System usability (15 minutes)
   - Ad hoc pen-and-paper questionnaire to assess comfort and ease of use of the novel dark-adaptometer

Subjects will have investigations (1-2) during the screening phase.
Retinal structure, visual and behavioural tests (3) will be carried out once at baseline.
Follow-up visits include dark-adaptometry testing (4), which will be carried out 3 times over 3 weeks. A minimum 1-day window is required after each testing phase.
System usability (4) will be assessed twice, respectively upon completion of the first and the last dark-adaptometry testing visit.

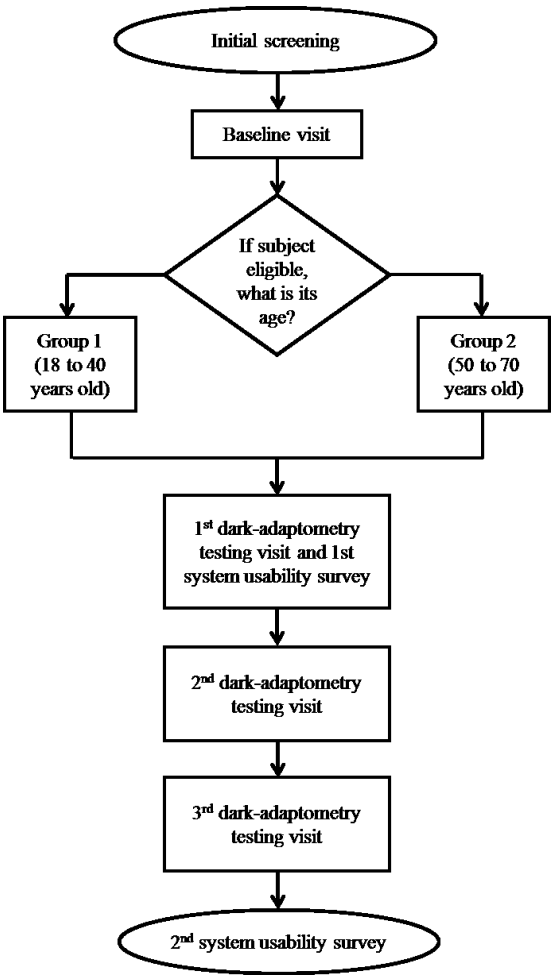

Figure 2. Flow-chart of the study design and steps

4.8 Detailed description of investigations

Refractive error

The refractive error of the study eye will be obtained by means of objective and subjective refraction techniques.

An initial estimation of the refractive error will be obtained by retinoscopy, an objective technique that uses a handheld device called retinoscope. The retinoscope projects a beam of light into the eye which is reflected at the back of the eye, producing the so called "retinoscopic reflex". The examiner will move the light of the retinoscope across the subject's eye assessing the movement (speed and direction) of the retinoscopic reflex. This movement will be neutralised using spherical and cylindrical lenses, which will represent an estimate of the refractive error. Immediately after retinoscopy subjective tests (duochrome and Jackson crossed cylinders) will be performed to determine the refractive correction which gives the subject the most comfortable vision.

Retinoscopy requires the subject to look at the examiner's light for a time period (approximately 5 minutes).

DVA

Distance visual acuity will be measured with glasses (i.e., best corrected) and without glasses (i.e., uncorrected) by using the Early Treatment Diabetic Retinopathy Study (ETDRS) 4 m charts. These charts contain 14 lines of 5 letters each, meaning that the space between the letters and the between lines decreases proportionally to the size of the letters. For the purpose of this study 2 ETDRS charts (right and left eye) will be used, placed over a standardised illuminated box (62.9 x 65.4 x 17.8 cm) to guarantee consistent illumination. VA will be measured monocularly (right eye always first) with the chart positioned 4 m away. The chart will be moved to 1 m if the subject cannot see the first 20 letters (4 lines). Letters correctly identified will be counted, adding 30 letters in those cases where the chart was not moved to 1 m. Results will be recorded as the number or letters read correctly as well as a logMAR score. If no letters are correctly identified at a distance of 1 m the examiner will record vision as "counting fingers", "hand movement", "light perception" or "no light perception".

SKILL card

The Smith-Kettlewell Institute Low Luminance (SKILL) card will be used to measure spatial vision under conditions of reduced contrast and luminance. The SKILL card consists of two near acuity charts mounted back to back. One side of the card contains black letters on a dark grey background (designed to simulate reduced contrast and luminance conditions) whilst the other side has a high-contrast, black-on-white letter chart. Using the appropriate near correction each card will be successively presented at a distance of 40 cm. The SKILL score will be recorded as the difference in performance (number of letters) between the two cards.

Contrast sensitivity

Contrast sensitivity will be measured using a Near Pelli-Robson Contrast Sensitivity Card. The card uses the traditional optotypes found in the original Pelli-Robson chart (i.e., 6 letters per line arranged in groups of 3 with a decreasing contrast) but features an overall size of 22.86 x 29.53 cm. Using the appropriate near correction, subjects will read the letters from a distance of 40 cm, starting with the highest contrast, until they are unable to read two or three letters in a single group. A score is assigned based on the contrast of the last group in which two or three letters were correctly identified.

Pupillometry

Pupil size will be measured manually (with a ruler) in the same room where the visual assessment was performed, under a known level of illumination.

Confrontational visual fields

To examine the visual field of the right eye the examiner will ask the subject to cover their left eye using the palm of their hand and instruct them to look at a pen located in from of them (held by the examiner). The examiner will use a slowly wagging finger to check the extent of the subject's visual field superiorly, inferiorly, nasally, temporally and on the diagonals. The subject must indicate when the examiner's finger comes into view. The same procedure is used to test the visual field of the left eye, covering the right eye.
This test will only pick up large visual field defects.

Direct ophthalmoscopy

Direct ophthalmoscopy is a non-invasive technique used to examine the posterior pole of the eye (fundus). A beam of light is shone into the eye through the pupil using an instrument called ophthalmoscope (a device about the size of a flashlight). Using an ophthalmoscope the examiner can detect pathological changes in the retina such as abnormal blood vessels or haemorrhages.

Psychomotor capability

Participants will undergo a brief psychomotor test by using the novel dark-adaptometer in order to confirm their ability to comply with the study procedure and be trained for the follow-up phase visits. An intermittent white light of 200 milliseconds duration and luminance starting at ~4.86 cd/m^2 is shone through the OLED display of the light-emitting goggles. Participants are asked to respond to the visible light stimuli with a button press on the handheld joystick of the novel dark-adaptometer. Luminance is decreased by 0.3 logarithmic units at each button press or increased by 0.1 logarithmic units at each missed button press. The test stops automatically when 3 dark-adaptation thresholds have been determined under the conditions described above. Participants must respond within a time interval of 750 milliseconds after each light onset in order to be considered capable to comply with the study.

Dark-adaptometry testing

Dark-adaptometry testing will be carried out by using the novel investigational system described in Section 4.3. Wearable goggles and joystick are given to participants in their switched-on status and hold by the subject under test respectively on the forehead, with an elastic strap, and on the dominant hand. For hygienic safety purposes, a non-allergenic disposable tissue will be placed on top of the inner gasket of the goggles that will be in contact with participants' face. Where a disposable tissue would not be available, the inner gasket of the goggles will be accurately cleaned with a sterile isopropanol solution. Participants will sit comfortably throughout the test and wear disposable earphones playing music to drown out external noise and any auditory signals from the embedded electronics when light stimuli are emitted. Testing is non-invasive and is semi-automatic in that the subject under test is required to collaborate actively during the procedure in order to start it and go through it. A responsible collaboration throughout the test will be repeatedly encouraged as testing results are strictly based on the subject's response. The examiner will start the test by triggering a white flash of light through a standard camera flash (e.g., Canon Speedlite 540EZ – Guide Number 54, 1.2 ms duration). The flash will be channelled to the study eye and aims to photo-bleach, hence reset the photoreceptors of the retina. Immediately after the onset of the photo-bleaching flash, the goggles are pulled down over the eyes by the user that will now be exposed to intermittent white light stimuli. Light stimuli are shone automatically inside the goggles after the photo-bleaching flash and until the end of the test. Dark-adaptometry testing will last for about 25-35

minutes and will involve one eye only. This will be randomly allocated by the examiner or will be the only eye meeting the inclusion criteria. As the brightness of the intermittent light stimuli changes throughout the test as well as the time at which they are presented, participants are asked to press either of the joystick's buttons to indicate when the light is visible. The intermittent light features an initial brightness of approximately 4.86 cd/m^2 and becomes dimmer and dimmer overall. The test lasts until participants will not be able to identify any new light stimuli.
The absolute threshold will be the last measured threshold and recorded as the dimmest light that the subject can detect when the rod sensitivity of the study eye is maximum. In a healthy adult, the absolute threshold is typically equal to a logarithmic light intensity around 10^{-6} cd/m^2 at a time of around 30 minutes from the start of the test.
The examiner will monitor in real-time the testing results on a laptop, which is USB-connected to the frame of the goggles from the same side of the study eye. A best fit of collected threshold measurements will be calculated; from this fit, a biphasic curve is expected to be observed.
The rod-cone break will be identified as the point of phase-change in the calculated curve, which coincides with the point when rods become more sensitive than cones. In a healthy adult, the rod-cone break is typically equal to a logarithmic light intensity around 10^{-3} cd/m^2 at a time of around 7 minutes from the start of the test.
The slope of the rod-mediated response will be also calculated from the best fit of the collected threshold measurements. Particularly, the slope of the (second) S2 and (third) S3 rod-mediated component of recovery will be identified: these coincide with a long section of near linear slope following the initial rapid nonlinear increase in rod sensitivity.

An ad hoc pen-and-paper system usability questionnaire will be presented to participants twice upon completion of the first and the last dark-adaptometry testing visit. Participants will rate comfort and ease of use of the system on a scale of 1-10 through questions targeting hardware and software implementation as well as testing duration and expectations.

4.9. Study schedule

Table 1 shows the schedule for each group of participants.

Table 1 Healthy adult volunteers schedule (Group 1 & 2)

Study Phase	Screening	Inclusion	Follow-Up Phase		
			Testing Phase		Testing Phase/ Exit
Visit	Screening	Baseline W0	W1-W3		
Min. window (in days) after each testing phase	N/A	0	N/A	1	1
Procedures					
Informed consent	X				
Medical history, family history and demographics	X				
Inclusion/Exclusion criteria	X	X			
Ocular History		X			
Refractive error		X			
DVA		X			
SKILL card		X			
Contrast sensitivity		X			
Pupillometry		X			
Confrontational visual fields		X			
Direct ophthalmoscopy		X			
Psychomotor capability		X			
Dark-adaptometry testing			X	X	X
Usability questionnaire			X		X

The end of the trial will be defined as the last dark-adaptometry testing visit of the last participant, followed by the collection of the 2nd system usability questionnaire.

4.10. Recruitment

Group 1 & 2
The PI will recruit healthy adult volunteers with no prior or current eye problems and good general health. They will be recruited from University of Liverpool students and staff, Royal Liverpool University Hospital students, and people accompanying patients attending St. Paul's Eye Unit.
Baseline examination takes place in general ophthalmology clinics of St. Paul's Eye Unit at Royal Liverpool University Hospital. Subjects deemed eligible for the study will be recruited after signing a detailed informed consent if they meet the inclusion criteria.

4.11. Primary outcomes

Primary outcome variables for Group 1&2:
- Rod-cone break
- Absolute threshold

4.12. Secondary outcomes

Secondary outcome variables for Group 1&2:

- Slope of the (second) S2 component of rod-mediated recovery
- Slope of the (third) S3 component of rod-mediated recovery
- Mean difference between corresponding threshold measurements, which are significant for shaping the dark-adaptation function, obtained at each dark-adaptometry visit;
- Score of the questionnaire used to assess participants' comfort and ease of use of the novel light-emitting system.

Based on the results, several correlations between primary and secondary outcomes will be investigated in each group.

4.13. Data collection/Project management

Anonymised data will be collected by the PI and stored in accordance with the Data Protection Act and data governance protocols of the University of Liverpool and Royal Liverpool University Hospital. A clinical database will be arranged by the PI and held for a period between 6-12 months into a locally installed electronic data recording system at the Clinical Eye Research Centre of St. Paul's Eye Unit, Royal Liverpool University Hospital.
A Project Steering Committee will meet at the beginning and at the end of the investigation. In addition to these meetings they will be issued with information about the real-time data collected by the PI when all recruited participants will have undergone at least one dark-adaptometry testing visit. The committee will evaluate system performance and variability of collected data based on the real-time plots recorded by the PI during the first dark-adaptometry visit. In the event that the assumptions underlying the sample size calculation are seen to be incorrect at the time of the interim analysis, they will have the option to advise further recruitment to the study.

Data variables:
Refractive error (-5÷0÷5 dioptres – spherical; 0÷3 – cylinder)
DVA (letters)
Low-luminance NVA (letters)
Contrast sensitivity (letters)
Pupil size (mm)
Visual field
Fundus

Data collected during the dark-adaptometry testing visits will be in the form of a “log threshold intensity vs time” plot together with a .csv file including a list of the data points.

4.14. Data analysis

Data analysis will be performed by the PI and reviewed by this study team members.

4.15 Adverse event reporting

All adverse events, whether related to the investigational system or not, will be recorded fully and all serious adverse events will be reported in accordance with regulation 16(10)(a) of the

Medical Devices Regulations 2002 (SI618) and Annex X of the Medical Devices Directive 93/42.
The PI will monitor the dark-adaptometry examination in real time and terminate it if a safety signal is identified and considered to be significant.

4.16. Study personnel

Medical staff	Prof Simon P Harding	Consultant Ophthalmologist
Scientists	Dr Alessandro Giuliano Prof Rachel Williams	Honorary Research Fellow Professor of Ophthalmic Bioengineering
Optometrist	Dr Noelia V Pitrelli	Research Officer

5. References

[1] Adler's Physiology of the Eye, (2002) edited by Kaufman, P. and Albert, A. Chapter 10. 10th Edition. Elsevier Health Sciences. St. Louis. Mosby Company.
[2] Ripps, H. (1982). Night blindness revisited: from man to molecules. Invest Ophthalmology Vis. Sci. 23: 582-609.
[3] Rosenfield M. and Logan, N. (2009). 2nd Edition Optometry: Science, Techniques and Clinical Management. Butterworth. Pp. 72-76.
[4] Schwartz, (2004) Visual Perception. 3rd Edition. Chapter 3. University Press Chicago. Pp. 23-60.
[5] Hecht, S. and Mandelbaum , J. (1938). Rod-cone dark adaptation and Vitamin A. Science, 88(2279): 219-221.
[6] Hecht, S. and Mandelbaum, J. (1939). The relation between vitamin A and dark adaptation. J.AM.A 112(19): 1910-16.
[7] Aubert, H. (1865). Physiologie der netzhaut (Breslau, E. Morgenstem). Pp. 394.
[8] National research council Washington, DC. 1987. Department of the Navy and school of aerospace medicine. Night vision: Current research and future directions - symposium proceedings (technical report 1985-1987).
[9] Sheard, C. (1944). J. Opt. Soc. Am. 34: 464.
[10] Russell, R.M., Smith, V.C., Multack, R. and Krill, A.E. (1973). Dark-adaptation of subclinical vitamin A deficiency and evaluation of therapy. The Lancet. 2: 1161-1164.
[11] Graham, C.H., Bartlett, N.R. et al. (1965). Vision and visual perception. Chapter 3. J. Wiley & Sons.
[12] Mote, F.A. (1955). Variability of measurements of human dark adaptation. J. Opt. Soc. Am., 45: 7-12.
[13] Morrison, S.A, Russell, R.M., Camey, E.A. and Oaks, E.V. (1978). Zinc deficiency: a cause of abnormal dark adaptation in cirrhotics. Am. J. Clin. Nutr. 31: 276-281.
[14] Rushton, W.A. (1963). Incremental threshold and dark adaptation. J. Opt. Soc. Am., 3: 104-109.
[15] Davson, H. (1980). Physiology of the eye. Chapter 8 (Photochemistry) Fourth edition. Churchill Livingstone.
[16] Graham, C.H., Bartlett, N.R. et al. (1965). Vision and visual perception. Chapter 2 (Eye illustration/Neural net) J. Wiley & Sons.
[17] Stabell, U., Stabell, B., Hisdal, B. and Nordby, K. (1990). Rod and Cone Dark Adaptation. Clin. Vision Sci., 5: 345-351.

[18] Hecht, S., and Shlaer, S. (1938). An adaptometer for measuring human dark adaptation. J. Optic. Soc. Am., 28: 269-275.
[19] Sloan, L.L. (1940). Instruments and technics for the clinical testing of light sense. IV. Size of pupil as a variable factor in the determination of the light minimum. Arch. Ophth., 24: 258-275.
[20] Graham, C.H., Bartlett, N.R. et al. (1965). Vision and visual perception. Chapter 8 (Adaptation) J. Wiley & Sons.
[21] Arden, G.B., Carter, R.M., Hogg. C.R. et al. (1983). Rod and cone activity in patients with dominantly inherited retinitis pigmentosa: comparisons between psychophysical and electroretinographic measurements. Br. J. Ophth., 67: 405-413.
[22] Underwood, B.A. (1990). Methods for assessment of vitamin A status. J. Nutr. 120, Suppl. 11: 1459-1463.
[23] Jeans, P.C. and Zentmire, Z. (1934). A clinical method for determining moderate degrees of vitamin A deficiency. J. Am. Med. Assoc., 102: 892.
[24] Jeans, P.C., Blanchard, E. and Zentmire, Z. (1937). Dark adaptation and vitamin A: A new photometric technic. J. Am. Med. Assoc., 108: 451.
[25] Domey, R.G. and McFarJand, R.A. (1961). Dark adaptation as a junction of age: individual predication. Amer. J. of Opht. 51: 1262-1268.
[26] Birren, J.E. and Shock, N.W. (1950). Age changes in rate and level of visual dark adaptation. J. App. Phy. 2: 407-411.
[27] Birren, J.E., Bick, M.W. and Fox, C. (1948). Age changes in the light threshold of the dark adapted eye. J. Gerontol., 3: 267-271.
[28] McFarland, R.A. and Fisher, M.B. (l955). Alterations in dark adaptation as a function of age. J. Gerontol., 10(4): 424-428.
[29] Wald, G. (1941). A portable visual adaptometer. J. Opt. Soc. Am., 31: 235-238.
[30] Henson, D.B. and Allen, M.J. (1977). A new type of dark adaptometer. Amer. J. Optometry and Physiological Optics, 54: 641-644.
[31] Haag-Streit. Adaptometer Goldmann/Weekers Handbook No. 518. Liebefeld-Berne
[32] Krill, A.E. and Klien, B.A. (1965). Flecked Retina Syndrome. Arch. Ophth., 74.
[33] Solomons, N. W., Russell, R.M., Vinton, E., Guerrero, A. and Mejia, L., (1982). Application of a rapid dark adaptation test in children. J. of Paed. Gastroen. Nutr., 1: 571-574.
[34] Kelsey, J.H. and Arden, G.B. (1971). Acquired unilateral loss of dark adaptation. Br. J. Opht., 55: 38-43.
[35] Thomton, S. (1977). A rapid test for dark adaptation. Ann. Opht., 9: 731-734.
[36] Baca, W., Fishman, G.A., Alexande, K.R. and Glenn, A.M. (1994). Dark adaptation in patients with Best vitelliform macular dystrophy. Br. J. Opht. 78(6): 430-432.
[37] Ernst. W., Faulker, C., Hogg, D., Powell, G. and Arden, G. (l983). An automated static perimeter/adaptometer using light emitting diodes. Brit. J. Opth., 67: 431-442.
[38] Bedwell, C.H. (1967). New instrumentation for visual field investigation: the visual field analyser. Ophthalmic Optician, 7: 566-568.
[39] Sommer, A., Hussaini, G., Muhilal, M.D., Tarwotjo, I., Susanto, D. and Saroso, M. (1980). History of night-blindness: a simple tool for Xerophthalmia screening. Am. J. Clio. Nutr., 33: 887-891.
[40] Ramos-Cortes, E., De Ocampo, G. and Villamora, E. (1973). PERI color test for dark adaptation and night blindness I. Normal: pre-school and early school age group. Philipp. J. Opht. 5: 1-7.
[41] Udomkesmalle, E. (1989) Assessment of marginal vitamin A deficiency in Thai school by impression cytology, dark adaptometry and serum retinols. (abs) XIII IVACG meeting, Kathmandu. Nepal, November 5-10.

[42] Vinton, N. and Russell, R. (1981). Evaluation of a rapid test of dark adaptation. Am. J. Clin. Nutr., 34: 1961-1966.
[43] Jackson, G.R., Owsley, C. and McGwin, G.J. (1999). Aging and dark adaptation. Vis. Res., 39: 3975-3982.
[44] Wigelsworth, A.E. (1941). Apparatus for determination of human dark adaptation. U.S. patent 2, 239, 164.

6. Dissemination

Results of the study will be publicised by mean of papers submitted to international peer-reviewed journals with high impact factor in the specialty of ophthalmology and proceedings of international conferences.