



Optotype recognition visual acuity in the domestic cat

Daria L.Clark¹, Robert A.Clark^{1,2*}

¹Family Eye Medical Group, 4100 Long Beach Blvd, Suite 108, Long Beach, CA 90807, (USA)

²Department of Ophthalmology, Miller Children's Hospital, Long Beach Memorial Medical Center, 2801 Atlantic Avenue, Long Beach, CA 90807, (USA)

E-mail : drraclark@yahoo.com

ABSTRACT

Behavioral studies estimate feline vision between 3 and 9 cycles per degree (20/67 to 20/200). To determine cat visual acuity using a directly comparable, human optotype recognition task, four cats were trained on a pseudo-random, two-choice discrimination task using HOTV optotypes. The minimum resolvable optotype was then determined by sequentially presenting smaller optotypes at longer distances. The smallest optotype and longest distance successfully completed were confirmed with a second test requiring a minimum 27 correct out of 36 consecutive trials, yielding a binomial probability greater than 0.001 of non-random occurrence. Two of the four cats completed all training and visual acuity testing: M1, a 6.5 year old male gray tabby with +2.00 OU refraction, tested for best visual acuity of 20/74 and F1, a 1.5 year old female gray tabby with +0.25 OU refraction, tested for best visual acuity of 20/33. These results demonstrate that a young cat with good focus is capable of recognition visual acuity of 20/33, in close agreement to the physiologic maximum. Older age and uncorrected focusing errors can degrade visual performance. Good lighting, high contrast targets, long viewing distances, and lack of time pressure resulted in better feline visual acuity measurements than previously described. © 2014 Trade Science Inc. - INDIA

KEYWORDS

Domestic cat;
Discrimination task;
Visual discrimination
learning;
Visual acuity;
Vision.

INTRODUCTION

Behavioral studies estimate feline grating acuity between 3 and 9 cycles per degree^[1-10], or about three to ten times worse than human vision. Cycles per degree can be converted into a Snellen visual acuity equivalent by dividing cycles per degree into 600^[11], converting those behavioral measurements to 20/67 (~ 30 mm Snellen optotype) and 20/200 (~89 mm Snellen optotype), respectively. Based on optical clarity, however, the cat's eye is capable of resolving gratings as fine as

20-30 cycles/degree, depending on pupil dilation^[12], or 20/20 to 20/30 Snellen acuity. Based on the retinal cone density of 1.7 minutes of arc, compared to human cone density of 1.0 minute of arc, the cat's retina has the potential for approximately 20/34 Snellen acuity^[13,14], marginally worse than the maximum optical clarity. There is a large discrepancy, therefore, between the predicted maximum feline acuity based on optics and retinal cone density and the actual feline acuity measured in behavioral experiments. This loss of acuity has been attributed to pooling of retinal cones into smaller num-

Regular Paper

bers of retinal ganglion cells, constraining the retinal sampling rate and decreasing the overall acuity^[15-17]. Diminished numbers of ganglion cells does not explain the three-fold range of experimental measurements of feline acuity, from 20/67 to 20/200, and also raises questions about the purpose for the high cone densities found in the feline retina. Why create a cone array with a high spatial resolution if that information is ignored during upper visual processing?

The prior behavioral studies on feline visual acuity all have important limitations that might explain the lack of precision and consistency in their results. The first limitation is the use of relatively low contrast targets in testing environments without adequate illumination^[3,4,6]. A minimum illumination of 64 cd/m² should be used to stimulate feline photopic cones^[18] and, for comparison, 80-320 cd/m² and 100% contrast (black on white) is considered optimal for human testing^[19]. The second limitation is a confined testing area that limits the cat's viewing distance^[1-8]. Cats have a poor accommodative ability for near visual targets^[20], and that limited accommodation should not be overstressed to focus near targets if the goal is to measure the best possible vision under optimum conditions^[21]. Therefore, the closest target should ideally be more than 100 cm from the eyes, instead of the 25 cm to 80 cm used in these studies^[1-10]. The third limitation is that these studies did not check the optical focus of the cats prior to testing their visual acuity^[5-9]. Refraction to determine the focus must be performed to eliminate the effects of poor focus from compromising visual performance^[9,22,23]. The fourth limitation is the quick sequential presentation of visual targets in an potentially stressful environment^[1-8]. Subjects require adequate time to view and process the targets prior to making a choice to attain their best visual acuity results. Because cats are not working animals by nature, suboptimal results may occur if they are forced to perform dozens of trials per day. Excessive experimentation can lead to loss of motivation and concentration, resulting in an inferior visual acuity measurement. In addition, pupillary dilation from stress, either from the closed environments or from electrical shocks for incorrect choices^[6,7] can blur the vision^[8]. The fifth limitation is dietary restriction to enforce a fast and steady work rate within the test environment^[3-7]. Hunger is unlikely to promote better performance on tests that require fine visual discrimination and target selection,

so while maintaining cats at 80-85% of their normal weight might motivate them to work harder for treats, such restrictions might impair their grating recognition performance.

Perhaps the most important limitation, however, is the character of the targets themselves. Visual acuity is not just detection, but also object recognition^[24]. In order to achieve a human equivalent measurement of visual acuity, the cat should discriminate between two separate, high contrast targets, a task that involves higher centers of visual processing that might enhance or diminish the visual acuity measurement compared with simple grating detection. Because of the cats' central role in human vision studies, including ongoing work in amblyopia^[5,25-27], retinal degenerations^[28,29], and other disorders^[30], accurate feline vision assessment is vital to furthering the understanding of normal human vision and its response to various disease processes. This study attempts to estimate feline recognition visual acuity using techniques directly comparable to human visual acuity testing.

MATERIALS AND METHODS

The subjects were four (three male and one female) spayed or neutered gray tabby mixed breed domestic cats with no known medical or ophthalmic problem. Prior to testing, the refraction was measured in each cat with streak retinoscopy through the undilated pupil^[22,31]. This study strictly adhered to the guidelines within the ISAE Ethical Treatment for Animals^[32]. The cats were maintained on their regular diet, without restrictions to food, activity, or treats, throughout the study. Additionally, vision testing was conducted based on subject interest. During the day, the cats roamed freely within a two-story home. They signaled readiness for participation by queuing outside the testing room in anticipation of performing for their treat. This "on demand" testing limited the total number of trials to 3-6 per day throughout the testing period.

To facilitate direct comparisons with Snellen visual acuity measurements, distances were recorded in feet rather than meters. Trials were conducted in a windowless, 10 ft. by 11 ft. uniform white room with light beige carpeting illuminated by two 13-watt compact fluorescent bulbs (60 watt incandescent equivalent), generating 140 cd/m² measured luminance. The testing room

was diagonally divided in half by a series of 2 ft. tall, thin wooden panels of varying lengths beginning at the far corner facing the entrance (Figure 1). The first wooden panel was 2 ft. long and placed at a 45-degree angle from the corner. This panel was not moved and provided a minimum distance from the walls to access the treats behind the visual targets. Two additional 4 ft. long panels were marked in 1 ft. intervals and extended from the corner panel towards the room entrance. These panels could be extended or overlapped to create a variable 6 ft. to 10 ft. room divider, in 1-foot increments. Depending on extension, the end of the divider was 6 to 9 feet from the room entrance and was defined as the choice point for target selection.

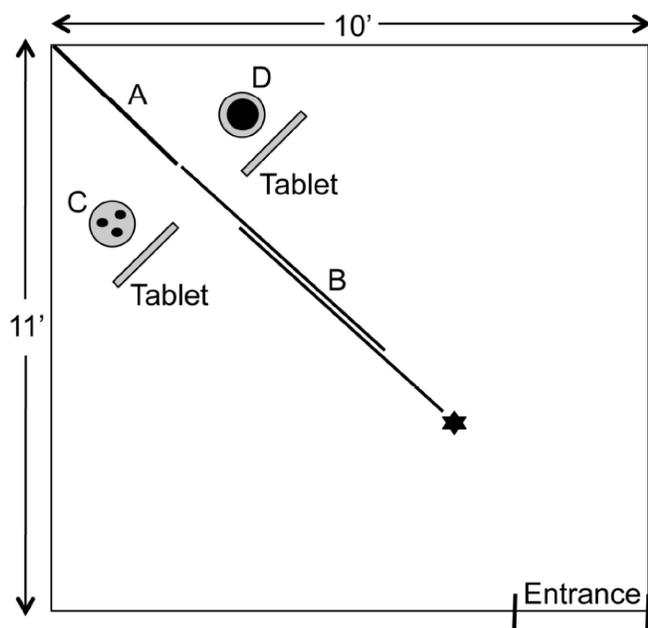


Figure 1 : Diagram of the windowless, 11 ft. x 10 ft. testing area. The room was divided in half diagonally by a series of 2' tall, thin wooden panels: 'A' marks a fixed 2 ft. long panel and 'B' marks two 4 ft. long sliding panels that could be overlapped to provide an adjustable 6 ft. to 10 ft. divider. The tablets were set up perpendicular to the barrier a variable distance behind the choice point (marked with the star). The treat container 'C' with the perforated lid was placed behind the tablet displaying the negative optotype and the treat container 'D' with a large central opening in the lid was placed behind the tablet displaying the positive optotype.

Two iPad 2 tablets (Apple®, Cupertino, CA) were used to present the optotypes. The tablets were set to maximum brightness (luminance equal to 410 cd/m² for white and 0.43 cd/m² for black for a contrast ratio of 99.9%)^[33] and were encased within identical black plastic and rubber stand cases oriented in landscape mode

tilted 15 degrees from vertical.

For optotype selection, the preverbal Landolt C and Tumbling E eye charts were rejected as too similar to gratings because they test for detection – find the gap in the optotype – instead of recognition. One of the simplest recognition charts is the HOTV eye chart, commonly used to screen toddlers as young as 30 months^[34,35]. "O" was chosen as the positive optotype because it matched the shape of the cats' usual dry food diet. "T" was chosen as the negative optotype because of its distinct vertical and horizontal elements, providing a clear contrast with the positive target. Targets were created as a single, centered black optotype on a white background in different sizes from 20/60 to 20/10 to exactly match a standard HOTV eye chart.

All trials were two-choice discrimination tasks with the location of the positive target following a pseudo-random Fellows sequence^[36]. To set up each trial, identical treats were hidden in plastic containers behind each tablet. The negative target hid a container with a lid that was perforated with small holes to allow scent to escape but no access, while the positive target hid a container with a lid with a large, central opening to allow access (Figure 1). After the targets concealing the treats were in place at the proper distance behind the choice point, the cats were admitted one at a time into the testing area. Initially, video surveillance was attempted but deemed unsuccessful because the cats appeared anxious when left alone in the room, sitting motionless looking back toward the room entrance, and did not try to choose a target. Instead, a human observer remained in the room near the entrance during each trial, positioned out of view when the cats faced the visual targets. This individual was careful to avoid any verbal or nonverbal cues, only providing stereotypic comments such as, "Find the circle" or "Go get your treat".

The cats were allowed unlimited time to view the targets, but were removed from the room without a treat if they lost interest in the task (e.g. laying on the ground, grooming, exploring the room, etc.) and no result was recorded for that trial. If the cat passed the choice point headed towards the positive target, the trial was recorded as a success; the cat received positive encouragement and was allowed to eat the treat. If the cat passed the choice point headed towards the negative target, the trial was recorded as a failure; the

Regular Paper

cat was removed from the room without any positive feedback or treat.

The visual acuity testing was divided into three phases. The training phase began with detection training to choose the 20/60 "O" optotype versus a blank, white screen. The visual targets were presented at the choice point (zero depth), 9 feet from the room entrance. Various combinations of treats were tested to determine a combination that produced the highest work rate. The best work rate was obtained using a few pieces of dry, solid cat treat intermixed with a few pieces of beef jerky, and that combination was used throughout the study. Successful training was defined as achieving a binomial probability of non-random occurrence at the 0.01 level: 7 consecutive correct choices, 9 correct out of 10 trials, 11 correct out of 13 trials, 13 correct out of 16 trials, or 16 correct out of 20 trials. After successfully completing detection training, recognition training proceeded using the same 20/60 "O" optotype versus a 20/60 "T" optotype at a 2 ft. depth from the choice point, utilizing the same success criteria.

Once the cats passed the training phase for both detection and recognition, the next phase was the optotype challenge. Using a 4 ft. depth, successively smaller optotypes were presented as the cats achieved the same success criteria. The cats failed an optotype size when they completed two consecutive Fellows sequences^[36] below 50% correct (less than 12 out of 24 consecutive trials correct). After failure at 4 ft., testing resumed with the smallest successful optotype at 4 ft., this time presented at increasing depth from the choice point based on the success criteria, until the failure criteria was met again.

The final phase was the optotype confirmation phase. Testing for the smallest successful optotype at the longest successful distance was repeated for three consecutive Fellows sequences^[36]. To confirm success, each cat needed to correctly identify 27 out of 36 consecutive trials, achieving a binomial probability of non-random occurrence at the 0.001 level. The Snellen equivalent vision was then calculated using the size of that optotype and that distance from the choice point (e.g. 20/20 optotype at 5 ft. translates to 20/80 acuity at 20 ft.). This final acuity represents a conservative estimate because the cats made their decision a variable distance before the choice point, a longer distance than was used for this calculation.

RESULTS

Two of the male cats could not complete training and were disqualified from the rest of the experiment. One cat became anxious when placed in the test room and would not look for the treat; the other cat would not consistently work for the treat. The remaining male (M1 – 6.5 years old, refraction +2.00 OU) and female (F1 – 1.5 years old, refraction +0.25 OU) cats completed the training and were used for the optotype challenge and confirmation phases.

During the detection phase of training, M1 reached success criteria with 9 out of 10 correct after 27 total trials over 9 days. F1 reached success criteria with 11 out of 13 correct after 61 total trials over 21 days. F1 initially appeared to try multiple guessing strategies – alternation, same side, and other side based on prior test failure – before learning to recognize the target. During the recognition phase of training, M1 reached success criteria with 11 out of 13 correct after 116 total trials over 26 days. M1 had initial difficulty transitioning to distinguishing between the two optotypes (recognition instead of detection), but gradually improved to successfully complete the training phase. F1 reached success criteria with 11 out of 13 correct after only 18 total trials over 6 days.

During the optotype challenge phase, M1 progressed to successfully pass the 20/15 optotype at 4 ft., then failed the 20/10 optotype at 4 ft. M1 attempted 20/15 at 6 ft., but failed again. At this point, his choices clearly deteriorated to an alternation strategy based on prior failure instead of optotype recognition. He was retested with the 20/15 optotype at 4 ft. and reached the failure criteria quickly. Due to his performance degeneration into strategic guessing, the decision was made to retrain M1 with the 20/40 optotype at 1 ft. to relearn the recognition task. The retraining took 55 trials to reach the success criteria. M1 then quickly progressed to successfully complete the 20/30 optotype at 2 ft., the 20/20 optotype at 2 ft., and finally the 20/15 optotype at 2 ft. At that point, he was deemed ready to proceed to the optotype confirmation phase using the 20/15 optotype at 4 ft.

F1 progressed to successfully pass the 20/10 optotype at 4 ft., the smallest optotype available on the standard HOTV eye chart. F1 then reached failure criteria with the 20/10 optotype at 7 ft., but successfully passed

the 20/10 optotype at 6 ft. She was deemed ready to proceed to the optotype confirmation phase using the 20/10 optotype at 6 ft.

During the optotype confirmation phase, M1 confirmed the 20/15 optotype at 4 ft. by correctly choosing 29 out of 36 trials (binomial probability = 0.00012). This optotype and distance translated to a 20/74 Snellen equivalent, or 8.1 cycles per degree. F1 confirmed the 20/10 optotype at 6 ft. by correctly choosing 31 out of 36 trials (binomial probability = 0.000005). This optotype and distance translated to a 20/33 Snellen equivalent, or 18.2 cycles per degree.

DISCUSSION

This study demonstrates that cats are capable of much higher recognition visual acuity than previously measured^[1-10]. A young cat with near-perfect focus and adequate lighting can resolve a high contrast 20/10 optotype (~ 4.4 mm in size) at a relatively long viewing distance of 6 ft., converting to a Snellen visual acuity measurement of 20/33. This value closely matches the cat's physiological limits of optical clarity^[12] and retinal cone density^[13,14], and provides evidence against the contention that upper visual processing created by the smaller numbers of retinal ganglion cells compared with retinal cones blurs the visual potential of the cat's optical system^[14].

The measurement of a higher feline acuity than previously suspected reinforces the importance of maintaining a physiologic environment to achieve optimal results in behavioral experiments. Motivated cats in brightly illuminated, *ad lib* environments, unsurprisingly, far surpassed the results obtained when cats were constrained within dark, enclosed testing areas with restricted movement. In addition, simple observation of normal feline stalking behavior provides ample evidence that a cat takes time to process visual information prior to making the decision to strike. The human observer within the testing room noted similar controlled, deliberate behavior as the cats approached the visual targets and choice point. Visual acuity testing in humans is not a speed or intelligence test, and accurate testing in young children or those with developmental issues requires patience. The goal of behavioral testing is maximize performance by allowing the subject to process all information within their environment before making

a choice.

M1 demonstrates the significance of ascertaining the refraction prior to testing visual acuity. In the cat, uncorrected refractive error has been shown to blur the optical system by approximately 25% per 0.50 diopters of defocus^[9]. Thus, the two diopters of hyperopia measured in M1 effectively doubles the size of minimum resolvable details by creating approximately 100% image blur compared with perfect focus. If F1's results represent the maximum potential visual acuity in a young cat with near-perfect focus, a doubling in the size of discernable details should be enough to decrease M1's optotype resolution from 20/33 to 20/66, close to his measured acuity. Accommodation can overcome some of that hyperopia^[20], but M1 clearly did not possess enough accommodative ability to overcome his hyperopia, resulting in his relatively poor performance on visual acuity testing.

This study has several important limitations. Only two subjects completed the experiment, reflecting the difficulty of training cats, but this small number is comparable to other animal behavioral experiments in literature. Furthermore, relatively few total trials were performed, creating more weight for each trial within the results. Allowing the cats to progress quickly down the eye chart while maintaining their interest in the experiment in a free roaming environment required careful planning and efficient execution. The confirmation phase, requiring more successful trials at a higher level of statistical certainty, was essential to validate the results.

Ideally, the experiment would have been conducted with a hidden observer and video surveillance. It is possible that the cats received some cue within the testing room, either verbal or nonverbal, from the human observer that influenced their choices despite a concerted effort to eliminate any clues. Repetitive testing failures at the same optotype level, however, provide strong evidence against any factor other than visual acuity affecting these results.

Another potential limitation was the use of a single optotype rather than simultaneously presenting multiple optotypes in a line. Studies in humans suggest better visual acuity measurements for single optotypes in both normal and especially amblyopic subjects^[37,38]. The study's goal, however, was to determine maximum visual acuity, not detection of amblyopia or other patho-

Regular Paper

logic conditions. The use of a single optotype made the test less confusing for the cats and prevented a detectable difference in luminance from influencing the results. Even the largest optotype, 20/60, only covered 1.2 % of the screen, so the luminance was overwhelmingly white on both tablet screens even during detection training.

In conclusion, this study demonstrates that young cats with ideal focus are capable of recognition visual acuity of 20/33. Optimum conditions, such as adequate lighting, time, and space, are required to produce accurate results during the physiologic testing of higher cortical functions like visual acuity. Conducting fewer trials per day and allowing more time for completion prevents factors such as intelligence, fatigue, apprehension, and inattentiveness from affecting the results and producing a sub-maximal performance. Uncorrected focusing problems and age can significantly degrade visual performance. These results provide evidence that the cat visual system may not be constrained by retinal ganglion cell density, but instead is capable of maximum resolution near the physiologic limits created by the optical clarity and retinal cone density of the feline eye.

REFERENCES

- [1] L.Harris; Contrast sensitivity and acuity of a conscious cat measured by the occipital evoked potential, *Vision Res.*, **18**, 175-178 (1978).
- [2] M.Berkley, D.Watkins; Grating resolution and refraction in the cat estimated from evoked cerebral potentials, *Vision Res.*, **13**, 403-415 (1973).
- [3] T.Pasternak, W.Merigan; The luminance dependence of spatial vision in the cat, *Vision Res.*, **21**, (1981).
- [4] T.Pasternak, et al.; The role of area centralis in the spatial vision of the cat, *Vision Res.*, **23**, 1409-1416 (1983).
- [5] M.Loop, et al.; Acuity, luminance, and monocular deprivation in the cat, *Behav Brain Res.*, **2**, 323-334 (1981).
- [6] R.Blake, S.Cool, M.Crawford; Visual resolution in the cat, *Vision Res.*, **14**, 1211-1217 (1974).
- [7] S.Jacobson, K.Franklin, W.McDonald; Visual acuity of the cat, *Vision Res.*, **16**, 1141-1143 (1976).
- [8] S.Hall, D.Mitchell; Grating acuity of cats measured with detection and discrimination tasks, *Behav Brain Res.*, **44**, 1-9 (1991).
- [9] Bonds; Optical quality of the living cat eye, *J.Physiol.*, **243**, 777-795 (1974).
- [10] J.Jarvis, C.Wathes; On the calculation of optical performance factors from vertebrate spatial contrast sensitivity, *Vision Res.*, **47**, 2259-2271 (2007).
- [11] M.Yanoff, J.Duker; 'Ophthalmology 3rd Edition', 1552, Mosby, Elsevier Health Sciences, Maryland Heights, Missouri (2008).
- [12] J.Robson, C.Enroth-Cugell; Light distribution in the cat's retinal image, *Vision Res.*, **18**, 159-173 (1978).
- [13] R.Steinberg, M.Reid, P.Lacy; The distribution of rods and cones in the retina of the cat (*Felis domesticus*), *J.Comp.Neur.*, **148**, 229-248 (1973).
- [14] R.Blake; Cat spatial vision, *Trends Neurosci.*, **11**, 78-83 (1988).
- [15] J.Stone; A quantitative analysis of the distribution of ganglion cells in the cat's retina, *J.Comp.Neur.*, **124**, 337-352 (1965).
- [16] Hughes; A quantitative analysis of the cat retinal ganglion cell topography, *J.Comp.Neur.*, **163**, 107-128 (1975).
- [17] Hughes; Cat retina and the sampling theorem: The relation of transient and sustained brisk-unit cut-off frequency to alpha and beta-mode cell density, *Exp.Brain Res.*, **42**, 196-202 (1981).
- [18] P.Hammond, G.Mouat; The relationship between feline pupil size and luminance, *Exp.Brain Res.*, **59**, 485-490 (1985).
- [19] J.Sheedy, I.Bailey, T.Raasch; Visual acuity and chart luminance, *Am.J.Optom.Physiol.Opt.*, **61**, 595-600 (1984).
- [20] M.Bloom, M.Berkley; Visual acuity and the near point of accommodation in cats, *Vision Res.*, **17**, 723-730 (1977).
- [21] L.Ostrin, A.Glasser; Accommodation measurements in a prepresbyopic and presbyopic population, *J.Cataract.Refract.Surg.*, **30**, 1435-1444 (2004).
- [22] K.Konrade, et al.; Refractive states of eyes and associations between ametropia and age, breed, and axial globe lengths in domestic cats, *Am.J.Vet.Res.*, **73**, 279-284 (2012).
- [23] L.Rose, U.Yinon, M.Belkin; Myopia induced in cats deprived of distance vision during development, *Vision Res.*, **14**, 1029-1033 (1974).
- [24] C.Kniestedt, R.Stamper; Visual acuity and its measurement, *Ophthalmol Clin.North.Am.*, **16**, 155-170 (2003).
- [25] Mitchell, J.Kennie, K.Duffy; Preference for binocular concordant visual input in early postnatal development remains despite prior monocular dep-

- rivation, *Vision Res.*, **51**, 1351-1359 (2011).
- [26] G.Gringas, D.Mitchell, R.F.Hess; The spatial localization deficit in visually deprived kittens, *Vision Res.*, **45**, 975-989 (2005).
- [27] J.Schroder, et al.; Ocular dominance in extrastriate cortex of strabismic amblyopic cats, *Vision Res.*, **42**, 29-39 (2002).
- [28] C.May, K.Narfstrom; Retinal capillary morphology in the Abyssinian cat with hereditary retinal degeneration, *Exp.Eye.Res.*, **99**, 45-47 (2012).
- [29] R.Blake; Visual acuity in cats with central retinal lesions, *Vision Res.*, **18**, 15-18 (1978).
- [30] D.Leonard, et al.; Ultrastructural analysis of hydraulic and abrasive retinal pigment epithelial cell debridements, *Exp.Eye.Res.*, **76**, 473-491 (2003).
- [31] C.Murphy, K.Zadnik, M.Mannis; Myopia and refractive error in dogs, *Invest Ophthalmol Vis.Sci.*, **33**, 2459-2463 (1992).
- [32] C.Sherwin, et al.; Guidelines for the ethical use of animals in applied ethology studies, *Appl Anim.Behav.Sci.*, **81**, 291-305 (2003).
- [33] R.Soneira; Apple iPad 2 and iPhone 4 LCD display shoot-out (accessed December 16th, 2012), available in updated form on at http://www.displaymate.com/iPad_ShootOut_1.htm, June 29th, (2012).
- [34] S.Cotter, et al.; Visual acuity testability in African-American and Hispanic children: the multi-ethnic pediatric eye disease study, *Am.J.Ophthalmol.*, **144**, 663-667 (2007).
- [35] M.Rice, D.Leske, J.Holmes; Comparison of the amblyopia treatment study HOTV and electronic-early treatment of diabetic retinopathy study visual acuity protocols in children age 5 to 12 years, *Am.J.Ophthalmol.*, **137**, 278-282 (2004).
- [36] B.Fellows; Chance stimulus sequences for discrimination tasks, *Psychol.Bull.*, **67**, 87-92 (1967).
- [37] Y.Morad, E.Werker, P.Nemet; Visual acuity tests using chart, line, and single optotype in healthy and amblyopic children, *J.AAPOS*, **3**, 94-97 (1999).
- [38] Y.Bonneh, D.Sagi, U.Polat; Local and non-local deficits in amblyopia: acuity and spacial interactions, *Vision Res.*, **44**, 3099-3110 (2004).