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Abstract

Pupil Size in Outdoor Environments

Stephanie A. King, MSPH, 2007

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Statement of the problem: This study was comprised of four research objectives: (i) determine the average pupil size in bright outdoor environments, (ii) determine the optimal method of pupillometry in bright outdoor environments, (iii) determine the limit of the smallest pupil size in bright outdoor environments, (iv) analyze methods of pupillometry in previous studies and determining the highest luminance levels measured in each.

Methods: The method of pupillometry used was digital photography with a fixed chin rest and camera platform and use of a Ganzfeld, a white screen which occupies the subjects' entire field of view. 86 subjects' pupils were photographed and then measured using computer imaging software. The range of luminance values were 789 $cd \cdot m^{-2}$ to 4253 $cd \cdot m^{-2}$.

Results: The average pupil size for this study was 2.39mm for an average luminance of 1473 $cd \cdot m^{-2}$. Pupil size measurements ranged from 1.44mm to 3.03mm. The data were stratified over luminance intervals, gender, eye color, and age.

Conclusions: A highly repeatable, precise, and accurate method of pupillometry in bright environments is digital photography with a fixed chin rest, camera mount, and Ganzfeld. Appropriate follow-on research includes measurements at higher luminance levels, pupil measurement while subjects wear protective lenses, researching infrared photography and retroreflection off the retina.

PUPIL SIZE IN OUTDOOR ENVIRONMENTS

by

Stephanie King

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Background

The primary purpose of the study was to determine the average pupil size in bright outdoor environments. Pupil size is a vital datum to determine risk and exposure assessment for ocular exposure hazards. Pupil size, the opening of the eye which allows light to illuminate the retina, determines the energy and radiant power delivered to the retina. Retinal health is an important component of the effectiveness on the battlefield and general health and well-being of military personnel. Military lasers and laser weapons utilizing radiation in the visible spectrum are becoming more prevalent, which brings retinal and ocular safety to the forefront. This has a direct bearing on the health and safety of military personnel in all Department of Defense service branches. Knowledge of the average pupil size in bright outdoor environments will assist in exposure assessment of the retina in bright ambient lighting, reevaluation of current laser safety standards, and risk assessment based on possible exposure as determined by the average pupil size.

In the late 1960's, the American National Standards Institute (ANSI) created a Laser Measurements and Hazards Evaluation Committee to create safety standards for laser risk and exposure hazards. A key component in their calculations included an average pupil size assumption to determine retinal exposures. After assessing the hazards associated with each pupil size from 2mm to 7mm, the 7-mm pupil size was determined to be the most conservative estimate for laser safety considerations.[1] A 2-mm pupil size corresponds to the size of the pupil in very bright conditions, and the 7-mm pupil size corresponds to a fully dark-adapted pupil diameter. The larger pupil allows more light to reach the retina. ANSI currently uses a 7-mm pupil size assumption in their calculations and estimates for laser safety standards.[2] In determining exposure estimates, ANSI uses irradiance at the corneal plane, or total laser power per unit area on the retina. ANSI assumes the most conservative estimate of a 7-mm diameter for a fully dilated pupil for pulsed and infrared lasers. All of the power will be delivered to the retina for a beam which enters the eye with a 7-mm pupil diameter. In the case of bright, outdoor environments, which the pupil diameter is less than 7mm, only a fraction of the power that could otherwise enter a 7-mm pupil will be delivered as a result of shielding by the iris.

Efforts to determine the average pupil size in bright environments, especially outdoors, were not of general interest as the most conservative estimate would protect laser operators in all conditions. Nonetheless, the importance of determining the average pupil size at the highest levels of luminance is still critical to determine the hazards associated with outdoor occupations where laser systems may be employed, particularly in a battlefield environment. Ocular injury from a laser may be irreversible or irreparable, thus threatening the health of military personnel which adversely affects force readiness and effectiveness. Currently the United States military uses the ANSI laser safety standards as their primary reference for their safety standards.

The 7-mm ANSI assumption may greatly over-estimate the risk of a laser-induced retinal injury in a daytime military setting. The 7-mm pupil diameter assumption is a realistic assumption for laser and other non-lethal weapon systems employed in nighttime conditions. Although the effectiveness of some laser systems may seem to be diminished in bright outdoor environments, particularly those operating in the visible spectrum, an inherent risk is still associated with these weapon systems. Nevertheless, the smaller pupil size in bright environments creates less risk of exposure to the individual from the military laser system.

This study examines the controversy surrounding the internationally recognized 7-mm pupil size present in the ANSI standards, the military safety standards with regard to a smaller pupil size, and a more accurate and relevant measurement of pupil size in outdoor environments with bright luminance.

Introduction

Research Questions

The purpose of the study was to determine the range of pupil size and the average pupil size in bright, natural outdoor environments. The second objective was to determine the best way to measure pupils in bright outdoor environments while maintaining accurately measured luminance levels. A third objective was to determine the highest levels of luminance in the classic pupillometry studies and examine the advantages and disadvantages of each methodology. The final objective was to determine the smallest size of pupil size in bright outdoor environments.

The objective of this study was to measure the pupil sizes in a range of out-of-door field conditions and to examine the range of pupil sizes for the same ambient condition. This is of interest to the military medical community to provide a baseline database for assessment of retinal hazards from military lasers in the field environment. Military personnel were recruited who were engaged in outdoor activities and training.

American National Standard for Safe Use of Lasers recommends a 7-mm aperture for measurements, but based exposure limits upon a 3-mm pupil for continuous exposures.[3] The Accessible Emission Limit (AEL) for a Class 2 laser was based upon the Maximum Permissible Exposure (MPE) for a 0.25 second (aversion response) exposure. ANSI classified lasers according to wavelength, power output, ability to due damage to tissue either directly or if the beam is scattered. The four classes are Class 1, Class 2, Class 3 and Class 4, with two sub-classes of 1M and 2M which refer to classifications based on optical magnification used in conjunction with the laser. The increasing number of each class corresponds to the increased risk for injury if the energy of the beam is absorbed by tissue. The Class 2 AEL is 1 mW, based upon the power entering a 7-mm aperture for an irradiance level of 2.5 $mW \cdot cm^{-2}$. This irradiance value is the MPE for a 0.25-second exposure, which is the approximate time for the aversion response from the laser, consisting of eye blink and head turn. In bright daylight, the typical pupil size was generally stated to be 2 to 3mm, or 5 to 7mm in shadowed or very dark areas. Use of the largest pupil size in safety standards would ensure the most conservative estimate for laser ocular hazards. The power entering a smaller pupil size would be reduced due to the smaller area of the pupil, i.e. by the square of the change in pupil diameter. Although the standards will reflect a more conservative assumption and estimate, the ocular hazard risk for those who work with lasers in bright environments remains unknown, especially since the average and smallest pupil size were not known in those conditions.[4] The purpose of this study is to address ocular hazard risk in terms of pupil size from lasers and radiation in the visible spectrum that may harm the retina.

Basic Optical Physics

Scientists employ two types of quantities to measure light: radiometric and photometric. Radiometric quantities measure optical radiation directly. Photometric quantities measure the visible radiation (light) as perceived by the human eye, i.e., by the visual response. "To quantify a photochemically initiated photobiological effect it is essential to specify the spectral quantity (wavelength distribution), the concentration (irradiance in $W \cdot cm^{-2}$) and the exposure duration at the target tissue."[5]

Relevant radiometric quantities include radiance, which describes the brightness of a source (in watts per centimeter squared steradian or $W \cdot cm^{-2} \cdot sr^{-1}$), and irradiance which describes the amount of radiation on a surface ($W \cdot cm^{-2}$). Parallel measurements exist as photometric quantities. Luminance (in lumens per meter squared steradian or $lm \cdot m^{-2} \cdot sr^{-1}$, which is equivalent to candela per meter squared or $cd \cdot m^{-2}$) is the amount of light from a source, and illuminance (in lux or $lm \cdot m^{-2}$) is the amount of light reflecting off a surface. No methods exist for translating one measurement to the other due to the myriad factors intrinsic to the photometric measurements. The principal unit for photometric measurements is the candela (equivalent to 1 $lm \cdot sr$). "The candela is the luminous intensity, in a given direction, of a source that emits monochromatic radiation of frequency $540x10^{12}$ Hz and that has a radiant intensity in that direction of 1/683 $W \cdot sr^{-1}$."[6] Even luminous intensity is a unit that is influenced by specific wavelengths of light and a mathematical model representing the typical spectral sensitivity of the human eye.

The terms scotopic, mesopic, and photopic refer not to a specific level of luminance of the retina, but a level of the eye's adaptation to light. The terms refer to the retinal and pupillary response as a result of the amount of light falling upon retina. Major components of the retina include two types of photoreceptors: rods and cones. Rods allow the brain and eye to detect black and white images and interpret images at very low light levels. Cones, the receptors most sensitive to color, require much higher levels of light in order to send a signal to the brain to form an image. Scotopic refers to the excitation and response of the rods as the primary photoreceptor. Photopic is a stimulation of the cones caused by higher luminance levels. Mesopic is a term which refers to the stimulation of both the rods and cones yielding somewhat equal response in conditions similar to those near dawn or dusk.[7]

The pupil size determines the amount of energy and power entering the eye and interacting with the retina. The energy transmitted to the retina is directly proportional to the area of the pupil. Previously published studies have found that pupil sizes range from approximately 8mm in scotopic conditions to 2mm in photopic conditions. The ratio of areas between an 8-mm pupil and a 2-mm pupil is 1:16, therefore an 8-mm diameter pupil allows 16 times more energy to reach the retina than a smaller 2-mm diameter pupil, as demonstrated in Figure 1.[8]

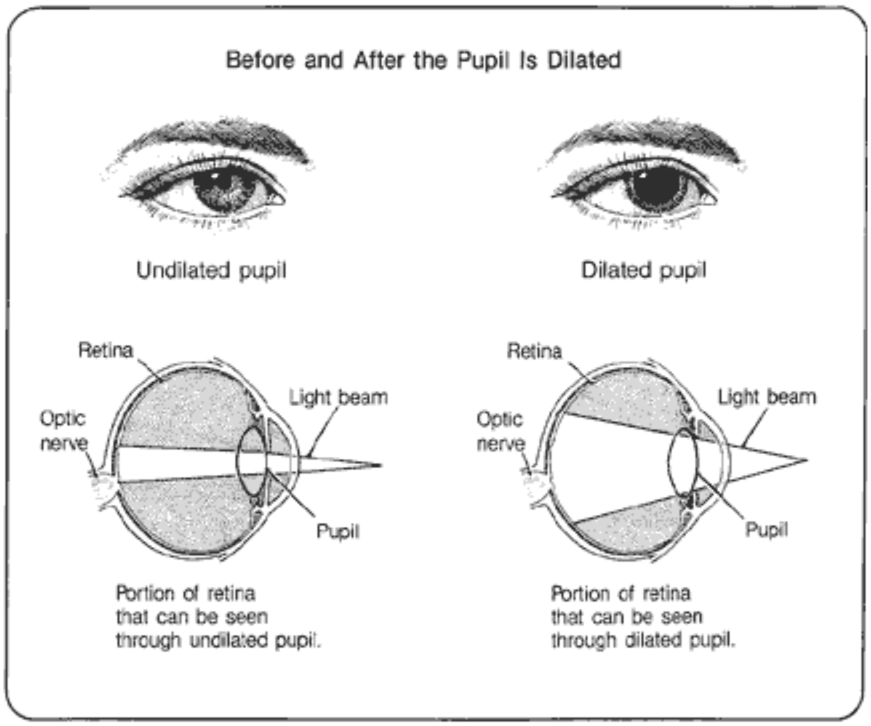

Figure 1: Diagram of spot size on the retina due to different pupil sizes.[9]
As the pupil constricts, the spot size of the retina decrease and less of the retina is exposed.

Luminance in outdoor environments can span more than several orders of magnitude as measured in $cd \cdot m^{-2}$. In a battlefield environment, soldiers may be subject to a wide variety of irradiance levels from a variety of surfaces. Table 1 lists various levels of luminance for selected surfaces, in both urban and rural environments.

Object Illuminated	**Luminance in Sunlight ($cd \cdot m^{-2}$)**	**Luminance in Cloudy Conditions ($cd \cdot m^{-2}$)**
Blacktop asphalt surface	3083	497
White paint	16102	1994
Signs	822	1987
Stucco building	4796	1888
Concrete	6167	1357
Grass	1644	353
White clouds	12676	6544
Blue sky	7537	N/A
White Ganzfeld	N/A	3101

Table 1: Measurements of ambient luminance values in outdoor environments.

Most of the light entering the eye does not enter from a direction parallel to the vertical axis, as shown in Figure 2. Light is emitted from many different directions and may enter the eye in many directions and orientations. The light enters the cornea, passes through the aqueous humor, through the pupil and then through the lens and vitreous humor before illuminating the retina. The pupil reacts by changing aperture diameter in order to maintain a relatively constant level of retinal illumination and adaptation level. In his 1969 study, Haines suggested it was important to know the relationship between the angle of light entering the eye and the apparent size of the pupil. The apparent pupil is the image of the pupil as viewed by an observer from the outside of the eye. The apparent pupil's image is virtual and erect. It is approximately 15% smaller than the actual pupil. When the pupil is viewed from along the optical axis, the apparent pupil size is maximized. Haines conducted a study which examined the size and shape of the apparent pupil when viewed at angles relative to the corneal plane.[10]

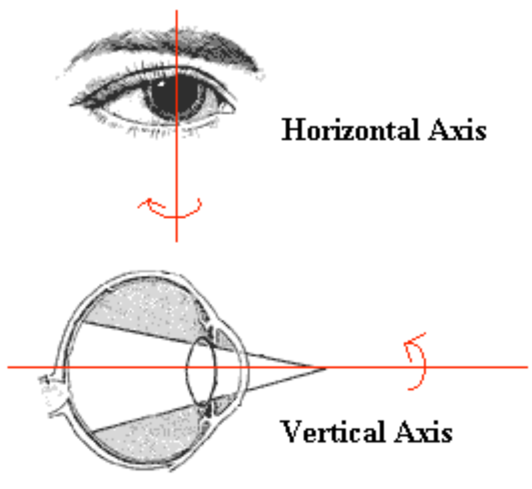

Figure 2: Diagram showing vertical and horizontal axes of the corneal plane

Haines used a film camera with a flash attachment to photograph the right pupil of his human subjects. The images on the film were magnified 10 times. Although Haines compared the apparent vertical diameter of the pupil to the apparent horizontal diameter, he did not compare the apparent vertical diameter of the pupil for each measurement angle to the diameter which corresponded to the zero degree angle. Haines calculated ratios, or correction factors, using the horizontal pupil diameters which compared the pupil size at the zero degree angle to the pupil diameters he measured at angles sweeping in discrete intervals from the nose to the temporal region. Haines did not calculate correction factors for pupil diameter in the vertical direction. These values will still be valid if the camera angle was changed to move in the vertical direction from chin to forehead. Note that this will be limited by the shape and angle of the eyelid and eye opening. The angle chosen for this experiment, ~28 degrees, had the smallest ratio, or correction factor, in addition to allowing for a complete unrestricted field of view for the subject. Haines also determined that from -30 degrees to +30 degrees from nasal to temporal regions with respect to the horizontal axis resulted in virtually no difference in apparent pupil shape and size, to include differences in horizontal measurement to vertical measurement. This result can therefore be applied to the optical plane sweeping from chin to forehead. Restricting the camera's motion to less than 30 degrees below the optical axis, the difference in size and shape of the pupil due to corneal refraction should be negligible.[11]

Pupil Physiology

The size of the pupil determines the exposure and power delivered to the retina. If the average pupil size in bright ambient light levels is determined, physicists and physicians will better be able to assess the extent of damage caused by a wide variety of non-ionizing optical radiation sources. The response of the iris, which is driven by the amount of light falling onto the retina determines pupil size, is the primary means of protection for the retina. Smaller pupil size decreases the area of the retina subject to effects from radiation. It is known that the amount of light creating an image on the retina is proportional to the area of the pupil, or the square of the pupil diameter, regardless of where on the retina the spot is located. As a result, this principle applies to the images formed on the peripheral regions of the retina as well as central retinal images.[12]

Retinal exposure is based on pupil size and the geometry of the eye in front of the pupil, consisting of the cornea and the aqueous humor in front of the lens. The iris muscles slightly overlap the circumference of the crystalline lens. Pupil size is important for visual acuity since it affects optical aberrations in the eye.[13] The pupil focuses light onto the macula, or more specifically the fovea, which is the most sensitive part of the retina. The macula has the highest concentration of cones of the entire retinal area. A smaller pupil size concentrates photons onto the macula yielding the highest visual acuity. Photons entering the eye at any angle other than parallel to the optical axis will result in a non-zero angle of refraction. Refraction focuses the beams of light onto the retina which allows for image focusing. The iris will block more of the highly angled rays from reaching the retina. The parallel rays and those close to parallel will create the most focused and clear image on the retina due to the smaller pupil size.

The pupil is the opening of the eye, and its size is controlled by the contraction of the pigmented iris muscles. Recent studies have shown that blue light, having wavelength range of 450-500nm and a component of light from the sun present in outdoor environments, is the primary wavelength range responsible for the retinal response, prompting the iris to make the pupil bigger or smaller.[14] The iris consists of circular muscles which contract to make the pupil smaller, while the radial muscles of the iris dilate the pupils. In scotopic conditions, the radial iris muscles are stimulated by a signal from the brain by the retina to contract. The sympathetic neurons control these signals. In mesopic and photopic conditions, the smooth iris muscle is stimulated to contract by the parasympathetic neurons. Thus, the pupil constricts to restrict the number photons reaching the retina.[15] Although it has not been determined what wavelength and intensity of photons cause pupil constriction and dilation or which type of receptors are responsible for pupil constriction and dilation, it is known that blue light causes greater pupil constriction when compared to light of other colors of the same luminance.[16]

In a 2003 study by Adrian, it was discovered that blue light produces smaller pupil sizes compared to other wavelengths of photopic luminance. “This observation has led… to the assumption that there is a scotopic input to the response of the pupil even at photopic luminance levels when cone vision prevails and the rod signals are neuronally suppressed. This inhibition prohibits the rods from contributing to the perceptive process, so the participation of rods in the response of the pupil is difficult to explain.”[17] Blue light results in a smaller pupil size upon illumination compared to light of other wavelengths of the same luminance value. Adrian discovered that in very bright, photopic conditions, pupil size is constant for wavelengths except for wavelengths longer than 520nm. His experiment included wavelengths of 624, 580, 521, 467, and 429 nm.[18]

Although the term "pupil" refers to the opening of the eye which allows light to enter the eye, terms require further refinement for the purpose of this research. "Pupil" refers to entrance pupil, specifically the opening of the iris that dilates and constricts. The actual size of the pupil behind the lens and cornea is different than the size of the pupil viewed on the outside of the eye. Apparent pupil size is the size of the pupil measured by an observer outside the eye. The apparent pupil size is the measurement of pupil size routinely used in vision research and is used in this study. The apparent pupil size is also the true optical aperture projected to the corneal plane. This projection defines the fraction of an incident laser beam that can enter the eye.[19]

Although a smaller pupil will decrease the amount of light reaching the retina, lid opening is also a factor. Like a smaller pupil, when the eyelid covers part of the pupil, the number of photons penetrating the retina is reduced.[20]

Classical Pupillometry Studies

Five major classical pupillometry studies were conducted between 1921 and 1952 to determine the average pupil size over a wide range of luminance values, from scotopic to photopic levels. The researchers conducted experiments that measured pupil size as a function of ambient luminance, or brightness, of the surrounding environment. Due to the fact the experiments were limited to indoor laboratory environments, their methods could not adequately determine the average pupil size in bright light conditions. Four of the classical studies of the variation of pupil diameter with adaptation level (i.e., ambient brightness) resulted in substantial difference between studies, as demonstrated in Figure 2.

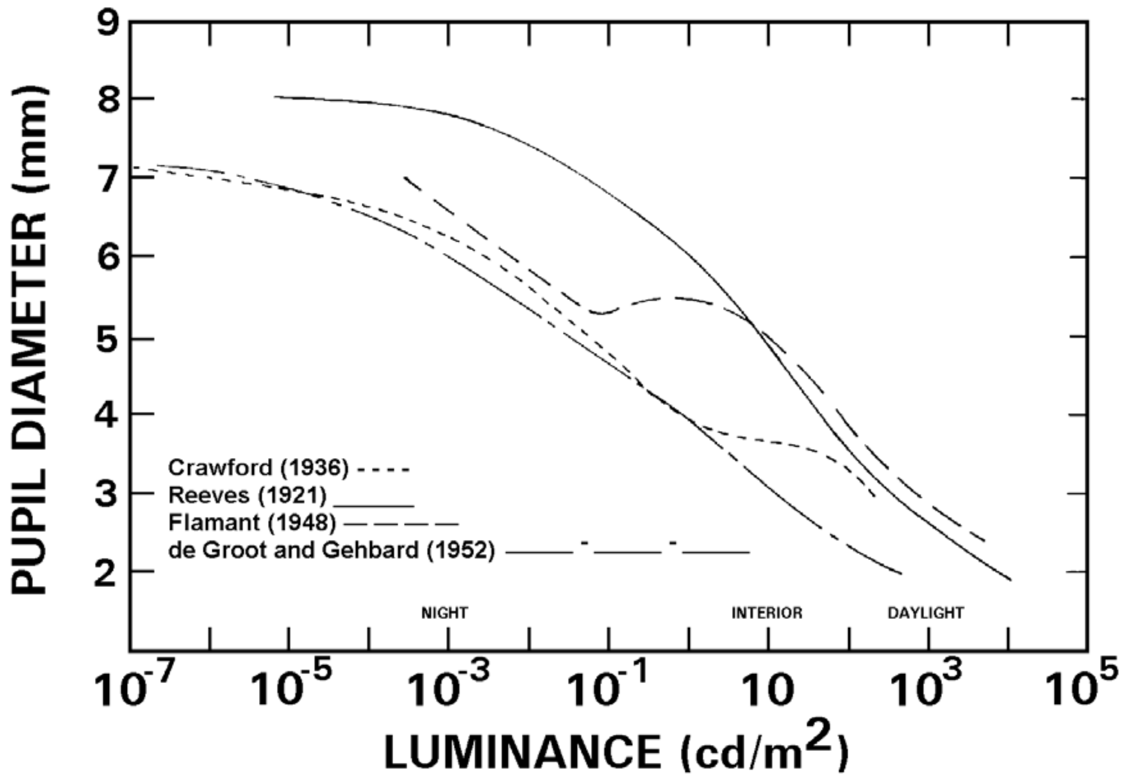

Figure 3: The variation of pupil diameter with ambient adaptation levels.[21]
This plot summarizes several major pupillometry studies.

A Ganzfeld is a screen placed in front of the subject which occupies the subject's entire field of view. Light, from a laboratory or outdoors, illuminates the screen and creates a uniform luminance for the subject to view. In a laboratory environment the luminance can be easily varied by lamps or other light sources. In outdoor environments a Ganzfeld is used to create uniform luminance levels in natural sunlight. Use of a Ganzfeld is one way to standardize illumination levels which results in the same retinal response and consequential pupil size for all subjects.

Reeves conducted a study attempting to relate the response of the pupil to varying light intensities in 1920.[22] Each measurement was taken after the subject's eyes had sufficient time to dark-adapt. Single- and double-eye exposures were examined. Reeves utilized flash photography to take photos of the dark-adapted pupils instead of the typical telescope with reticule. He assigned a range of luminance levels to each subject, for a total of 6 subjects and approximately 9 data points for each subject. He examined one eye closed versus both eyes open, measuring pupil size against luminance in each case. He did not specify if a Ganzfeld was used in his experiment or if a smaller visual target was used. In Reeves' experiment, he repeated his procedure several times over several luminance levels, "from total darkness to a just tolerable reflection of direct sunlight from white drawing paper."[23] He published data for luminance values of 1100 $cd \cdot m^{-2}$ (355 milliLamberts or mL) and 6400 $cd \cdot m^{-2}$ (2000 mL).

Reeves also constructed a plot of pupil sizes versus increasing luminance from 3×10^{-6} $cd \cdot m^{-2}$ (10^{-6} mL) to 300 $cd \cdot m^{-2}$ (10^{2} mL). A total of 8 data points were collected between 0 and 6000 $cd \cdot m^{-2}$ (2000 mL). The pupil diameter at 300 $cd \cdot m^{-2}$ (100 mL) was measured as 2.8 mm and at 6400 $cd \cdot m^{-2}$ (2000 mL) was it was measured as 2.0 mm. No pupil sizes were measured between 300 and 6400 $cd \cdot m^{-2}$. The values for the pupil diameter at higher luminance levels for one eye open and both eyes open are virtually the same above luminance values of 3 mL (10 $cd \cdot m^{-2}$). The second part of the experiment involved 8 subjects and pupil measurement using motion picture photography while luminance gradually increased. At the time, scientists knew two processes were involved in changing pupil size: a retinal response and the resultant pupillary response. This hypothesis led scientists to theorize that the retinal response that this special sensitivity of the blue rods may be primarily responsible for pupil size and subsequent retinal exposure. Reeves discovered that the smaller the pupil is at the time of the increasing luminance, the longer the time required the pupil to further constrict.

The 1952 study of de Groot and Gebhard remains the most comprehensive study to date on pupil size and luminance. This was the last major study conducted until this paper on pupil size and luminance. de Groot and Gebhard conducted a study to examine the relationship between the size of the natural human pupil and the flux density of the light incident on the cornea. They also recognized the need for more data in the higher luminance levels. They also determined that the actual stimulus to vision is the retinal image, not the luminance of an external source.[24] If the area of the pupil is known, the actual luminance on the retina may be determined. Rays at the edge of the pupil are less efficient and effective at focusing an image on the retina than the rays entering the center of the pupil. Fewer rays enter the eye at the edge of the pupil due to the shielding by the iris. A large area of the retina is illuminated but with a lower concentration of photons. A clear image is not produced due to the lack of cones away from the center of the retina. More rays enter the eye parallel to the optical axis, creating a clear, focused image on the fovea. Off-axis radiation leads to aberration, or lack of sharp, clear focus. Also, more off-axis radiation is admitted with larger pupils. Small pupils, therefore, create clearer images at higher visual acuity than large pupils. When a relatively large amount of light enters the eye, the pupil constricts as a method of refining the image.

In comparing the studies, de Groot and Gebhard examined the studies based on luminance and the number of subjects. For example, at 3 x 10^{-4} cd·m^{-2} (10^{-4} mL), a total of 24 subjects were examined. At 3 cd·m^{-2} (1 mL), 34 subjects were tested. For luminance values of 300 cd·m^{-2} (100 mL) and greater, the number of subjects varied: 18 subjects for 300 cd·m^{-2} (100 mL), eight for 1000 cd·m^{-2} (316 mL), three for 3200 cd·m^{-2} (1000 mL), and only one subject for 6400 cd·m^{-2} (2000mL). Based on the varying results from this comparison study, a need clearly exists for further studies in pupillometry at the higher luminance levels. According to de Groot and Gebhard, "there is no physiological or anatomical reason that we have been able to discover why the pupil cannot continue to contract more or less rapidly to less than two millimeters."[25]

	Reeves	**Holladay**	**Crawford**	**Moon & Spencer**
Year of Study	1921	1926	1936	1944
Method	Flash photography	Pinhole pupillometer	Infrared photography	Flash photography
N	6	4	10	7
Highest Luminance (cd·m^{-2})	6366	637	226	6366
Smallest Pupil Diameter (mm)	2.00	2.00	2.94	2.00
Other	No data points from 342 to 6366 cd·m^{-2}	No Ganzfeld	N/A	N/A

Table 2: Comparison of several classical pupillometry studies.[26]

Crawford in 1936 attempted to measure pupil sizes across a wide range of luminance levels.[27] He criticized methods used in previous studies. He considered Couvreux and Holladay's methods undesirable as they used a double-pinhole pupillometer.[28] Lythogoe and Reeves used photography and flashlight exposure as a method of pupillometry, which produced more accurate and precise results.[29] He therefore utilized infrared photography as his sole method of pupillometry. Although a flash in the visible spectrum was utilized, the pupil size was not affected since the duration of the flash was $1/20^{th}$ of a second or less. Crawford's study demonstrated that pupil diameters remain unchanged if the flash duration was less than $1/20^{th}$ of a second.[30] The Ganzfeld was placed 75 cm away from the subject's face. The subjects were exposed to five levels of increasing luminance, with an upper limit of 230 $cd \cdot m^{-2}$ (21 $cd \cdot ft^{-2}$). The pupil diameter at the highest luminance measurement was 2.94 mm.

The studies of Flamant and Crawford both demonstrated an interesting pupillary behavior in the mesopic region from about 0.1 to about 1.0 $cd \cdot m^{-2}$.[31] This may be attributed to a transition from rod-dominant to cone-dominant vision, but there may have been other factors, such as using different types light sources, unfixed camera or head position or light position that affected the outcome. It is very likely that the differences in measured pupil sizes were due to using different conditions within their respective laboratories. If the measurements were taken with a Ganzfeld, the entire retina would be stimulated, but if the subjects viewed a large source that subtended an angle of only 10 to 30 degrees, a much more limited area of the retina would have been stimulated. Another possible source of error may have been an incorrect measurement of luminance. It was noted that the differences between reported results of the different studies are greatest for the highest luminance values.

Several additional studies were conducted before the wide-spread use of clinical pupillometers. One of the earliest experiments which examined the effects of a dazzle source on the eye was Holladay in 1926.[32] The dazzle-source used in the experiment was a "large gas-filled tungsten lamp enclosed in a 10 or 12-inch white diffusing ball." The eye viewed the dazzle-source, and the examiner investigated the effects of the eye using a telescope at an unspecified angle from the subject's line of vision. In the field of view within the telescope, the examiner measured the subject's pupil size against a millimeter scale. Holladay measured pupil size against luminance of the dazzle-source. He also tested the effects of sudden increases in field brightness on pupil size and contrasted the results against the influence of long-time exposures. His data was limited to lower luminance levels, a maximum of 2mL (~ 6 $cd \cdot m^{-2}$).

Holladay used double pin-hole pupillometer to measure pupil diameter. He did not utilize a Ganzfeld, rather the cross-section of light of a specified luminance was the same size as the pupil. His study limited the luminance level to 685 $cd \cdot m^{-2}$ (200 mL), which corresponded to a 2.0-mm pupil size. He also determined that contraction of the pupils increases of depth of focus. Conversely, pupil dilation decreased the resolving power of the eye and decreased its visual acuity.[33]

In their 1944 experiment, Moon and Spencer also examined pupil size based on increasing luminance. In the experiment the subject was seated before the Ganzfeld. A photograph was taken of the subject's eye, and the pupil diameter was measured. The luminance was increased, and another pupil diameter measurement was taken. Eight pupil diameters were measured from each subject over varying luminance levels for seven subjects. The steady-state, dark-adapted pupil was 7.5 mm. Luminance was increased until the pupil contracted to its minimum size, 2mm, which was measured at approximately 6400 $cd \cdot m^{-2}$ (2000 mL).[34]

Moon and Spencer also compiled data from the classical pupillometry studies: Moon (1942), Crawford (1936), Stiles (1929), Reeves (1920), and Blanchard (1918). They determined slit-lamp measurements were inferior to those obtained photographically. Possible explanations for this result exist. First, the subject was forced to concentrate on the adjustments necessary to focus the pupillometer, which resulted in changes in accommodation, which is a known factor for affecting pupil size. Next, there was a general interference with normal vision by the apparatus. Infrared photography by Crawford resulted in smaller measured pupil diameters than Blanchard's slit-lamp pupillometer method.[35]

All of the previous studies concentrated on measuring pupil size in the indoor laboratory setting where luminance could be carefully controlled. The greatest variation in range of luminance values was in the measurements made at the highest luminance levels, which are characteristic of outdoor environments. Robust data at higher luminance levels do not exist in the classical studies. Pupil sizes measured in bright outdoor environments would provide sufficient data to fill in the void left behind in the classical studies at higher luminance levels while providing sufficient data in a realistic environment.

Modern Pupillometry Studies

Several types of pupillometry exist: video recording, handheld clinical pupillometers, digital photography, and videokeratography. The pupil may also be measured by the use of aberrometers and auto-refractors. Clinical handheld pupillometers include the Colvard pupillometer, the infrared Iowa pupillometer, and the Procyon pupillometer. The infrared Iowa pupillometer was the precursor and prototype for the Colvard pupillometer, a monocular handheld device. The Procyon pupillometer is a binocular pupillometer with a digital display. In the case of these handheld pupillometers, the subject presses the eye cup against his eye and looks into the pupillometer. An infrared beam illuminates the eye without affecting the pupil size. The infrared image of the pupil is converted into a visible image that is measured against an internal reticule by the investigator. These pupillometers are most frequently used in laser eye surgery, as they were designed to measure pupils in scotopic conditions to prevent the halo effect that can result due to laser refractive surgery. The halo effect is the result of laser refractive surgery on the cornea when the area affected does not cover the entire dilated pupil. This results in images observed by patients in scotopic conditions having a halo of light around them. Clinical handheld pupillometers are easy to use, inexpensive, and they yield results very quickly.

Videokeratographs, aberrometers and auto-refractors detect abnormalities of the surfaces of the eye, estimate the refraction of the eye, or map the refractive index of the cornea, respectively. Some of these devices also possess a secondary capability as a pupillometer.

Rulers may be used to measure pupils directly. Similarly, pupil cards and templates may measure pupil size directly, as they are cards with preprinted pupils at specific sizes to compare to the subject's pupil by holding the card next to his or her eye.

Video recording utilizes light in the infrared wavelength to take a series of pictures of the eye without affecting the pupil size, since the retina does not respond to IR radiation. Infrared radiation illuminates the eye and passes through the pupil. A shadow of the iris is cast, outlining the edges of the pupil. The video camera, which is sensitive to radiation in the infrared spectrum, records the images of the pupil. The individual frames of the video are analyzed and the pupil size is measured.

Digital photography is the simplest pupillometric method. Photographs are taken of the eye in ambient light conditions with a flash to illuminate the retina. The pupils in the photographs are measured using computer image software.

Many studies have compared the different types of pupillometry. The most accurate and reliable method for outdoor pupillometry was determined to be digital photography for many reasons: (i) it resulted in the least interference with allowing the pupil to react to the ambient luminance from outdoor environments, (ii) it allowed for binocular measurement, highest repeatability and accuracy, (iii) it afforded lasting documentation, and (iv) it involved most simple and fastest set-up and measurement.

The first study by Boxer Wachler compared videokeratography and the Infrared Iowa pupillometer.[36] The study measured pupils of 7 subjects, of ages 28 to 42 years. The highest level of luminance the pupils were exposed to was 344 $cd \cdot m^{-2}$. Pupils measured by the videokeratograph were smaller as compared to the results from the Infrared Iowa pupillometer. Videokeratography is not clinically recommended for measuring pupils prior to refractive surgery, and is not practical for an outdoor pupillometry study due to its complexity, size, and interference by the ambient light conditions. In another study, Mantry compared the Colvard pupillometer to the auto-refractor.[37] The highest light level was 15 lux (a luminance unit that is not directly convertible to luminance, but if 15 lux was illuminating a white target, it would approximate to about 5 $cd \cdot m^{-2}$), and 200 eyes were measured. 5 $cd \cdot m^{-2}$ corresponds to a dark room. 100 $cd \cdot m^{-2}$ is approximately the light level in an office. 1000 $cd \cdot m^{-2}$ is the approximate luminance from a cloudy day. A bright, sunny day would produce a luminance measurement of 4000 $cd \cdot m^{-2}$ or higher. The average pupil diameter in this study was 6.2mm.

Twa compared many methods of pupillometry.[38] Based on his findings, the methods from best to worst in terms of accuracy and repeatability in bright conditions were: digital photography, video recording, Colvard pupillometry, a ruler, and a template to measure pupils. The three levels of luminance utilized in this study were 0.63 lux, 5 lux, and 1000 lux (to “simulate afternoon sun”). The investigators used computer software to analyze the data from the video recording and digital photography. No significant differences in pupil size were found between video recording and digital photography. The investigator did not control for the distance between the eye and the digital camera, which created great variability in pupil size.[39] Again, the handheld clinical pupillometer fared better than the auto-refractor. Schnitzler and Bradley in two separate studies also compared the Colvard pupillometer with digital photography, although the level of luminance utilized was 0.42 $cd \cdot m^{-2}$, which is much lower than bright outdoor environments.[40] Again, the subject’s head was not placed in a fixed position. For scotopic conditions the handheld clinical pupillometer was determined to be superior to digital photography for measuring pupil size, but pupils exposed to higher luminance levels were not measured. Kohnen compared the handheld clinical pupillometer to measure pupils in scotopic and mesopic conditions, the aberrometer and the videokeratograph at three different luminance levels : 0.06 lux, 0.88 lux, and 6.61 lux (or approximately 0.02 $cd \cdot m^{-2}$, 0.3 $cd \cdot m^{-2}$, 2.2 $cd \cdot m^{-2}$ if illuminating a white target).[41] Wickremasinghe also compared the aberrometer and the Procyon pupillometer. Again, in both studies, the handheld pupillometers yielded better results than the videokeratograph and the aberrometer. Pupil cards, rulers, and templates fared worst of all across all studies.[42]

A study by Kurz was conducted to determine if binocular pupillometry differed from monocular pupillometry.[43] The illuminance levels were 0.03 lux, 0.82 lux, and 6.4 lux (0.01 $cd \cdot m^{-2}$, 0.3 $cd \cdot m^{-2}$, 2.1 $cd \cdot m^{-2}$, respectively, if illuminating a white screen). If each pupil is subject to different light levels, the larger pupil constricts to match more closely to the smaller pupil in an approximate averaging method.[44] In higher ambient illuminance levels (greater than 6.4 lux, or approximately 2.1 $cd \cdot m^{-2}$), differences in monocular and binocular pupillometry are significantly different. Binocular measurements may represent how eyes work more realistically than monocular measurements.

Handheld pupillometers produced the best results for measuring pupils in scotopic conditions, but digital photography and infrared video recording demonstrated the best results in photopic conditions. Digital photography was chosen for this study for many reasons, to include ease of measurement, subject comfort, portability into the field, high accuracy and repeatability, and strong evidence of accuracy and repeatability from published studies.

Pilot Studies and Methods Development

A number of pilot studies were conducted at the United States Army Center for Health Promotion and Preventive Medicine (USACHPPM) at the Aberdeen Proving Ground in Edgewood, MD to refine the pupillary photographic measurement method. The first pilot study involved photographing USACHPPM scientists' eyes with a NIST-calibrated ruler adjacent to the eye to find out if this would be an accurate measuring tool. The ruler was directly placed under the lower eyelid as close to the pupil as possible in order to directly measure the pupil diameter. The camera was positioned directly in front of the subject and the entire face was photographed. Accommodation was not a confounding factor considered at this point. Ambient light measurements were not taken. After the digital photos were taken and downloaded to a computer, it was found that the millimeter marks on the NIST ruler were too wide for a precise reading. This method of pupil measurement was judged not to be appropriate for this field study, since it required subject involvement in placing the ruler and the precision was only +/- 0.5mm for this pilot study.

The second pilot study involved characterizing light meters for use in the study as well as determination of the brightness (luminance) levels of various objects outdoors in bright sunlight. The purpose of this study was to measure illumination and target luminance levels of objects troops might be observing on the battlefield that would affect their pupil size. Viewing a dark forest or asphalt would result in a different pupil size than looking at lightly colored buildings and brightly illuminated objects. In this pupillometry study, a very bright field-of-view was necessary. In an effort to standardize the scene luminance, a Ganzfeld was set up and light measurements were taken at different points throughout the afternoon to determine the luminance levels for possible use in the pupil-size experiment. A Minolta Luminance Meter, 1-Degree Digital was used for luminance measurements.

The third pilot study tested a Ganzfeld configuration and potential challenges with digital photography, particularly with camera and Ganzfeld position. A white poster board which measured 1.5 meters by 2.5 meters with a matte finish was used as the Ganzfeld and was placed on an easel for potential subjects to look at during the photography. The digital cameras, Kodak DX7440 Zoom and Canon PowerShot SD550, were mounted on a tripod one at a time. The camera was offset from the front of the subject's eye by approximately 30 degrees temporal along the vertical optical axis. First the camera lens was placed level with the subject's eyes. Obstruction from squinting, eyelashes, dark eye color and reflection were revealed as major factors. Direct measurements of the pupil, such as those with the NIST ruler, were not acquired during this phase. The subjects merely stood in front of the Ganzfeld and were asked to look at the screen, then a photograph of the ocular region of each participant was taken. A specific head position was not established or researched at this time. The camera was moved in approximately 1-cm increments toward the nose and also downward along the optical axis approximately 15 degrees for each successive measurement. An optimal position for the camera was found to be along the optical axis and lower than the eye's line of sight, pointed upward toward the subject's face. This served two purposes: it ensured the entire pupil was photographed while unobstructed by the eyelids, and the eye's view of the Ganzfeld was unobstructed by the position of the camera.

The fourth pilot study examined the capability of the digital camera to photograph in the infrared spectrum. A number of infrared LEDs of wavelengths 700-950nm were tested in the laboratory to determine the wavelength of each diode and to determine If the contrast of the pupil edge was superior to photography with visible light. Photographs of LEDs at different wavelengths were taken with the digital camera. This experiment was conducted not only to determine the range of wavelengths the camera could detect, but to experiment with photographic techniques that could utilize radiation in the infrared spectrum to photograph pupils. Photography in the near-infrared would eliminate glare from the pupil due to the Ganzfeld and the flash. Emissions from each diode were detected by the camera in a darkened room. It remains unknown how one could use infrared photography in outdoor environments to photograph pupils. Unfortunately, in bright outdoor environments, IR photography proved unsuitable for use in this study due to the amount of infrared radiation produced by the sun and environmental masking of infrared reflections from the subjects' pupils. One could not produce a good reflection from the iris to photograph. It was determined that the ideal method of pupil measurement in outdoor environments for this study was digital photography in the visible spectrum.

A fifth pilot study was conducted to determine the best way to standardize the pupil diameter measurements, standardize the subjects' position relative to the camera, and maximize measurement repeatability. A chin rest and forehead rest were constructed from commercial optical lens holders and were mounted onto a tripod. The chin and forehead rest were mounted onto a tripod using a clamp and a narrow board. The camera was mounted on the same tripod using a slanted riser so the photographer could take pictures of the eye at an upward angle to minimize interference of the image with the upper eyelid. The entire apparatus could be adjusted up or down on the tripod based on the subject's height. This experiment determined the strengths and flaws in potential apparatus designs for the pupil-size experiment.

Next, a pilot study was conducted to determine the best way to enhance the images taken by the camera. First, various settings were adjusted on the camera to include flash on and off, different custom settings such as "portrait" and "close-up." The distance to the eye from the camera was changed, the angle of the camera in pitch and yaw were measured as images were taken, and light measurements were taken of the system to determine if the camera and its color contrast affected the pupil size when the subjects focused on the white Ganzfeld. The contrast and brightness were adjusted on the downloaded images in order to best see the edges of the pupil for measurement. Utilization of the flash produced the best images when compared to photos taken without the flash. It had been determined by Loewenfield and Lowenstein that flash photography did not affect the pupil size while using digital photography as a method of pupillometry.[45] Therefore it was decided to use the flash as it would produce high-quality images of the ocular region and not impact the pupil diameter measurements.

A significant problem arose while photographing the pupils of eyes with dark irides. The reflected image of the Ganzfeld from the corneal surface partially masked the edge of the pupil for measurement. Thus an additional pilot study involved photographing dark-colored eyes under four conditions: (i) with Ganzfeld, (ii) without

Ganzfeld, (iii) with flash, and (iv) without flash. The lighter shades of brown irides produced very good images with and without the Ganzfeld, but only with the flash on. The pupil was indistinguishable from the iris in eyes with deep brown irides. Reflection from the sun and brightly illuminated objects inhibited the view of the pupil without the Ganzfeld. Reflection from the Ganzfeld itself produced significant reflections on the surface of the eye, preventing accurate measurement of the pupil edge. Retinal reflection (retinal reflex) was attempted with the flash for the subject with the darkest brown iris. The subject was asked to look directly at the flash with head and body positioned square with the camera. Since the reflection of the Ganzfeld off the cornea could not be completely eliminated, it was necessary to ensure that the reflection of the Ganzfeld covered the entire pupil so the edges of the pupil could be distinguished and the pupil accurately measured (Figures 25-29). This effect was not apparent in the pupil measurements of blue and other light-colored eyes. Additionally, the flash served to reduce the intensity of the reflection from the Ganzfeld. If the luminance of the sunlight was brighter than average for a given experimental trial, a white Ganzfeld was replaced by a gray one to reduce visual discomfort and minimize the obscuration of the pupil by squinting of the subject. This further reduced the glare from the cornea. The gray Ganzfeld was approximately 30% reflectance of the white Ganzfeld.

Additionally, a pilot study determined an adequate position for the chin rest, camera, and Ganzfeld within the total experimental configuration. Factors included minimizing the camera angle and flash from the ocular line-of-sight, while the subject had a maximum view of the Ganzfeld subject to bright outdoor light, ensuring comfort for the subject, and ease and transportability for set-up of the experimental apparatus in the field.

Next, a pilot study was conducted on contact wearers, and it was determined that the contact lens did not significantly change the size of the pupil in the digital photographs. The pupils covered by contact lenses could be measured the same way as eyes without contact lenses.

Finally, a rehearsal of the data collection method determined the luminance limits of the Ganzfeld based on comfort the subjects' comfort. This luminance level was approximately the limit where eyes could not be photographed due to discomfort or extreme squinting. The luminance of the white poster board exceeded 8000 $cd \cdot m^{-2}$, then the gray poster board was used.

The uncertainty of the final pupillary measurement attributable to a variation in position of the corneal plane relative to the face (constrained by the head and chin rest) was investigated by sampling a number of subjects at USACHPPM. This was accomplished by measuring the variation of the corneal plane along the camera-eye axis. These measurements showed that the cornea-to-camera-lens distance for all subjects tested did not vary by more than 2.5 mm. The resulting difference of the pupil diameters in images produced by the digital camera at the maximum and minimum distances was less than 1%.

Methods

Pupils were photographed using digital photography with a commercial digital camera and a chin/face rest. The subjects volunteered to participate in the study, then they each answered a questionnaire, positioned their face firmly against the chin/face rest, stared at a Ganzfeld illuminated by natural ambient sunlight, and remained motionless while three photographs were taken. The images were downloaded to a computer and analyzed to determine average pupil size in bright, outdoor environments.

Subjects were recruited from two events: a monthly five kilometer (5K) race at the National Naval Medical Center (NNMC) and the Army and Navy physical fitness testing events at the Uniformed Services University of the Health Sciences (USUHS) in Bethesda, MD. These events were chosen because of close proximity to USUHS, the large number of military personnel participating in each event, and similar demographics between the students at USUHS compared to the active duty military. Additionally, each event took place in the middle of the day which would be more likely to provide an adequate level of luminance for pupils to constrict. A total of 87 subjects were recruited. A total of 26 were drawn from the 5K race and 61 subjects from the physical fitness testing volunteered to be subjects.

Before each event began, an announcement was made to include a brief description of the experiment and a request for volunteers to participate in the study. The principal investigator, a United States military officer, did not wear a uniform while collecting data, in order to prevent any inadvertent persuasion over enlisted personnel. Subjects volunteered to participate in the study after their respective athletic event ended.

The camera and apparatus for the experiment were assembled on a portable table near the finish lines of the 5K race and the physical fitness testing.

Each subject received an individual briefing approximately one minute in length describing the experiment in detail and outlining the risk from participation in the experiment. The risk to the volunteers was limited to a brief flash exposure from the digital camera. The questionnaire inquired about age, gender, military branch, recent head trauma, recent eye injury, recent eye dilation, eye color, and skin color. No questions were asked in regard to use of contact lenses or any other type of corrective lenses or eye surgery. Volunteers were accepted as subjects in the study if they were 18 years of age or older, had not had their eyes dilated in the past 24 hours, had not sustained any acute head trauma or head injury requiring hospitalization or medical treatment over the past 48 hours, or had not sustained any eye trauma over the past 48 hours. The study included subjects of all eye colors, any type of ametropia or imperfect vision, and those using any type of prescription medications. The principal investigator's contact information and informed consent information was given to each of the subjects.

A portable table held the equipment for the experiment. A gray or white foam core poster board served as the Ganzfeld for the subjects, and was positioned at the edge of the table (Figure 5). The use of the gray or white poster board was dependent on both the ambient luminance level and the current weather conditions. If the luminance of the white poster board exceeded 8000 $cd \cdot m^{-2}$, then the gray poster board was used. They gray poster board was approximately 30% of the reflection of the white poster board. Additionally, luminance levels over 8000 $cd \cdot m^{-2}$ do not appear frequently in nature or on the battlefield. On both days of testing, the weather was overcast, which resulted in a maximum luminance level of approximately 4250 $cd \cdot m^{-2}$. Therefore the white poster board was used in the early afternoon. The table was oriented so the Ganzfeld received the greatest amount of sunlight, but also in a position such that the subject did not cast a shadow on the Ganzfeld. The luminance for the experiment ranged from 789 $cd \cdot m^{-2}$ to 4253 $cd \cdot m^{-2}$, thus all measurements were recorded using the white Ganzfeld. The Minolta Luminance Meter, 1-Degree Digital was the spot photometer used in the experiment. The spot photometer was calibrated per manufacturer's instruction and was within the calibration certificate date.

Subjects sat in a chair directly across from the Ganzfeld on the opposite side of the table facing the camera. The eye-camera distance was maintained by the chin rest mounted to the same piece of wood as the camera (Figures 4-8). The camera was pointed upward toward the subject's face.

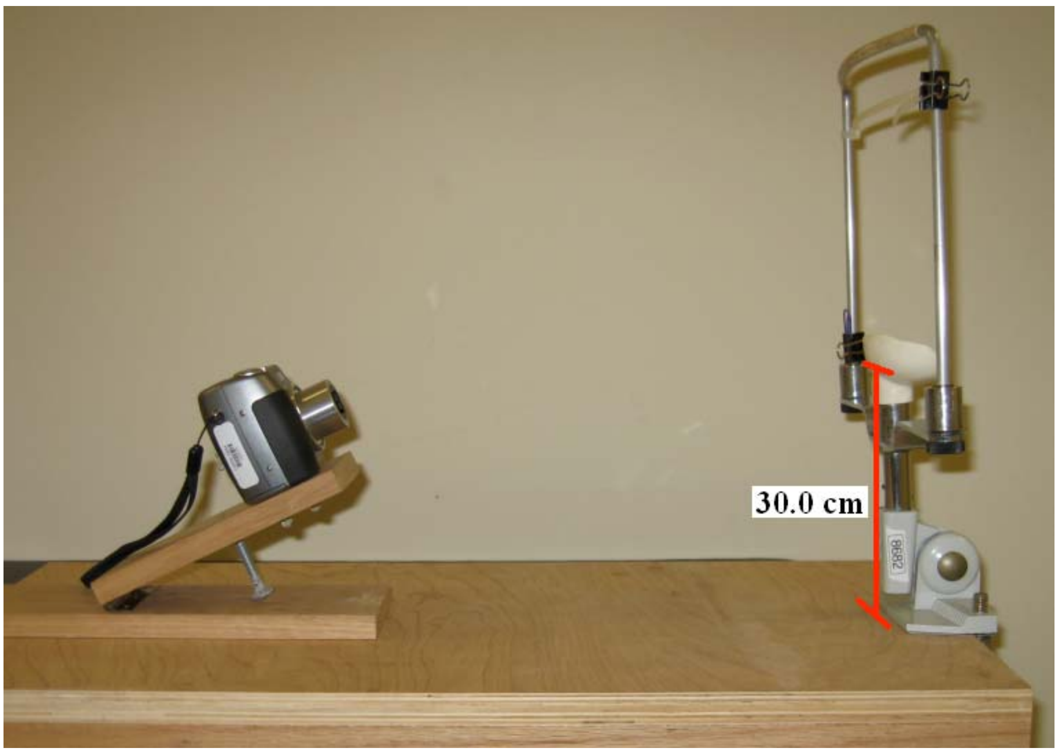

Figure 4: Distance from base to the chin cup of the chin rest.

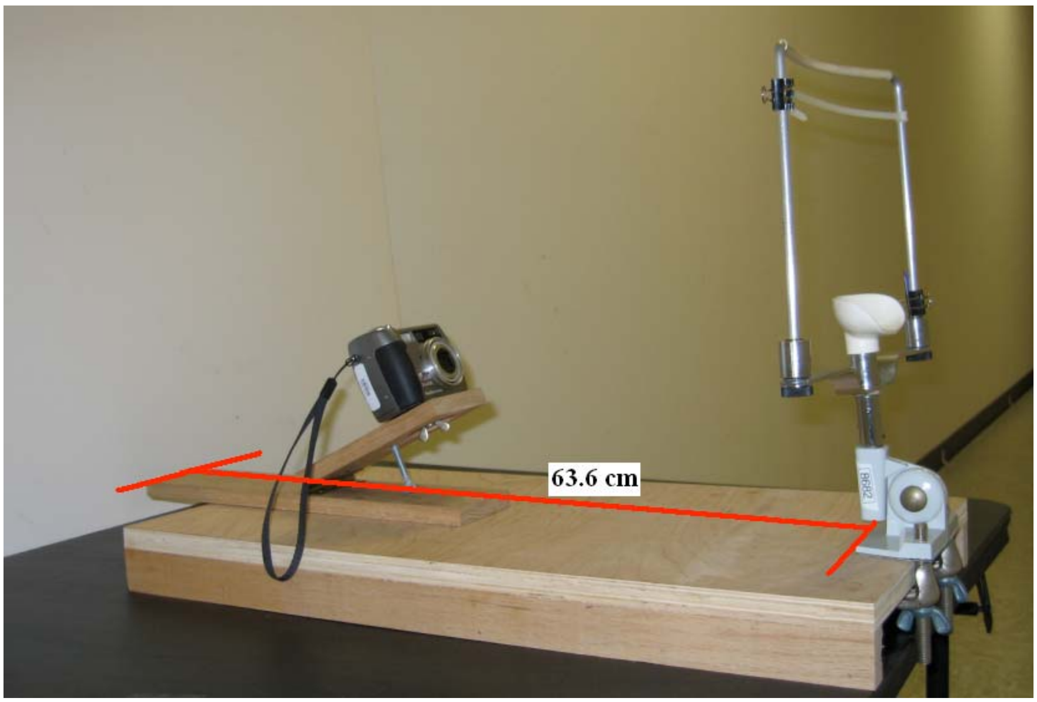

Figure 5: Distance from Ganzfeld to base of chin rest.

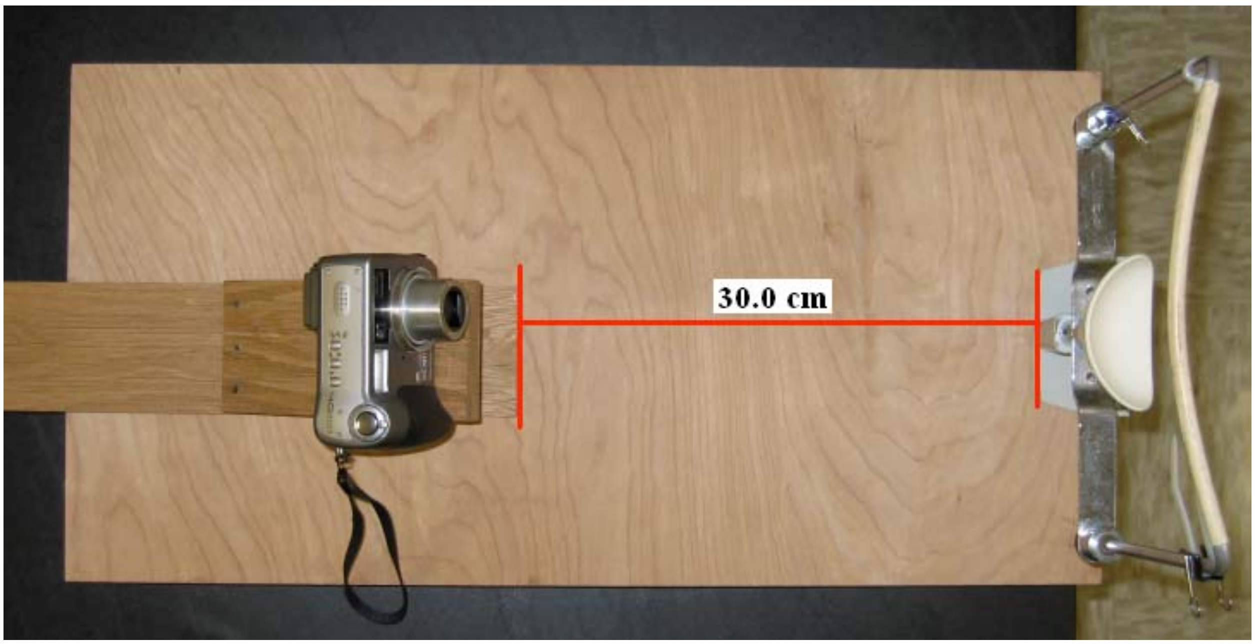

Figure 6: Distance from chin rest to the camera platform.

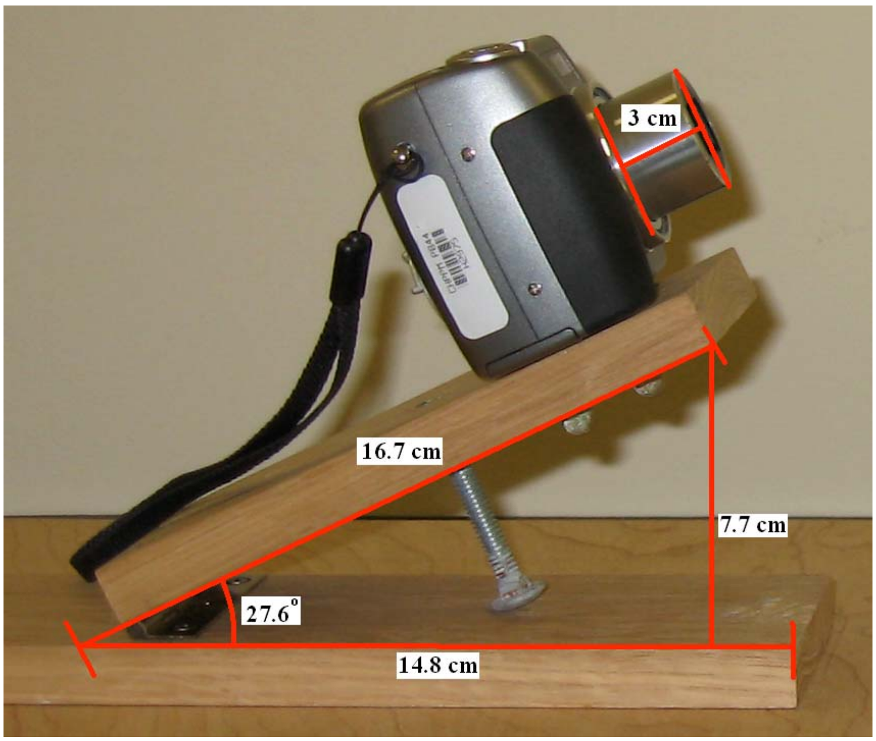

Figure 7: Dimensions of camera platform.

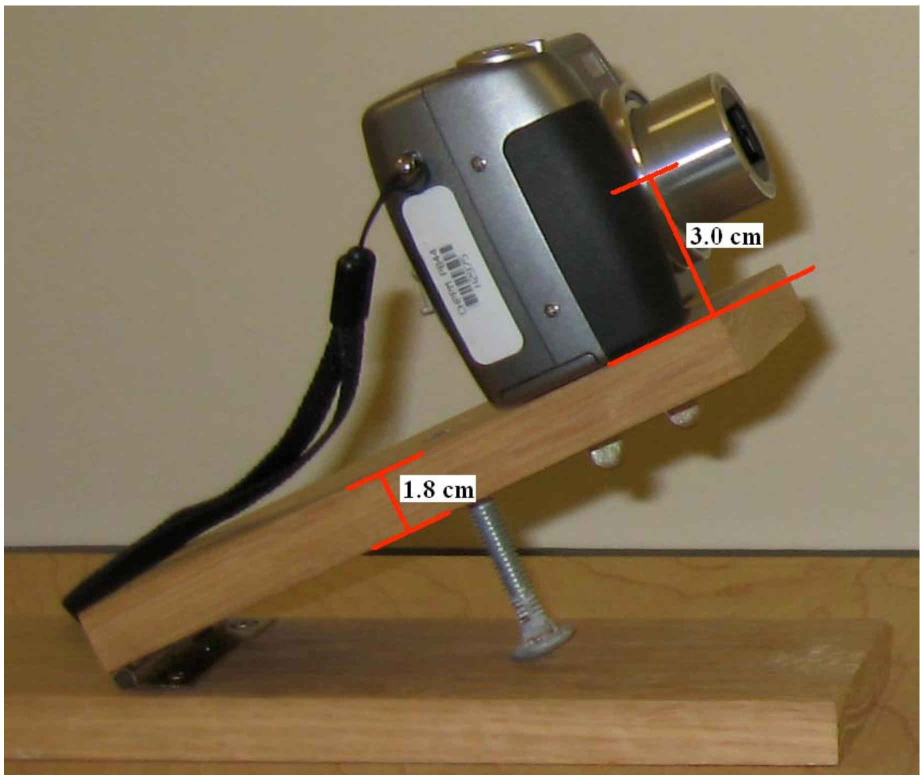

Figure 8: Side view of camera platform.

After signing the consent form and completing the questionnaire, the subject was assigned an identification code based on the order they volunteered to participate. Next the subject sat in the chair at the table and placed his or her face into the chin rest. If the subject wore glasses, he or she was instructed to remove them for the duration of the experiment. The subject was instructed to focus on the pink dot on the Ganzfeld which controlled for accommodation. On the count of "three" the subject opened his or her eyes as wide as possible and looked directly at the pink dot. A photograph of the ocular region was taken at that time. The flash required eight seconds of time in order to recharge. After eight seconds, the process was repeated. This process was repeated for a third photograph to complete the data set. Three measurements of the luminance of the Ganzfeld using the spot photometer were recorded.

The settings on the Canon PowerShot SD550 Digital Elph camera were selected to maximize quality of the photographs and limit the photographic area to the ocular area only, as specified by the USUHS Institutional Review Board (IRB) to preserve privacy and anonymity for the voluntary subjects. The data could not be traced back to the volunteering subject since only the eyes were photographed and assigned an identification code. Maximum quality was defined as easily distinguishing a clear border between the iris and the pupil in each of the photographs. The camera settings were optimized for the study to give the optimal presentation of the pupil. Finally, the camera was zoomed to 3X, the maximum optical zoom value. These settings reduced reflection of the Ganzfeld off the cornea, allowed the camera to zoom in close to the ocular region to capture as many details of the pupil size and shape as possible, and allowed for the highest resolution of the camera.

After the photographs were acquired on the day of the experiment, they were downloaded to a computer for analysis. The photographs are property of USACHPPM. Each photograph was stored in a folder corresponding to the subject's identification label. The computer screen contrast and brightness were adjusted for maximum image quality. Each image was converted to a 24-bit uncompressed, lossless bitmap format. This format ensured resolution would not be lost due to storage in the "lossy" formats that use algorithmic averaging to reduce file size. This resolution could have caused blurring which would in turn introduce error in the measurement of pupil diameter. The criteria for usable photographs included specific requirements. First, the full diameter of the pupil had to be completely exposed and evident. Also, clear distinction between the pupil edge and the iris was evident, which proved to be particularly important for dark-colored eyes. Partially closed eyes were permitted, so long as the full horizontal diameter of the pupil was exposed and measurable. A raster graphics software program was used to measure the diameter of the subjects' pupils, using the following methodology.

The photographs of the eyes were magnified to 800% so the image of the pupil occupied the entire screen. Vertical red lines were drawn in the raster editing program at the outside edges of the pupil. The edge of the red line correlated with the edge of the pupil, but did not include any part of the pupil (Figure 11). A grid was overlaid onto the image, each grid square was termed a "micro grid square" (MGS). A horizontal line was drawn between the two vertical red lines outlining the pupil (Figure 12, 13). Every ten MGS were represented in differing colors to as to assist in counting the number of MGS.

Figure 9: Raster image of right eye, 100% zoom.

Figure 10: Raster image of right eye, 800% zoom.

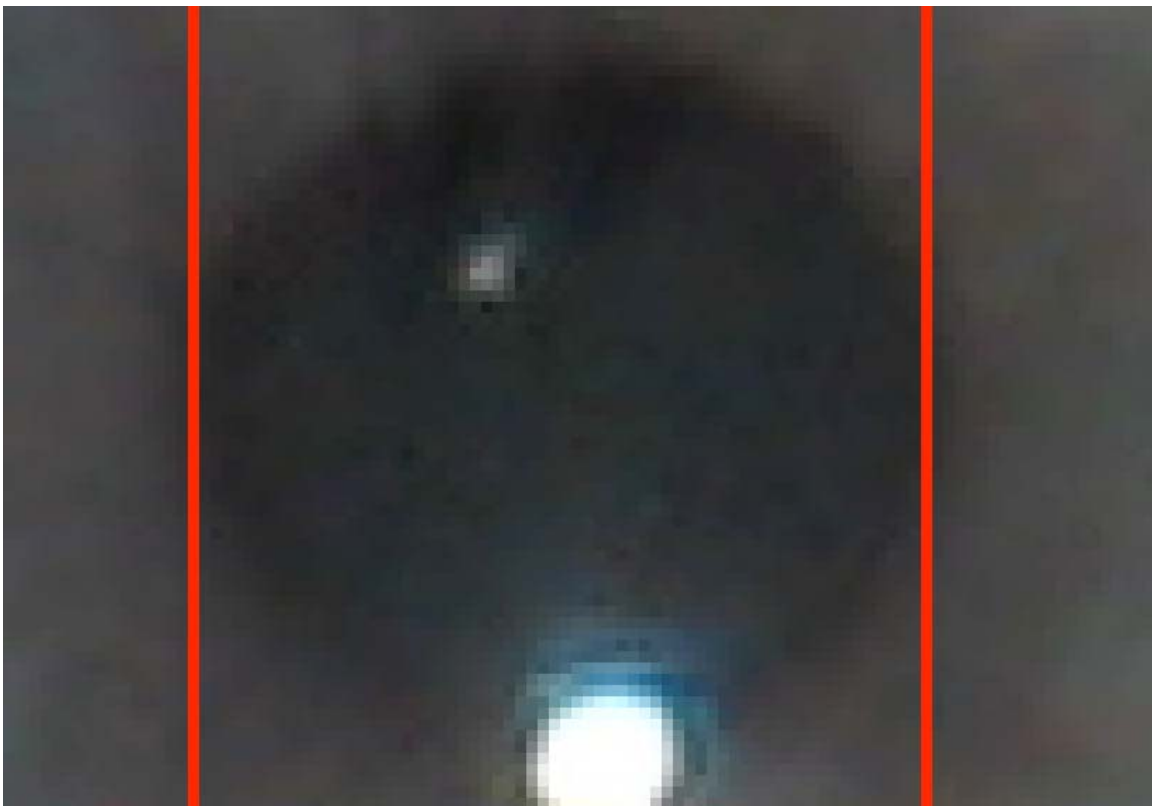

Figure 11: Red vertical lines outlining the pupil.

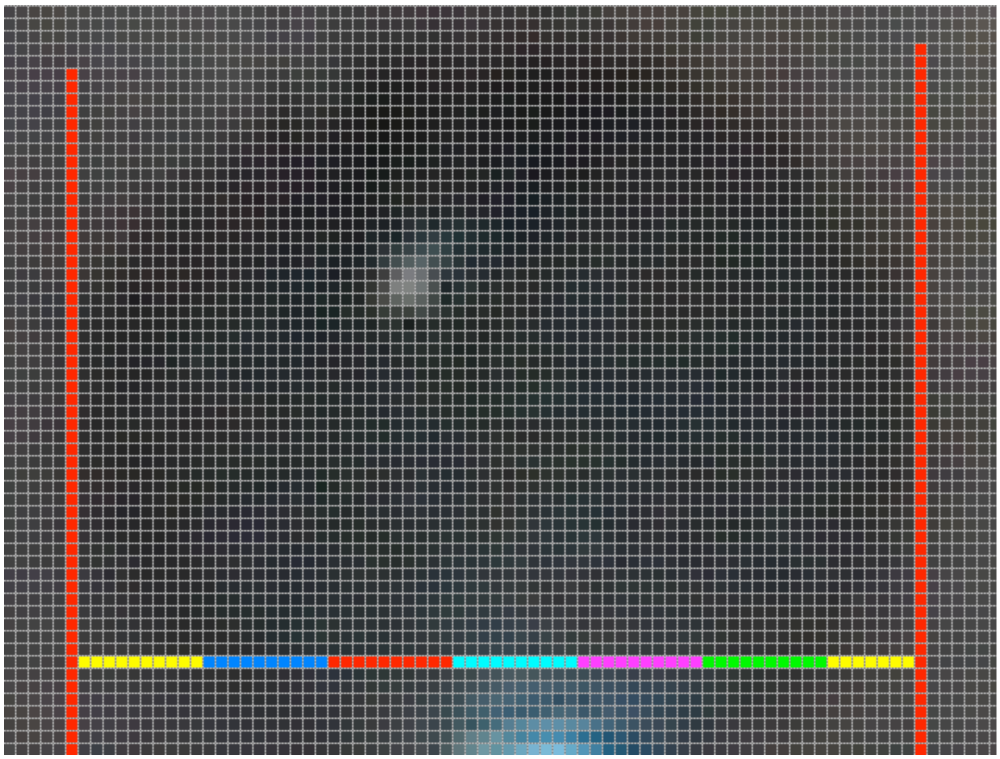

Figure 12: Right eye measurement process.
800% zoom after measurement in "grid" mode.

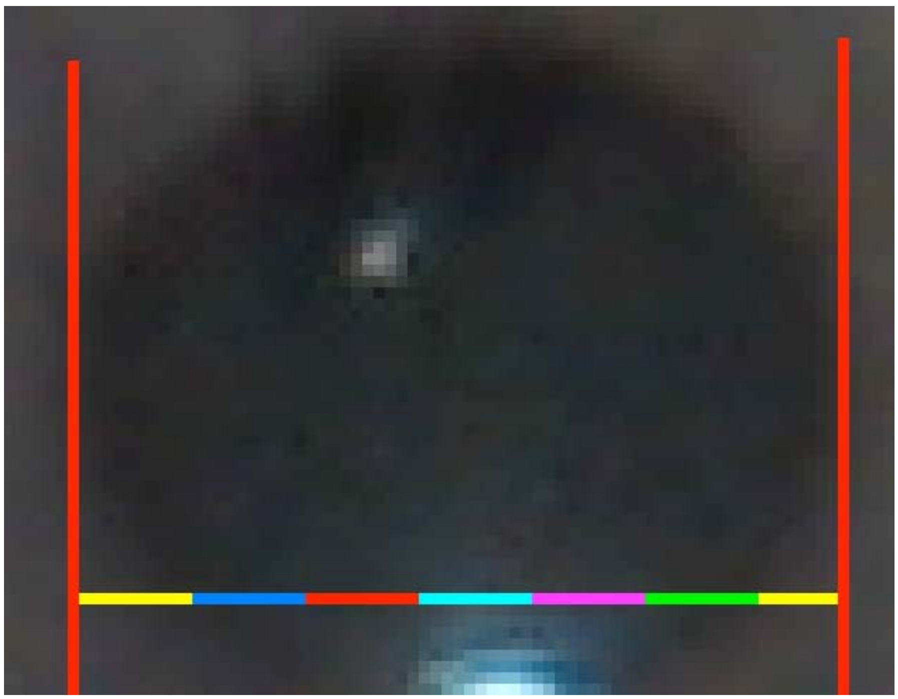

Figure 13: Right eye measurement process
800% zoom after measurement with "grid" mode turned off

The final number of micro grid squares (MGS), the pupil diameter, was recorded for each eye photographed for each subject.

The next step was to convert pupil diameter measurements from MGS to millimeters. A sheet of graph paper in millimeter increments was measured using a NIST ruler and calipers. Photographs were taken of 12 subjects' facial profiles with their faces in the chin rest. Once the photographs were taken, the maximum and minimum distances from the eye to the camera were measured. These measurements determined the greatest difference in distance between the cornea and the lens of the camera. This difference in eye distance to the camera was 2.5 mm (or 60 MGS). Once the maximum and minimum distances between the eyes and the camera was determined, the graph paper was placed at the two maximum and minimum head positions on the chin rest and photographed in the same manner using the same profile orientation of the chin rest. The graph paper images acquired in the two positions were measured in the same manner as the pupils. Millimeter increments for each of the graph paper photographs in the two positions were converted to MGS. In the maximum and minimum positions where the graph paper was photographed, each millimeter measurement resulted in the same number of MGS where 1 mm equals 25 MGS. There was no difference in mm-to-MGS calibration between the maximum eye-to-camera distance and the minimum eye-to-camera distance. The conclusion was that there was no difference in pupil size based on head position in the chin rest. Several measurements of the millimeter increment of graph paper were taken, and several photographs of measured millimeter increments graph paper were measured to determine if there was a difference in head position. The variation of measured pupil diameter due to difference in head position between subjects and between photographs was negligible.

Data Analysis

The statistical analysis on the collected data sets was performed using the SPSS Statistical Package version 12.0.1. The average pupil size in the study was 2.39 mm (Table 3). A total of 87 pupils were measured, 26 subjects from the running race and 61 subjects from the physical fitness test (Table 4). The range of luminance measured from 789 $cd \cdot m^{-2}$ to 4253 $cd \cdot m^{-2}$ and one outlier measurement of 7957 $cd \cdot m^{-2}$. The average pupil size of the outlier was 1.82 mm. From this point forward, the outlier will not be included any statistical calculation or discussion. The smallest average pupil size was 1.44 mm, and the male subject was 25 years old. The luminance for that pupil size was 3340 $cd \cdot m^{-2}$. The p-value for all statistical tests was $p = 0.05$.

	N	*Mean (mm)*	*Std Dev*	*Min (mm)*	*Max (mm)*	*95% CI for Mean Lower Bound*	*95% CI for Mean Upper Bound*
All pupil measurements	86	2.39	0.29	1.44	3.03	2.33	2.45

Table 3: Descriptive statistics for pupils measured over luminance range.

	Men	*Women*	*Dark Pupils*	*Light Pupils*	*Age 20-29 years*	*Age 30-39 years*	*Age 40+ years*
N all pupil measurements	64	22	50	36	52	24	10

Table 4: N in each strata for all pupil measurements.

Many factors are known to change pupil size, such as specific drugs and pharmaceuticals, head trauma, and more. In the literature, it remains unclear if age, eye color, or gender affects pupil size. The data is stratified over the aforementioned variables in addition to luminance interval. Statistical tests were conducted to determine if statistically significant differences exist for each strata as well as mathematically describe the overall data set.

The stratification of the ages was determined by two factors: (i) distribution of ages of subjects measured and (ii) knowledge that the range of pupil size decreases as a function of age beginning in the forties.[46] The age range of subjects in this study was somewhat smaller. If a relationship between pupil size and age was to be seen, at least one division needed to be made between the smallest age and the point at which biological effects are known, such as the forty-year old age group. The ages were divided into ten-year increments: twenties, thirties and forties. The ages were not normally distributed, as shown in Figure 14.

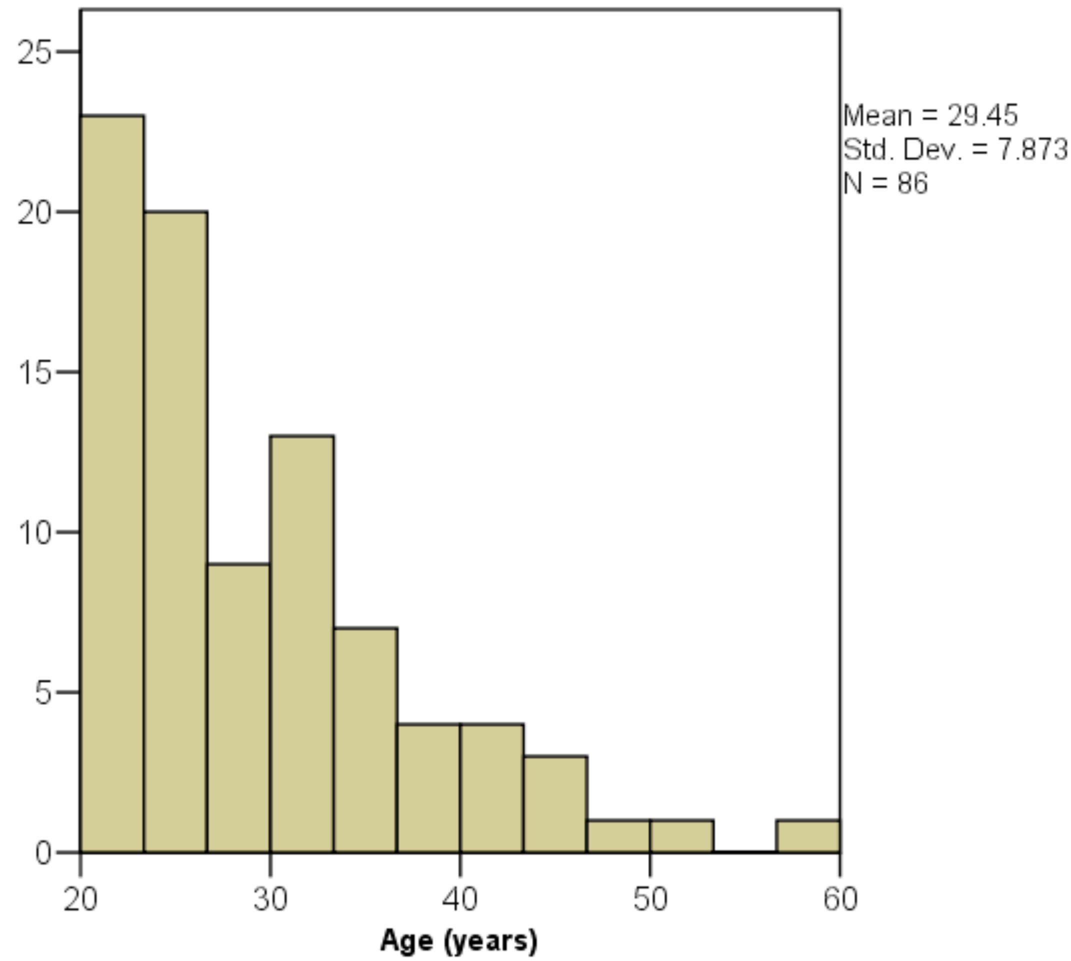

Figure 14: Histogram of age distribution of sample population.

Due to the fact that 61 subjects came from a physical fitness test at a military medical school, the high number of subjects in their twenties was expected, followed by a higher number of subjects in their thirties. Finally, the subjects in the higher age groups primarily were recruited during the running race on the navy base. This distribution may be representative of a military population. Many younger people fill the lower-ranking positions and fewer older people fill the fewer numbers of higher ranks.

Similar to the age distribution, the measurements and range for luminance were not normally distributed. Due to two cloudy days on which the measurements were made, the measurements of luminance are lower for standard outdoor light levels. Since three measurements of luminance for each subject were recorded, the measurements were averaged. Each averaged measurement of luminance corresponds to one subject whose pupils were measured. The mean measurement of luminance was 1470 $cd \cdot m^{-2}$, though the data is not normally distributed (Figure 15). The luminance measurements ranged from 788 $cd \cdot m^{-2}$ to 4250 $cd \cdot m^{-2}$ with one measurement at 7960 $cd \cdot m^{-2}$. Three intervals of luminance in which pupil sizes were analyzed were created based on a logarithmic scale: less than 1000 $cd \cdot m^{-2}$, between 1000 to 2000 $cd \cdot m^{-2}$, and greater than 2000 $cd \cdot m^{-2}$. The majority of pupil measurements were taken between 1000 to 2000 $cd \cdot m^{-2}$. Pupil changes, among other physiological responses, occur in response to stimulus changing on a logarithmic scale.[47] Additionally, the data was stratified against luminance intervals in a similar manner as many other pupillometry studies in the literature.

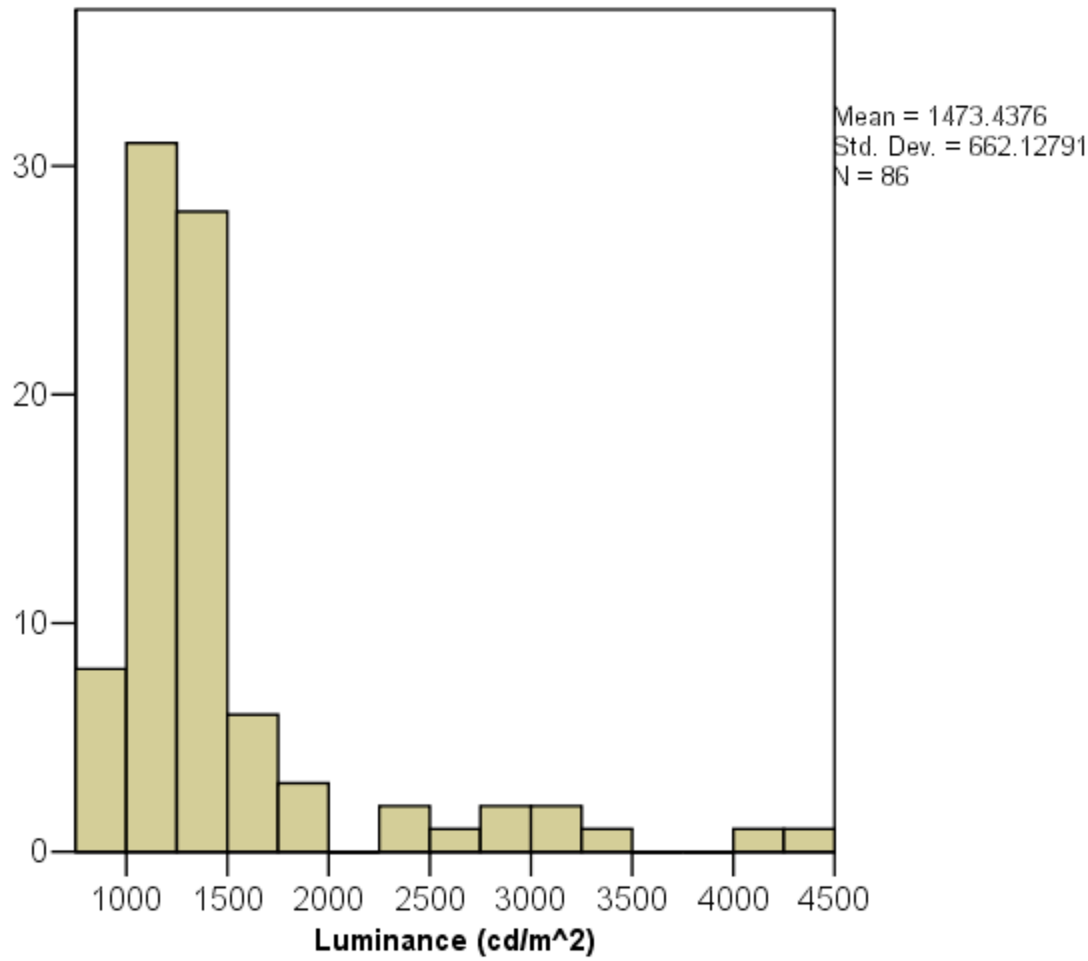

Figure 15: Histogram of luminance measurements for all subjects.

Three pupil size measurements for each eye were recorded for each subject. The left and right eye pupil size measurements were averaged together so each subject had one corresponding average pupil size. No statistical difference existed between the three measurements for the left eye and right eye, and as a result the left and right eye pupil size measurements could be averaged together. Across the range of luminance measurements, the distributions pupil measurements were normally distributed. The distributions passed the Kolmogorov-Smirnov normality test ($\alpha = 0.2$ for left and right eyes, Figure 16).

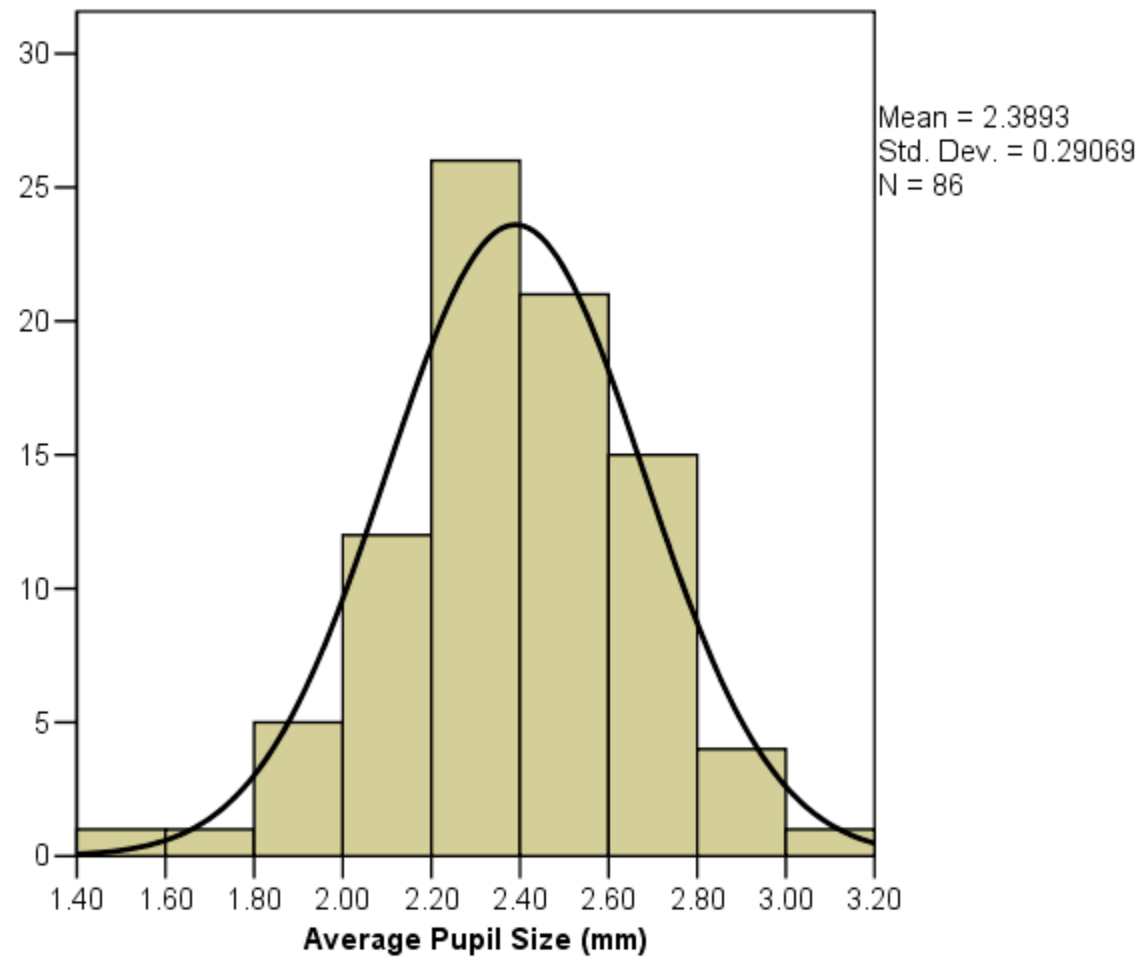

Figure 16: Histogram of all average pupil measurements.
The histogram represents average pupil sizes over all luminance measurements, ages, and genders.

It was also determined that a linear relationship existed between pupil sizes and luminance, even at higher light levels. The F-test statistic for linear regression for pupil sizes was $\alpha = 0.002$ and less than the test statistic $p = 0.05$ which determined that a linear relationship between luminance and pupil size exists for this study. The line was fitted using a least-squares fit method, with a slope of -0.000159 and a y-intercept of 2.62 (Figure 17). The slope seems reasonable given the range of outdoor luminance values. According to this linear model, if an eye is subject to 4000 $cd \cdot m^{-2}$ the corresponding pupil size will be approximately 1.98mm. Although at the upper range of luminance values for outdoor environments, an 8000 $cd \cdot m^{-2}$ luminance measurement would result in a 1.38mm pupil size, which may be physiologically improbable. Although the linear equation for this data set exists at the $p = 0.05$ level at a slope of -0.000159, this may suggest the minimum pupil size for outdoor environments was almost reached.

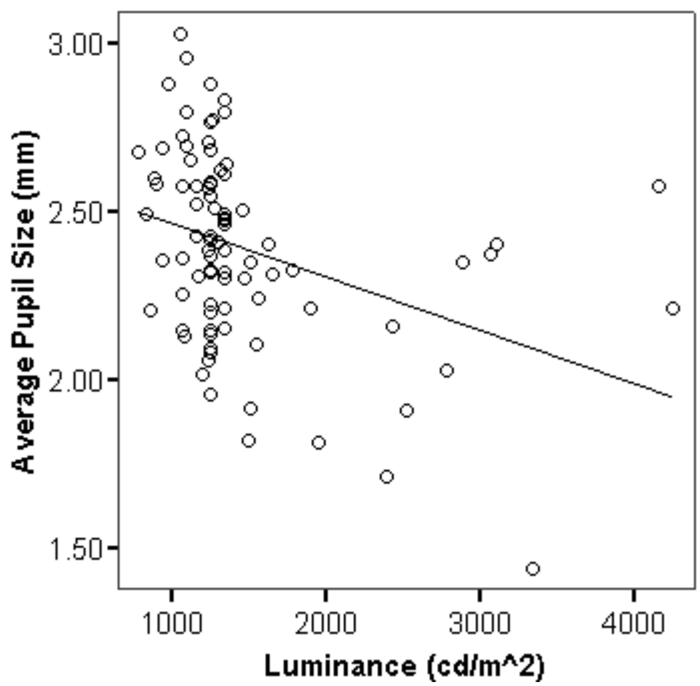

Figure 17: Linear relationship between pupil size and luminance. The straight line was a least-squares fit.

The means were compared between age, gender, and eye color to determine if a significant difference existed between those factors. The first factor to be examined over the range of luminance values was eye color. The questionnaire completed by the subjects at the time of the data collection asked them to document their eye color from several choices: blue/gray, dark brown, amber, hazel, and green. If the subject was unsure of his or her eye color, the principal investigator determined the appropriate color via observation. The aforementioned colors were sorted into one of two categories, dark irides and light irides. Dark irides included dark brown, amber, and hazel. Light irides included blue/gray and green. Though no statistical different existed between the two categories of eye color, the light-colored eyes had a larger range of pupil sizes and luminance values even though the number of light-colored eyes was smaller, as $\alpha = 0.055$ when compared to $p = 0.05$ (Table 5; Figures 18, 19, 20).

	N	*Mean (mm)*	*Std Dev*	*Max*	*Min*	*t-test α Statistic*
Dark	50	2.34	0.287	2.88	1.44	0.055
Light	36	2.46	0.284	3.03	1.71	

Table 5: Descriptive statistics stratified against eye color.

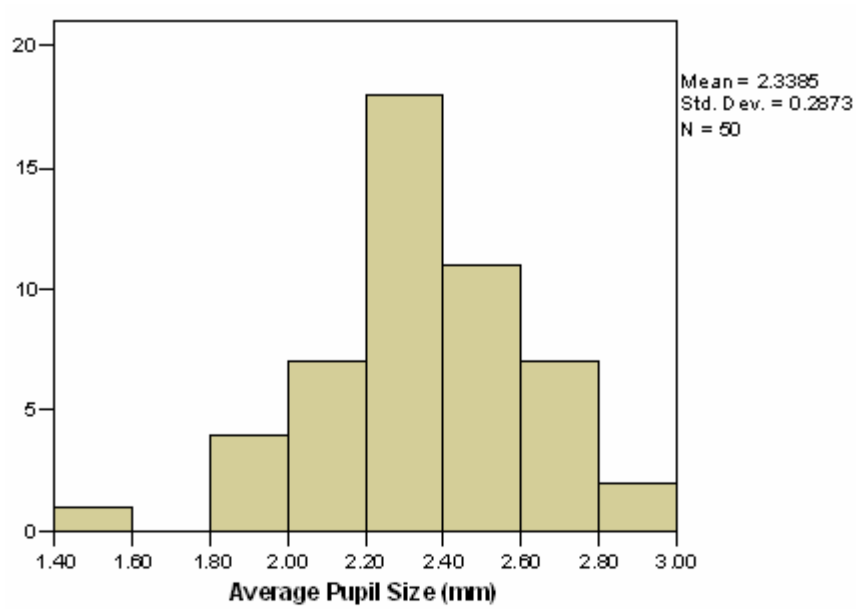

Figure 18: Histogram of average pupil size for dark-colored eyes. The average pupil sizes span all levels of luminance.

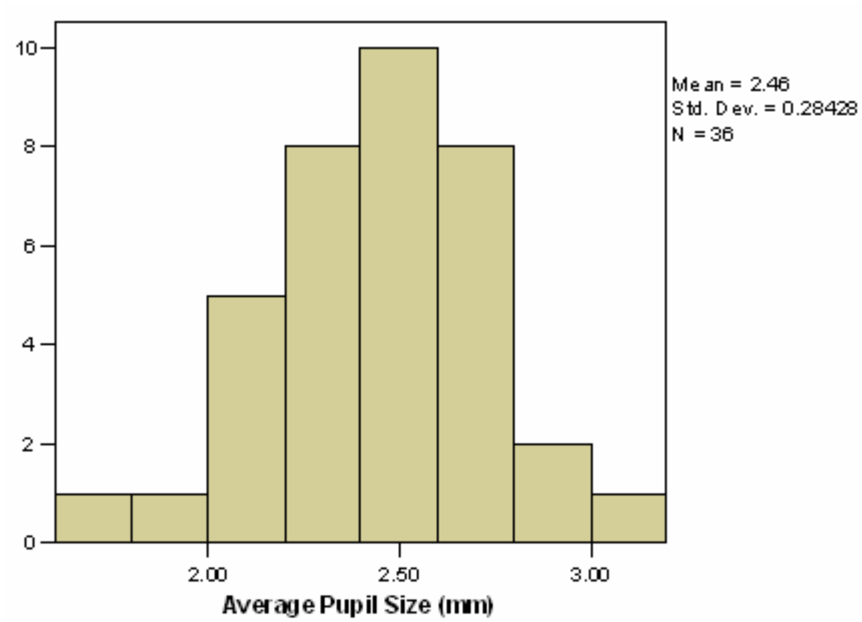

Figure 19: Histogram of average pupil size for light-colored eyes. The average pupil sizes span all levels of luminance.

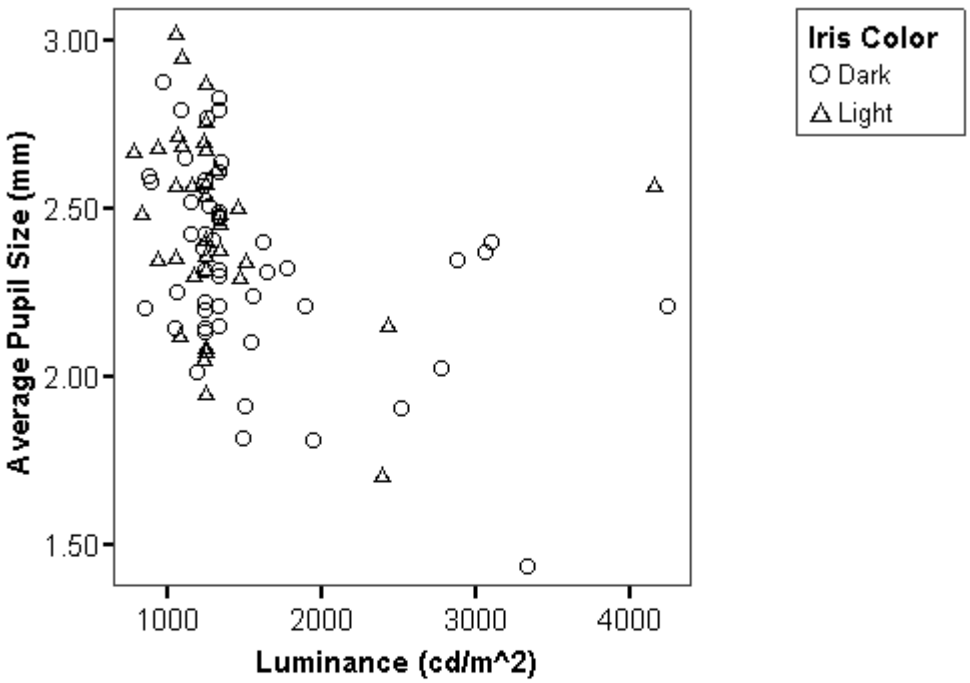

Figure 20: Scatterplot of average pupil sizes stratified against iris color.

For both the left and right eye pupil measurements no significant statistical difference existed between the mean pupil sizes for eyes with dark-colored irides and light-colored irides, compared to a p-value of 0.05. Also, both eye colors for the right and left eyes were normally distributed across the range of luminance ($\alpha = 0.2$ and $\alpha = 0.2$, respectively for the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality). In the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality an α-value greater than the p-value of 0.05 is desired to prove normality. Eyes of both dark and light irides were sampled mostly in the 1000 to 1500 $cd \cdot m^{-2}$ range of luminance.

The data was next stratified using gender. A significant measurable difference in the means exists between male and female subjects in the study. For the pupil size measurements, the statistic $\alpha = 0.000$ was less than the p-value of 0.05, which demonstrates a significant statistical difference in pupil size between male and female subjects in this study. The mean of the pupil measurements of the female subjects was statistically larger by 0.3mm than the mean of the pupil measurements of the male subjects. The distributions of male and female average pupil sizes over the range of luminance values are normally distributed, as determined by the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality ($\alpha = 0.2$ and $\alpha = 0.2$, respectively). Although the distributions of luminance for each gender are almost identical, the pupil size distributions for each gender were not (Figures 21, 22). The mean of the luminance for female subjects was 1458 $cd \cdot m^{-2}$ and the mean luminance for male subjects was 1478 $cd \cdot m^{-2}$. Therefore a strong argument exists proving the statistical difference in average pupil size between genders over the same range of luminance. This result still may hold true even though three times as many male subjects participated in this study than female subjects.

	N	*Mean (mm)*	*Std Dev*	*Max*	*Min*	*t-test Statistic*
Female	22	2.60	0.197	3.03	2.31	0.000
Male	64	2.32	0.284	2.88	1.44	

Table 6: Descriptive statistics stratified against gender.

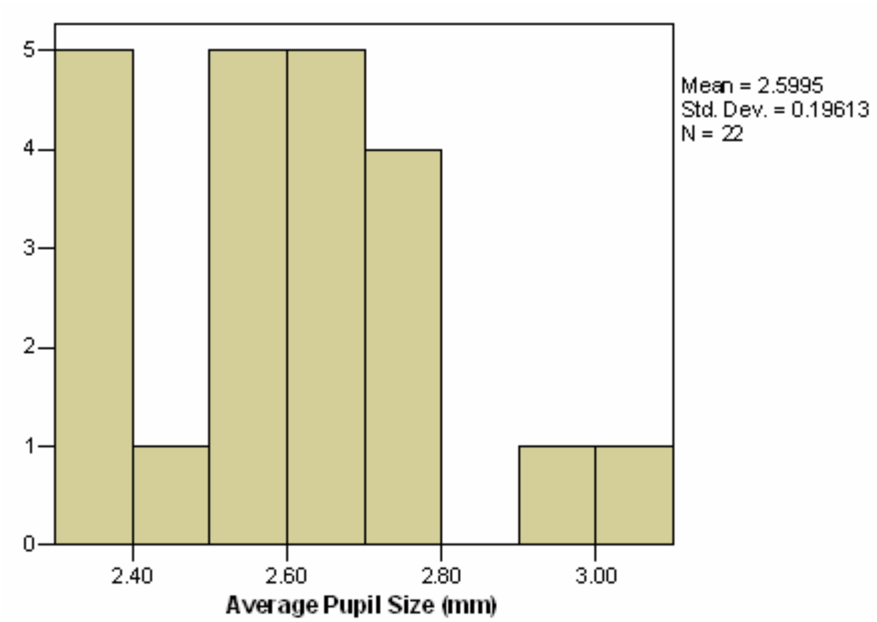

Figure 21: Histogram of average pupil sizes for female subjects.

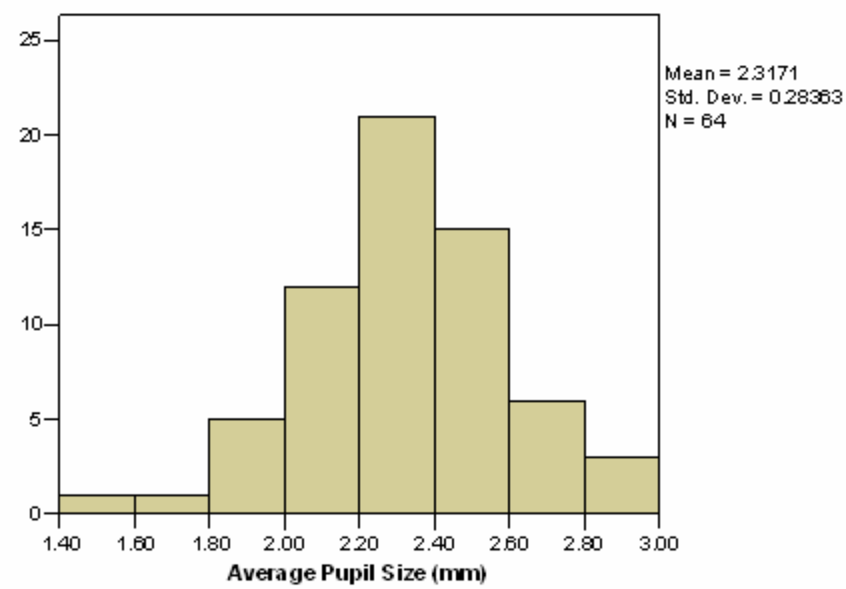

Figure 22: Histogram of average pupil sizes for male subjects.

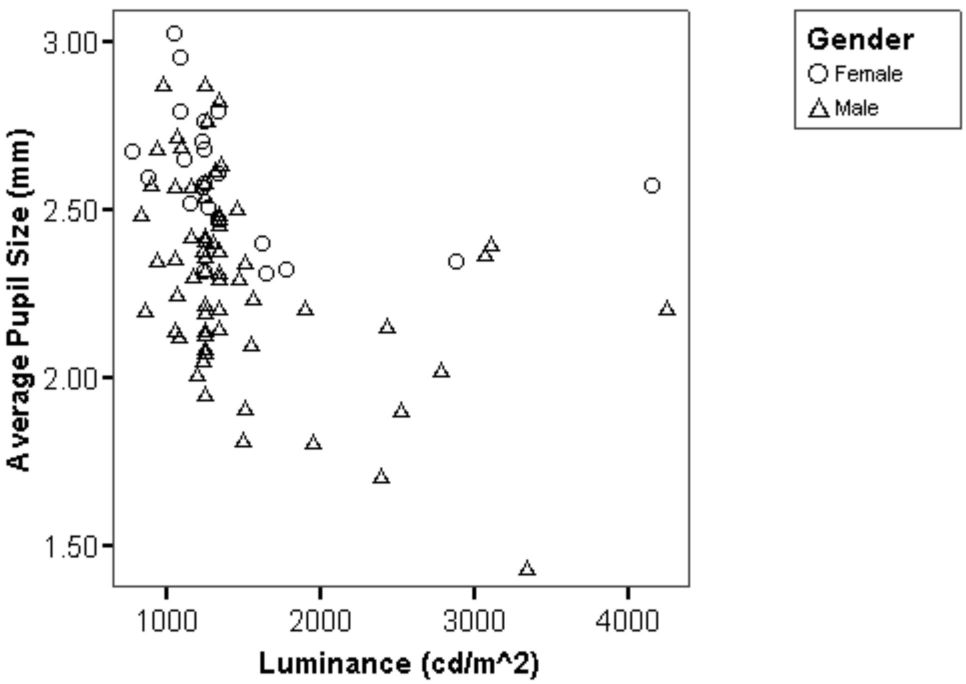

Figure 23: Scatterplot of average pupil size stratified against gender.

Finally, the data were stratified by age. The data were categorized into three age groups: 20-29 years, 30-39 years, and 40 years of age and over. Most of the subjects fell into the 20-29 age group range due to the fact that the military population sampled consisted of people mostly in this age range.

Of the right eye pupil measurements, the average pupil size of each age group is significantly different than each other, when compared to $p = 0.05$ (Table 8). An inverse relationship between age and average pupil size exists in this sample. The mean pupil size for subjects 40 years of age and older was 0.33 mm smaller than the average pupil size for the subjects in their twenties (Table 7). The distributions of luminance for the twenties and thirties age group was very similar, but the distribution for the forties age group had a much higher mean luminance value (1928 $cd \cdot m^{-2}$ compared to 1360 $cd \cdot m^{-2}$ and 1530 $cd \cdot m^{-2}$ for the twenties and thirties, respectively). This may explain a smaller pupil size, but perhaps not a difference of 0.33 mm. The stability of the pupil size as a function of age may also be a factor.

	N	*Mean (mm)*	*Std Dev*	*Max (mm)*	*Min (mm)*
20-29 yrs	52	2.49	0.281	3.03	1.44
30-39 yrs	24	2.29	0.177	2.67	2.06
40+ yrs	10	2.10	0.310	2.58	1.71

Table 7: Descriptive statistics stratified against age.

	20-29 yrs	*30-39 yrs*	*40+ yrs*
20-29 yrs	---	0.002	0.000
30-39 yrs	0.002	---	0.033
40+ yrs	0.000	0.033	---

Table 8: t-test statistics to compare the mean of pupil sizes for each age strata.

The distributions of pupil sizes stratified for age over the entire range of luminance values are normally distributed, as determined by the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality, despite the fact that the age group of 40 years and older consisted of only 10 subjects. Generally the statistical tests used in this study are valid of the number of subjects or data points is 20 or higher

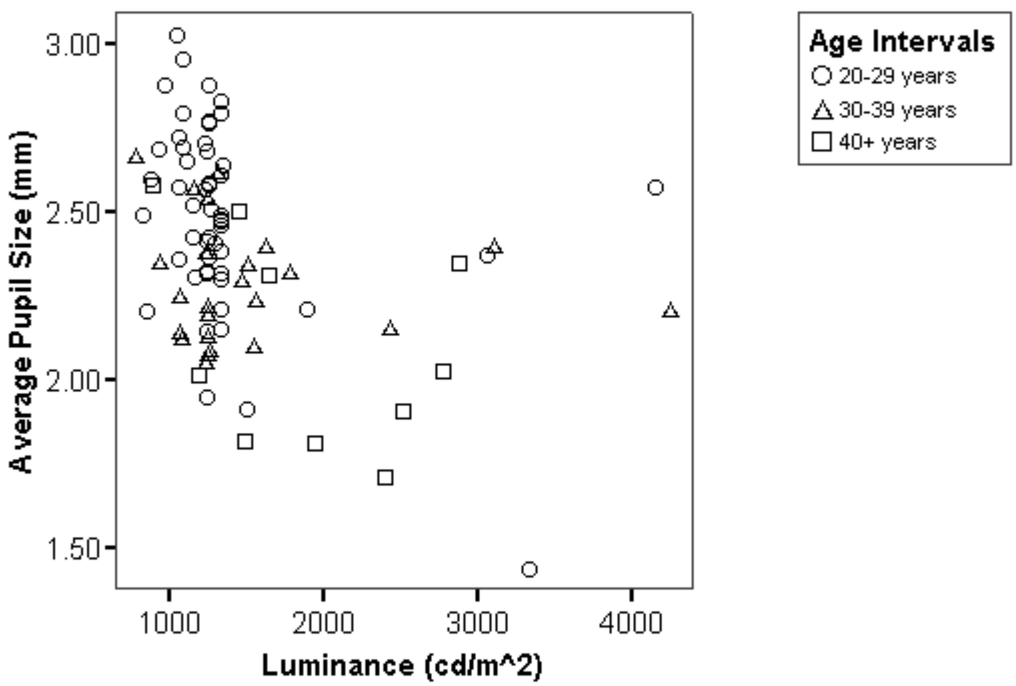

Figure 24: Scatterplot of average pupil sizes stratified against age groups.

Analysis for Luminance Intervals

The next step in the analysis was to categorize the luminance measurements into intervals and examine pupil size data over smaller changes in light level. The three intervals were 788 $cd \cdot m^{-2}$ (lower limit) to 1000 $cd \cdot m^{-2}$, 1000 to 2000 $cd \cdot m^{-2}$, and 2000 $cd \cdot m^{-2}$ to 4253 $cd \cdot m^{-2}$ (upper limit). The results of the statistical analysis upon stratification of the data based on luminance are expected. Higher luminance levels resulted in smaller pupil sizes. Brighter conditions constricted the pupil size. The stratification of the luminance intervals was based on how the luminance levels were stratified in the classical pupillometry studies, which was typically broken down on a logarithmic scale.

The lowest interval of luminance, 788 to 1000 $cd \cdot m^{-2}$, was a result of the consistent cloudy weather during the photography. Nevertheless, the levels of luminance were still consistent with outdoor luminance levels. The average pupil size in this region was 2.56mm.

The highest interval of luminance was 2000-4252 $cd \cdot m^{-2}$, which corresponds to slightly overcast or slightly cloudy daytime conditions outdoors. The average pupil size in this interval was 2.13mm, or 0.42mm smaller than the mean pupil size in the lowest interval of luminance measurements. Table 9 summarizes the number of subjects in each strata as well as the mean, standard deviation, and range of each. Table 10 summarizes the statistically significant differences in the means of the average pupil sizes for each stratification interval.

Luminance ($cd \cdot m^{-2}$)	*N*	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*
788-1000	8	2.56	0.21	2.88	2.21
1000-2000	68	2.41	0.27	3.03	1.81
2000-4253	10	2.12	0.35	2.57	1.44

Table 9: Descriptive statistics for luminance interval stratification.

Intervals in $cd \cdot m^{-2}$	*788-1000*	*1000-2000*	*2000-4253*
788-1000	---	0.131	0.006
1000-2000	0.131	---	0.002
2000-4253	0.006	0.002	---

Table 10: t-test statistics to compare means for luminance intervals.

For the highest interval of luminance, a significant difference in average pupil size exists between that interval and the lower two intervals. One explanation might be the ages of the subjects measured in each level of luminance. The average age of subjects in the 788-1000 $cd \cdot m^{-2}$ and 1000-2000 $cd \cdot m^{-2}$ intervals was 29 years. The average age of subjects in the 2000-4253 $cd \cdot m^{-2}$ interval was 35 years of age. The difference in average age is only 6 years, but the distributions suggest that the older subjects and their subsequent smaller pupil sizes will lower the mean in that interval of luminance.

788-1000 $cd \cdot m^{-2}$ Luminance Interval Stratification

For each luminance interval, the data was further stratified against pupil color, gender, and age. For all stratified data within this interval, the average pupil sizes were virtually the same, with the largest difference between them 0.12 mm. The sample sizes for each strata were very small as well. With such small samples sizes, one may expect a greater difference in the mean values of average pupil size when the data is stratified, but that is not the case with this sample population. Due to the small sample sizes, statistical tests for normality and differences in the means may not be used and the results from these tests may not be valid since the number of data points is less than 20. Therefore analysis must be limited to qualitative means. According to the Student's t-test for comparing means, no significant statistical difference in the average pupil size exists between dark and light irides (Table 11), between male and female subjects (Table 12), and any age group (Tables 13, 14). This may not actually be the case since only two female subjects were tested in this range of luminance.

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*	*t-test statistic*
Dark	4	2.57	0.27	2.88	2.21	0.929
Light	4	2.55	0.16	2.69	2.35	

Table 11: Descriptive statistics for eye color strata for 788-1000 cd·m^{-2}.

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*	*t-test statistic*
Female	2	2.64	0.05	2.68	2.60	0.582
Male	6	2.53	0.24	2.88	2.21	

Table 12: Descriptive statistics stratified against gender.

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*
20-29 yrs	5	2.57	0.25	2.88	2.21
30-39 yrs	2	2.51	0.23	2.68	2.35
40+ yrs	1	2.56	---	---	---

Table 13: Descriptive statistics stratified against age.

	20-29 yrs	*30-39 yrs*	*40+ yrs*
20-29 yrs	---	0.781	0.981
30-39 yrs	0.781	---	0.850
40+ yrs	0.981	0.850	---

Table 14: t-test statistics to compare the mean of pupil sizes for each age strata.

1000-2000 cd·m^{-2} Luminance Interval Stratification

Like the lowest luminance intervals and the overall data set before stratification, the average pupil sizes of eyes with dark-colored irides and light-colored irides are not significantly statistically different (Table 15).

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*	*t-test Statistic*
Dark	39	2.36	2.56	2.84	1.81	0.064
Light	29	2.48	0.27	3.03	1.95	

Table 15: Descriptive statistics for eye color strata for 1000-2000 cd·m^{-2}.

When the data for the 1000-2000 cd·m^{-2} is stratified against gender, a statistically significant difference between the means of the average pupil size for each gender exists (Table 16). The test statistic, compared to p-value of 0.05, indicates that the means are not equal. The average pupil size for female subjects was 0.27mm larger than the average pupil size of the male subjects. In this case, the number of male and female subjects was quite large in each case, thus the statistical test may have merit when compared to the lowest luminance interval. Even though the number of male subjects is greater than female subjects, the mean of the average pupil sizes can be compared.

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*	*t-test Statistic*
Female	18	2.61	0.21	2.32	3.03	0.000
Male	50	2.34	0.25	2.88	1.81	

Table 16: Descriptive statistics stratified against gender.

The final stratification of the data was using age as a variable, which used the same age groups as the overall data analysis. No significant difference existed in average pupil size between the 30-39 year age group and the 40 and older age group

(Tables 17, 18). Conversely, a significant difference in average pupil size exists when comparing the twenties age group to the thirties and forties age groups using a p-value of 0.05. The sample size of the forty and over age group was quite small, yet the standard deviation was close to that of the twenties age group, indicating that the spread of the data may be similar, but again, the small sample size of the oldest age interval may indicate that the statistical tests may not be valid. The range of pupil sizes for the forty and over age group was smaller than the other age groups.

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*
20-29 yrs	44	2.51	0.25	3.03	1.92
30-39 yrs	19	2.27	0.17	2.62	2.06
40+ yrs	5	2.09	0.31	2.51	1.81

Table 17: Descriptive statistics stratified against age.

	20-29 yrs	*30-39 yrs*	*40+ yrs*
20-29 yrs	---	0.000	0.001
30-39 yrs	0.000	---	0.093
40+ yrs	0.001	0.093	---

Table 18: t-test statistics to compare the mean of pupil sizes for each age strata.

2000-4253 cd·m^{-2} Luminance Interval Stratification

The majority of the pupil size data points were in the 1000-2000 cd·m^{-2} luminance interval. Like the 788-1000 cd·m^{-2} data set, this data set for the highest luminance values was also very small, but the results varied from those of the lowest luminance interval.

Again, as expected, the average pupil sizes for the dark and light-colored eyes were virtually the same. The sample sizes were small, but one may still examine the mean values for the average pupil sizes. The greatest difference between the means was 0.05 mm (Table 19).

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*	*t-test Statistic*
Dark	7	2.10	0.35	2.40	1.44	0.866
Light	3	2.15	0.43	2.57	1.71	

Table 19: Descriptive statistics for eye color strata for 2000-4253 cd·m^{-2}.

A difference in the means of the average pupil sizes exists in the gender stratified data set for this luminance interval. A difference of almost half a millimeter in pupil size was calculated from the data of the female subjects and the male subjects for both eyes (Table 20). This result corresponded to the overall data set when stratified against gender, though the difference was approximately 0.30mm as compared to 0.44mm in this data set.

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*	*t-test Statistic*
Female	2	2.46	0.16	2.57	2.35	0.123
Male	8	2.03	0.33	2.40	1.44	

Table 20: Descriptive statistics stratified against gender.

The results of the 2000-4253 cd·m^{-2} data stratification differed from the other luminance intervals. The sample sizes in this luminance interval were very small, similar to the lowest luminance interval. The literature suggested an inverse relationship between pupil size and age exists. These data do not suggest that, although the number of data points in this luminance interval is very small for each age interval (Tables 21, 22).

The average pupil size for the twenties age group was smaller than that of the thirties age group. The forty and over age group still had the smallest average pupil size, consistent with the overall data set and the other stratified data sets. Perhaps this was an artifact of small sample size, as the standard deviation of the twenties age group is quite large, and the pupil size range was large as well. The luminance measurements for each of the three average pupil sizes in the 20-year age group range from approximately 3000 to 4100 cd·m^{-2} but the difference in the maximum pupil diameter and the minimum pupil diameter was 1.14mm.

	N	*Mean Pupil Diameter (mm)*	*Std Dev*	*Max Pupil Diameter (mm)*	*Min Pupil Diameter (mm)*
20-29 yrs	3	2.13	0.60	2.57	1.44
30-39 yrs	3	2.26	0.13	2.40	2.16
40+ yrs	4	2.00	0.27	2.35	1.71

Table 21: Descriptive statistics stratified against age.

	20-29 yrs	*30-39 yrs*	*40+ yrs*
20-29 yrs	---	0.734	0.712
30-39 yrs	0.734	---	0.187
40+ yrs	0.712	0.187	---

Table 22: t-test statistics to compare the mean of pupil sizes for each age strata.

Discussion of Error Analysis in Methods

The design of the experiment attempted to control for as many variables as possible while still preserving the natural illumination conditions in bright outdoor environments. Controlled variables included: (i) age of the subjects, (ii) constant and uniform luminance exposure from the Ganzfeld, (iii) camera and head position in the experimental set-up, and (iv) time of day the experiment was conducted and data was collected. The age distributions of the subjects who participated in the study may represent a general cross section of the military population. The majority of the subjects were in their twenties. Soldiers in this age group would likely be the majority of deployed combat personnel utilizing weapons with laser technology on the battlefield. Next, the Ganzfeld consisted entirely of one texture and color thus providing constant and uniform luminance levels to the subjects' eyes. The experiment took place in the early afternoon hours over three days, approximately 12:00 PM to 3:00 PM. This allowed for maximum luminance exposure to the Ganzfeld. Although weather could not be controlled, the varied weather conditions allowed for a wider range of luminance values, most of which were lower luminance for outdoor conditions. Nevertheless, a wider range of luminance measurements, especially in the higher ranges, would have been desirable.

The first step in determining the error in the measurements was to calculate variation in distance between the subject's eye to the camera lens in the calibration technique. Difference in distance between the eye and the camera lens could affect the size of the pupil in the photographic images.

As previously described, the calibration method for converting micro grid squares (MGS) into millimeters included calculations for error analysis. Briefly, 12 subjects were photographed from the side to determine the closest and furthest eye distances to the camera. This is a relative distance of eye-to-camera while maintaining a constant distance of the camera to other facial features, such as the chin and forehead. Differences in number of MGS to millimeters for each extreme head position represent the uncertainty in each measurement based on differences in head position. When comparing the number of MGS for each millimeter-increment for both the minimum and maximum eye-to-camera distances, the values were the same. Therefore, the error in the measurements due to difference in head position is minimal, and in this case, head position is not a factor in error analysis. The error in the average pupil size of this experiment is less than 1% based on the relative eye-to-camera position.

The error in the luminance measurements by the spot photometer was listed as 4%, +/- 1 digit in the last display position by the manufacturer. In the stratification of the data, the luminance measurements were placed into intervals. Fortunately, the luminance measurements on the end of each luminance interval are not affected by the error of the spot photometer. When the error is added or subtracted to the end values of each luminance interval for each strata, the luminance intervals are still contained within each strata. Next, the luminance measurements are large enough that error inherent to the spot photometer is minimal when compared to the luminance measurement. None of the luminance measurements will change from one interval of the luminance stratification to another when the uncertainty of the measurements was calculated.

Several of the pupils in some photographs could not be measured due to photographic error. For one photo, the zoom feature of the camera malfunctioned and a whole-face photograph was captured. These photographs were destroyed as they were not in compliance with the approved protocol. The left eyes in five photographs were immeasurable because a raindrop landed on the lens of the camera where the left eye image would have been captured. Finally, two subjects blinked in the photographs, and a replacement photograph was acquired. The total data set included 81 average left eye pupils measured from 87 subjects, and 86 average right eye pupils measured from 87 subjects.

The quality of the photographs was limited by the resolution of the digital camera used. The resolution for the camera used in the experiment was 7.4 mega pixels. Second, the accuracy of the measurements was limited by the capabilities of the imaging software. The photographs could be zoomed only to 800%, and measurements were limited by the size of the MGS of the software. Finally, the contrast and brightness features were limited on the software. The software had a small range of contrast and brightness which were not quantifiable.

The pupils of dark irides were somewhat difficult to measure as a result of the flash reflection and reflection of the sun off of the Ganzfeld. Fortunately, the reflection of the Ganzfeld covered the entire pupillary area, so the edges of the pupil could still be determined. This problem occurred only with eye with dark irides. Light colored eyes were not affected. The following photographs (Figures 25-29) demonstrate the difficulty of determining the edges of the pupil through the reflections of the Ganzfeld off of the cornea. The edge of the pupil and iris is very difficult to clearly define.

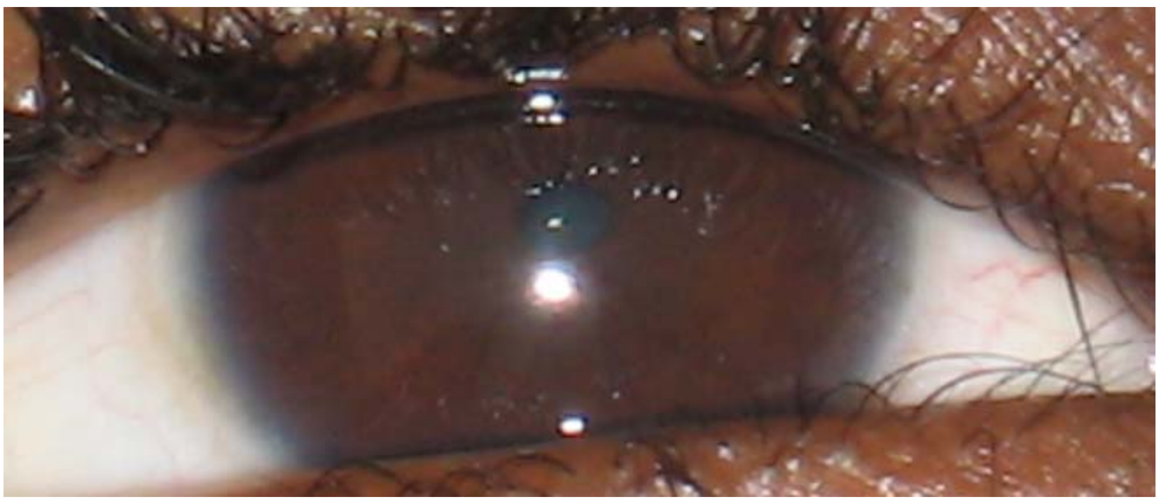

Figure 25: Reflection of Ganzfeld on cornea.

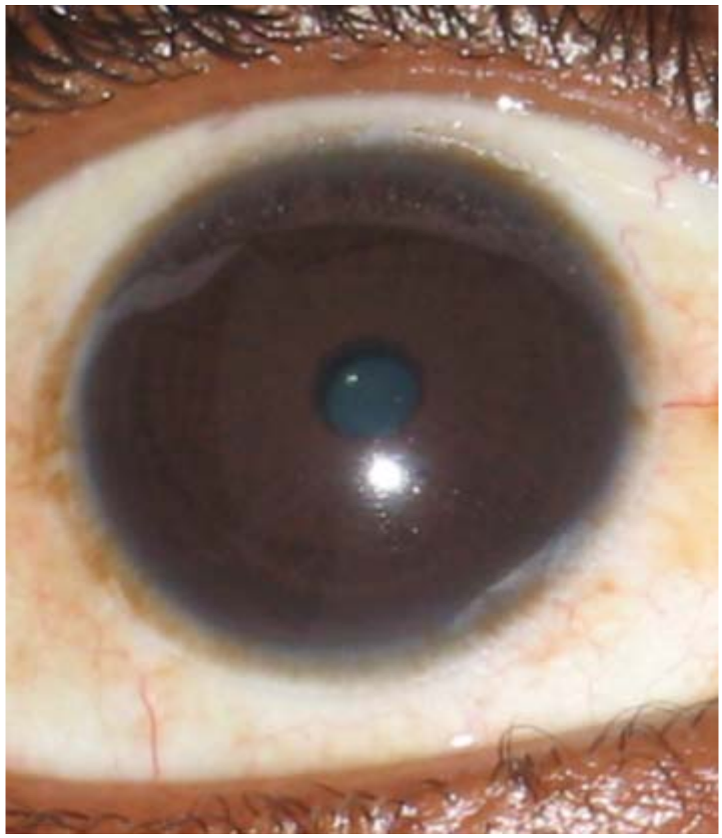

Figure 26: Reflection of Ganzfeld on cornea.

Figure 27: Reflection of Ganzfeld on cornea.

Figure 28: Reflection of Ganzfeld on cornea.

Figure 29: Reflection of Ganzfeld on cornea.

Not all pupils in the study were perfectly circular. Many of them were irregularly shaped. Although many slight variations in pupil size were discovered, the measurement of pupil diameter only consisted of a measurement across the widest horizontal diameter. The elliptical nature of the pupil shapes was not quantified in this study. None of the images of the pupils were rotated in any direction in order to measure the diameter. Additionally, the surface areas of the pupils were not measured. The following figures (Figures 30-33) are examples of photographs of irregularly-shaped pupils measured in the study.

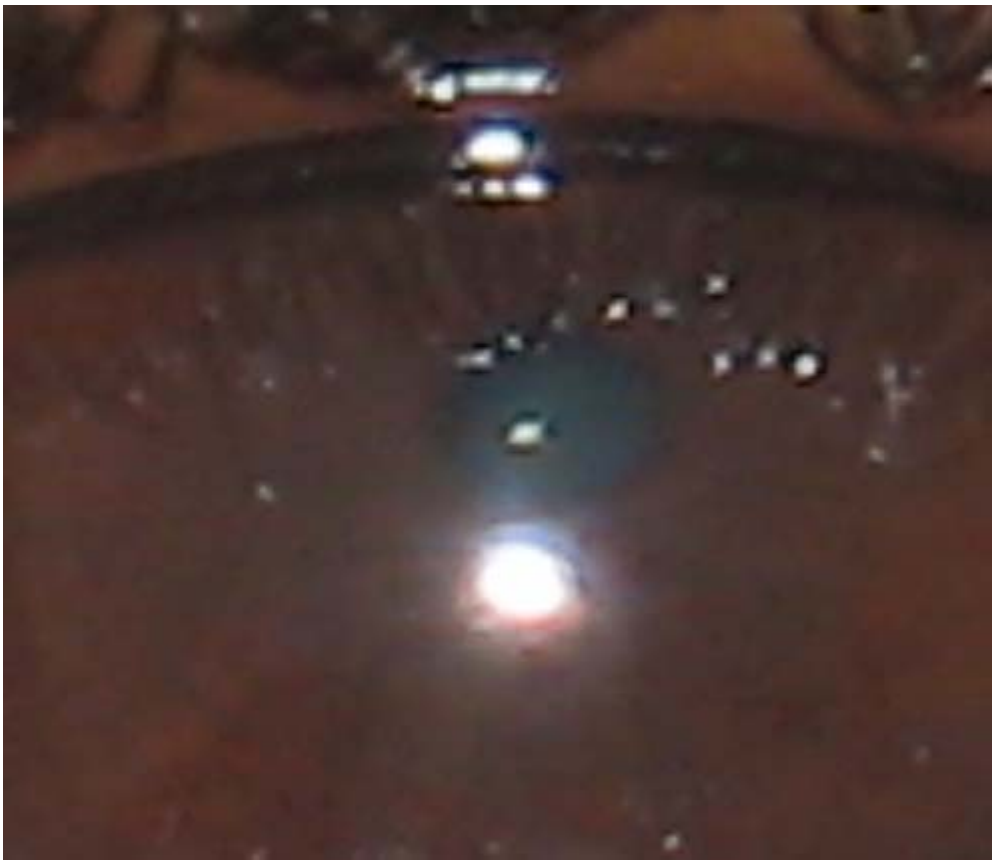

Figure 30: Irregularly-shaped pupil.

Figure 31: Irregularly-shaped pupil.

Figure 32: Irregularly-shaped pupil.

Figure 33: Irregularly-shaped pupil.

Conclusion

The research questions this study attempted to answer were: what is the average pupil size in bright outdoor environments, what is the best way to measure pupils in outdoor environments, what were the highest levels of luminance in classical pupillometry studies that pupils were exposed to, and what is the smallest pupil size in bright outdoor environments?

The overall average pupil size across all measurements of luminance was 2.39mm for an average luminance measurement of 1473 $cd \cdot m^{-2}$. The luminance levels in the study spanned three intervals, each with a corresponding average pupil size. The first interval of luminance 788-1000 $cd \cdot m^{-2}$ had an average pupil size of 2.56mm. The next interval of luminance 1000-2000 $cd \cdot m^{-2}$ had an average pupil size of 2.42mm. The highest luminance interval 2000-4253 $cd \cdot m^{-2}$ had an average pupil size of 2.13mm, the smallest average pupil sizes. The smallest average pupil size measured was 1.44mm. One outlier measurement of 7957 $cd \cdot m^{-2}$ yielded pupil sizes of a 1.82mm average.

This study may have found the limit of pupil size. It would be helpful measure pupil sizes at higher luminance levels and conduct the statistical test for linear regression to examine the correlation between luminance and pupil size.

Significant statistical differences between gender and age across all luminance intervals exist at the p-value 0.05 level, but not between eye color over any luminance interval. Women's pupils were significantly larger across all intervals of luminance. A correlation between age and pupil size also exists. The oldest subjects, those in the 40 years and older age group, had the smallest average pupil sizes across all intervals of luminance; however, there was inadequate numbers to draw a general conclusion from this sample that pupil size diminishes with age (although this is accepted by most clinicians).

Digital photography proved to be the best method for pupillometry in bright sunlight in outdoor environments. This method demonstrated high repeatability and accuracy. This method was easily transportable and very quick to set-up. Measurements were quick to acquire, and wait time for subjects was minimized with this method. The method is quite simple. Accommodation was controlled, it allows for a wide luminance range, and one has lasting documentation of the measurements.

Future work still needs to be done in a variety of areas. More pupil sizes need to be measured at luminance higher than 2000 $cd \cdot m^{-2}$. Infrared photography in bright outdoor environments may still hold potential and is worth exploring, but the utilizing of the retroreflection "red-eye" phenomenon holds more promise for pupillometry by means of digital photography. Finally, if one possesses the resources, an experiment using the same subjects over a wide range of high luminance levels would be worthy. A study such as that might also be able to adequately answer the research questions posed in this study. Nonetheless, a solid methodology for measuring pupil sizes in bright outdoor environments has been developed and produced interesting and relevant results.

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