

Contrast gain control before and after cataract surgery; a case study

D.I.A. MacLeod\* & S. Anstis\*\*

\*E-mail: [dmacleod@ucsd.edu](mailto:dmacleod@ucsd.edu), Telephone: (858) 534-3975, Fax: (858) 534-7193.

\*\*E-mail: [sanstis@ucsd.edu](mailto:sanstis@ucsd.edu), Telephone: (858) 534-5456, Fax: (858) 534-7190.

**Purpose:** Cataracts may greatly reduce the contrast of the retinal image, but the visual consequences of this contrast loss could be mitigated by neural adaptation (provided that the contrast of the stimulus is sufficient for it to be visible at all). We sought evidence for such neural adaptation. **Methods:** We investigated the suprathreshold perception of contrast, as well as contrast sensitivity, in one observer. We measured the attenuation of perceived contrast using binocular matches of contrast to reference stimuli viewed by the fellow eye. In addition the contrast differences needed for discrimination at a range of baseline contrast levels were measured in each eye. Such data were collected in the days immediately following surgical cataract removal, starting immediately after the eye patch was removed, as well as pre-operatively. **Results:** Pre-operatively, suprathreshold stimuli viewed by the cataract eye were judged at least a factor of three higher in contrast than expected on the basis of contrast threshold, so that contrast matches between the two eyes were more accurate than expected on the basis of the threshold difference. This suprathreshold contrast boost persisted post-operatively, so that now gratings could look higher in contrast to the operated eye than to the fellow eye. Contrast discrimination thresholds conformed to Weber's Law, and were similar for the two eyes. **Conclusions:** The binocular contrast matches reveal a neural 'contrast constancy' process that was able to compensate partly for the optical loss of contrast introduced by the cataract, producing a close approach to accurate contrast matching preoperatively and an 'overshoot' of perceived contrast post-operatively.

## Introduction

Cataract, or clouding of the lens of the eye, is one of the commonest causes of visual impairment. It is age-related, occurs more often in women than in men, and affects from half to three-quarters of all people aged 70 or over throughout the world ([1]; [2]; [3]). But from our point of view, a cataract offers an instructive experiment of nature. It grossly impairs visual acuity, especially reducing sensitivity to high spatial frequencies, and often lasts over a period of months or years. Relatively simple surgery can rapidly restore visual function almost to pre-cataract levels. Finally, the intact fellow eye provides an excellent control. But what is the experiment? We asked whether the pronounced long-term blur produced by the cataract would lead to *compensatory neural adaptation*. In normal vision, Webster, Georgeson and Webster [4] have observed short-term neural adaptation to blur. They found that a slightly-blurred test picture looked apparently sharper following adaptation to a strongly-blurred image, and it looked apparent more blurred following adaptation to a sharply focused image. We predicted that the visual blur and reduced retinal contrast imposed by a cataract over a period of up to two years might lead to long-term adaptation in the form of a compensatory neural boost in perceived contrast for those patterns that have enough contrast to be visible at all. Such an adaptation has been observed in a case of congenital bilateral cataract [5], where perceived contrast at high spatial frequencies was exaggerated post-operatively. The perceptual contrast losses encountered at very high spatial frequencies in normal vision exhibit a similar compensation. The loss of contrast is much less marked above the contrast threshold than at threshold: threshold sensitivity may be markedly reduced, largely owing to optical contrast losses, yet observers assess contrast much more accurately above threshold than their insensitivity at threshold might suggest [6]. We investigated the possibility that this *contrast constancy* above threshold also arises as an adaptation to cataract. Both these adaptations might be measurable both before and after the corrective surgery. Pfoff and Werner [7] and Delahunt, Webster, Ma and Werner [8] have examined the effects of cataract surgery on contrast sensitivity and chromatic mechanisms respectively, finding evidence of neural plasticity in the latter case.

In their investigation of contrast constancy, Georgeson and Sullivan [6] measured contrast thresholds for sinusoidal gratings over a range of spatial frequencies, and obtained a standard contrast sensitivity function, namely a U-shaped curve that was lowest (best performance) around 1—2 cycles per degree of visual angle (cpd). They then exposed a standard grating of (say) 1 cpd at 1 log unit above threshold, and invited observers to match to it the apparent contrast of different spatial frequencies. The curves of equal apparent contrast were not U-shaped curves above and parallel to the threshold curve, but instead were almost straight horizontal lines. They dubbed this phenomenon ‘contrast constancy’ since it demonstrates the visual system’s ability to perceive object contrast independent of size or spatial frequency, even though these affect both retinal contrast and detectability. Fletcher and Munson [9]: reviewed by Suzuki & Takeshima [10] reported analogous auditory curves of constant loudness.

Like other perceptual constancies, contrast constancy is evidence that the visual system infers the stable properties of objects from the changing properties of retinal images.

Georgeson and Sullivan suggested an underlying mechanism, in which proportionate increases in contrast produce greater neural returns at spatial frequencies where sensitivity was poor. Such a disproportionate increase in perceived contrast above threshold might also be helpful as an adaptation to contrast losses associated with cataract. If so, neural compensation for the optical losses created by the cataract should be more evident in suprathreshold contrast matching than at threshold.

## Methods

Investigative procedures, including informed consent procedures, were approved by the UCSD Institutional Review Board of UC San Diego and were in compliance with the tenets of the Declaration of Helsinki.

*Medical history.* In 1981 our subject, the second author, was diagnosed with bilateral open-angle glaucoma and was put on a routine regimen of eye drops. By 2003 the glaucoma had progressed to such an extent that a trabeculectomy was performed on the right eye to reduce the intraocular pressure. Probably as a side effect of the surgery, a cataract developed in the right eye over the ensuing two years until the patient's acuity had fallen to 20/400. This low level of acuity is classified as legal blindness: the patient could just barely make out the largest E on a standard optometric chart, and he could just barely resolve his own fingers at arm's length against a low-contrast background. In December 2005 the cataract lens in the right eye was removed and replaced by an implanted intraocular lens. This produced an immediate and striking improvement: acuity in the operated eye rose from 20/400 to 20/25 in post-operative measurements made the day after surgery. (Subsequently, corneal abrasions and a wavy corneal surface caused the acuity in the operated eye to fall back to 20/60).

*Experimental details.* Pre-operative measurements were made five days before the surgery. The post-operative measurements were begun as soon as possible, 24 hours after the surgery, shortly after the eye patch was removed. Exposure of the operated eye to visual stimuli in the clinic after patch removal was kept to a minimum, in order to minimize any perceptual learning by the operated eye, and we estimate it totaled 30—60 minutes. The eye patch was replaced and the observer was driven to the lab. The patch was then removed from the right eye and the observer made a series of dichoptic contrast matches.

*Forced-choice contrast sensitivity functions at threshold.* Before and after surgery we measured contrast thresholds in the feasible range of spatial frequencies, using one eye at a time. In the forced-choice procedure, on each trial a grating stimulus appeared for 200 msec either on the left or on the right half, randomly determined, of the monitor screen in a 12° high by 8° wide field illuminated at 50cd/m<sup>2</sup>. By pressing a key the observer indicated on which side he saw the grating. The contrast was adjusted by a staircase procedure over 100 trials at each frequency to define the contrast sufficient for detection with 75% reliability.

*Suprathreshold contrast matches.* To allow the observer to compare suprathreshold contrasts for left and right eyes, two vertical sinusoidal gratings were flashed up simultaneously, side by side on the screen for 200 ms. A vertical septum ran from the

observer's nose to the vertical midline of the monitor, so that the left eye viewed the left-hand grating and the right eye viewed the right-hand grating. Separate fixation points for the two eyes, each close to the screen midline, were binocularly fused to ensure that the two gratings were never fused binocularly but were always seen side by side. The observer pressed the left or right response button to indicate that the left or right grating appeared to have the higher contrast. The grating on the right side of the screen, seen by the operated eye, had a fixed reference contrast, and the computer adjusted the contrast presented to the left eye for the next trial in accordance with the subject's responses, thus converging (over 100 trials) on a contrast that was equally likely to be considered too much or too little to match the reference.

*Contrast discrimination.* Finally, the precision of contrast discrimination was assessed separately for each eye by removing the septum and patching one eye. A grating of fixed contrast appeared on one side of the screen, randomly selected. On the other side, grating contrast was varied using a parallel adaptive search algorithm that tracked (on interleaved trials) either the contrast judged higher on 83% of trials, or the contrast judged higher on only 17% of trials. Half the difference between these values, based on 100 trials, was our measure of the contrast difference threshold.

## Results

### 1. Contrast Sensitivity Functions at Threshold

*Pre- and post-operative forced-choice contrast sensitivity functions.* We measured separate CSFs for the cataract and fellow eyes both before and after surgery (Figure 1). Before surgery, the cataract eye showed massive losses in contrast sensitivity with a contrast threshold higher (worse) than the fellow eye by 0.6 log units at 2 cpd, 1 log unit at 3.5 cpd, and 1.33 log units at 6.5 cpd. Surgery produced a vast improvement in the cataract eye, in effect shifting the CSSF up and to the right. However, despite the improvement its performance was still well below the fellow eye. Below 5 cpd the contrast sensitivity of the cataract eye was nearly half a log unit worse than for the fellow eye, and this performance gap increased steadily with increasing spatial frequency. The difference spectrum (post-op minus pre-op; dashed curve in Fig. 1) shows the improvements produced by the surgery, which were greatest at higher spatial frequencies.

### 2. Pre-operative suprathreshold contrast sensitivity as a function of frequency

*2a. Full contrast reference in the cataract eye.* Dichoptic contrast matches were made by exposing a grating of full contrast (a contrast of 1.0) and spatial frequency 0.25, 0.5, 1, 2 or 2.5 cpd to the cataract eye, and gratings of adjustable contrast but matching spatial frequency to the other eye. The resulting contrast matches are shown in Figure 2a. For comparison, the top edge of the graph would represent veridical matches, where a grating would look the same to both eyes, and the lower curve shows the hypothetical contrast matches that would be predicted from the thresholds alone (Fig. 1) if there were no neural boost in the cataract eye. The upper curve shows the actual results. At each spatial frequency examined, the apparent contrast of full contrast gratings seen with the cataracts

exceeds what would be expected from the poor contrast sensitivity of the cataract eye assessed at threshold. The implied boosts in supra-threshold contrast were 0.4, 0.52, 0.53, 0.61 and 0.41 log units (mean = 0.49 log units) at spatial frequencies of 0.25, 0.5, 1, 2 and 2.5 cpd respectively.

*2b. Near-threshold reference contrast.* To investigate whether the improvement in the impaired eye's contrast sensitivity above threshold occurs only at high contrast, or is present over most of the supra-threshold contrast range, dichoptic contrast matches were also made at a reference contrast of 0.3 in the cataract eye, a value not far above threshold at the highest spatial frequencies of interest. Again the lower curve of Figure 2b, a vertically shifted version of the one in Figure 2a, shows the hypothetical contrast matches that would be predicted from the thresholds alone if equal multiples of threshold contrast appeared the same. At the lower spatial frequencies for which this low contrast reference is easily visible, the results (upper curve in Fig. 2b) show a boost of comparable magnitude to that seen at full contrast, about 0.5 log units. At 1 cpd, the highest frequency we could test, the boost is diminished, perhaps because the threshold condition is approached.

### *3. Pre-operative contrast matches as a function of reference contrast.*

To further characterize the way that perceived contrast varies with physical contrast above threshold, we selected a spatial frequency of 0.5 cpd, a frequency for which the cataract eye retained a useful range of contrasts above threshold. Dichoptic contrast matches were made by simultaneously flashing a grating of 0.5 cpd to each eye. Various reference contrasts were presented to the pre-op cataract eye in random order, while the contrast of the other eye's grating was adjusted by the observer over successive trials until the contrasts seen by the two eyes appeared to match. The resulting contrast matches are shown in Figure 3. In Fig. 3, the upper dashed line of unit slope shows the hypothetical, veridical contrast matches that one might expect if no cataract existed and if visual processing had been optically and neurally normal in both eyes. The lower dashed line of unit slope predicts the hypothetical results if equal multiples of contrast threshold appear the same, as expected if the two eyes differ optically (because of the cataract) but not neurally. In that case the relative contrast sensitivity of the two eyes would be determined by the optical contrast loss, and would be the same above threshold as it is at threshold.

The actual results shown in Fig. 3 lie along the inflected curve. At the very lowest contrasts, which are close to threshold, the matching curve lies close to the lower curve, implying equal multiples of threshold match, or that the eye-specific contrast loss of 0.8 log units reflected in the thresholds produces a corresponding asymmetry in the near-threshold matches. But the curve quickly bends up, to approach the line of equal physical contrast when threshold is substantially exceeded.

For medium or high contrasts ( $> -0.75$  on the log contrast x-axis, or  $\geq 0.2$  in contrast) presented to the cataract eye, the physical contrast required by the fellow eye for a match remains less than the reference contrast, but by a relatively small amount (0.25 log units). This suggests that a 0.8 log unit optical loss is offset in this contrast range by a compensatory neural boost of  $0.8 - 0.25$  or nearly 0.6 log units (a factor of 4), and that

this compensation is present over nearly the whole suprathreshold contrast range. This behavior is an instance of contrast constancy [6].

#### *4. Post-operative contrast matches as a function of reference contrast.*

If the visual system continues to boost contrasts for the cataract eye post-operatively, then contrasts in the operated eye should continue after surgery to appear greater than expected from the threshold sensitivities. To test this, the dichoptic contrast matches were repeated post-operatively. The resulting contrast matches are shown in Figure 4. The upper line of unit slope shows veridical contrast matches.

In this case, the near-threshold region was not fully explored, as the lowest tested contrasts were well above the post-operative threshold. Just as in Figure 3, the post-operative contrast matches show proportionality between test and reference at safely suprathreshold levels. Now, however, the matches obtained conform fairly closely to the line indicating veridical matching of physical contrasts. In fact, the two lowest contrast gratings (0.1 and .2, or -1 and -.7 in log contrast) now looked slightly higher in contrast to the cataract eye than to the fellow eye. Evidently the suprathreshold boost that was insufficient, even at this low frequency, to compensate fully for pre-operative contrast loss in Figure 3, was sufficient or even too much to compensate for the slight remaining post-operative loss.

#### *5. Post-operative suprathreshold perceived contrast as a function of frequency*

The pre-operative dichoptic contrast matches indicate a supra-threshold compensation that did not vary markedly with spatial frequency in the testable range. To test if this remained so postoperatively, we made dichoptic contrast matches using a reference grating of contrast 0.2 in the operated eye. This is a relatively low contrast, but is detectable at frequencies not detectable pre-operatively, for instance 5 cpd, as well as at lower frequencies that were pre-operatively detectable. The resulting contrast matches are shown in Figure 5. The horizontal line at  $y = -0.7$  represents equality in physical contrast (veridical matches), whilst the lower curve shows the hypothetical contrast matches that would be predicted from the threshold difference in post-operative contrast sensitivity (Fig. 1) if there were no neural boost in the cataract eye—that is, if equal multiples of the contrast threshold were perceived as equal in contrast. The actual results (upper curve) indicate a boost of about half a log unit at the lowest spatial frequencies (0.25 to 1 cpd), as previously seen (which in this case is enough to make the matches approximate physical contrast matches), but there is no evidence of a boost at the newly testable frequency 5 cpd. We return to this point below.

#### *6. Pre-operative contrast discrimination.*

In Figure 3 when a grating presented to the cataract eye is increased from a just visible contrast of 0.15 to an only slightly greater contrast of 0.2, the contrast required in the fellow eye to match it goes up at least threefold. If these changes in physical contrast—by a small factor in the cataract eye, and a larger factor in the fellow eye—are not only equal in terms of subjective contrast but are also equally detectable, it would follow that contrast discrimination by the cataract eye must be correspondingly more precise, in the sense that the cataract eye detects smaller percentage changes in contrast than the fellow eye. To investigate this point we measured forced-choice contrast discrimination

threshold for each eye in turn, at a range of contrast levels. A superiority of the cataract eye is expected in the contrast range where the contrast matching curve of Figure 3 is steepest, near contrasts of .15 or .2, or about -0.75 in log contrast on the x-axis. The results (Figure 6) clearly contradict this expectation. Contrast discrimination thresholds for the two eyes are almost identical. For each eye, Weber's Law for contrast is closely approximated, with a Weber fraction of about .075—that is, the contrast difference needed for contrast discrimination is .075 times the reference contrast across the whole measured range of reference contrasts. We discuss the implications of this below.

## Discussion

Let us summarize our results.

*Before* surgery, the cataract grossly reduced contrast sensitivity, particularly at high spatial frequencies (Figure 1). It also reduced perceived contrast of gratings, especially at low contrasts. More interestingly, dichoptic contrast matches (Figures 2 and 3) revealed a pronounced compensatory neural boost in perceived contrast for the cataract eye, though never enough to make a given grating look as high in contrast to the cataract eye as to the normal eye.

*After* surgery, the cataract eye showed a huge and immediate improvement in threshold contrast sensitivity, although it did not quite catch up with the normal fellow eye. In addition, our results (Figure 4) suggest that apparent contrasts using the cataract eye remained at least a factor of three higher than expected on the basis of contrast threshold sensitivity measures. Under some conditions (Figure 5) this was now enough to make a given grating look higher in contrast to the cataract eye than to the normal eye.

Evidently a contrast constancy process was able to compensate partly for the optical loss of contrast introduced by the cataract: the right eye's neural apparatus differs from the left eye's in some way that reduces the loss of suprathreshold apparent contrast in the right eye. Our observations place little constraint on the nature of this difference. One significant point, however, is that it did not alter contrast discrimination; the Weber fraction, or just noticeable different between two contrasts, was unaffected. Where Weber's Law applies to contrast discrimination, optical contrast losses are no disadvantage, so it is not surprising that the cataract eye performs as well as the fellow eye. More surprising, perhaps, is the absence of the superiority near threshold that the neural boost idea had led us to expect. Our results on this point are not, however, inconsistent with the hypothesis of a neural boost, for the following reason.

A boost in contrast gain that is applied at a relatively late stage of processing will not necessarily behave like an increase in supra-threshold stimulus contrast. Such a boost will bring no benefit in forced-choice contrast discrimination if the random "noise" that prevents reliable judgment of small differences originates early in visual processing, before the stage at which the boost is applied. In that case, the noise as well as the signal will be boosted, and these effects will cancel. Taking this idea further, one could abandon the assumption, implicit in all our discussion, that contrast matching is done by equating neural signals that have a simple dependence on the stimulus, and that undergo the

postulated boosting. Instead, the noise-contaminated afferent neural signals from the two eyes could merely provide the data on the basis of which a central neural processor makes an estimate of contrast. On this view, equality of the afferent signals would not be required for a binocular match in contrast: experience could inform the central processor that a weak signal from the eye with cataract might imply the same contrast as a stronger signal from the fellow eye. The concept of a boosting or change in gain may therefore be too simple.

Finally, in section 5, we noted that Figure 5 suggests no compensatory enhancement of contrast at spatial frequencies that were pre-operatively undetectable by the cataract eye. This might appear surprising, since it is at the higher frequencies that the cataract eye has been most disadvantaged and is most in need of help. But then, enhancement is of little use when there is nothing to enhance. Our observations here are consistent with other evidence that minor losses can be compensated in neural development, while major ones lead to failure to exploit even the impoverished information available. Reductions of acuity of neural origin due to optical deprivation [5] are a case in point.

In conclusion, this investigation takes advantage of the opportunity offered by the second author's cataract surgery to demonstrate that the loss of contrast introduced by a cataract can be partially restored in perception (provided there is enough contrast for the target to be seen at all). The neural adaptation process responsible remains operative for at least a few days post-operatively.

## **Acknowledgments**

Supported by NIH grant EY01711

## References

1. Weale, R.A., *Age and the transmittance of the human crystalline lens*. J Physiol, 1988. **395**: p. 577-87.
2. Young, R., *Age-related Cataract*. 1991, New York: Oxford University Press.
3. Brown, N.P., *Classification and pathology of cataract*, in *Oxford textbook of ophthalmology*, E.D.S. JM, Editor. 1999, Oxford University Press: New York. p. 474-482.
4. Webster, M.A., M.A. Georgeson, and S.M. Webster, *Neural adjustments to image blur*. Nat Neurosci, 2002. **5**(9): p. 839-40.
5. Fine, I., et al., *Long-term deprivation affects visual perception and cortex*. Nat Neurosci, 2003. **6**(9): p. 915-6.
6. Georgeson, M.A. and G.D. Sullivan, *Contrast constancy: deblurring in human vision by spatial frequency channels*. J Physiol, 1975. **252**(3): p. 627-56.
7. Pfoff, D.S. and J.S. Werner, *Effect of cataract surgery on contrast sensitivity and glare in patients with 20/50 or better Snellen acuity*. J Cataract Refract Surg, 1994. **20**(6): p. 620-5.
8. Delahunt, P.B., et al., *Long-term renormalization of chromatic mechanisms following cataract surgery*. Vis Neurosci, 2004. **21**(3): p. 301-7.
9. Fletcher, H., Munson, WA, *Loudness, its definition, measurement and calculation*. Journal of the acoustical Society of America, 1933. **5**: p. 82-108.
10. Suzuki, Y. and H. Takeshima, *Equal-loudness-level contours for pure tones*. J Acoust Soc Am, 2004. **116**(2): p. 918-33.

## Figure 1

**Pre- and post-operative contrast sensitivity functions.** Pre-operative contrast sensitivity functions for the normal eye (upper curve, filled squares) and the cataract eye (lower curve, open circles). Contrast sensitivity for the normal eye is approximately flat for spatial frequencies ranging from 0.5 to 2.5 cpd. Contrast sensitivity for the cataract eye is massively impaired, being 0.6 log units below the fellow eye at 0.5 cpd and with the deficit steadily increasing up to 1.4 log units (a factor of 25) at 2.5 c.p.d. Middle curve (filled circles) shows CSF of cataract eye 24 hours post-surgery. Comparison with the open circles shows an enormous improvement, indicated by the dashed curve and crosses which show improvement approaching a factor of 10 at high frequencies. But performance is still well below the fellow eye, with a deficit of approximately 0.4 log units at low frequencies.

## Figure 2: Pre-operative dichoptic contrast matches.

A. Matches to unity contrast in the cataract eye. Horizontal top edge of graph ( $\log y = 0$ ) shows hypothetical veridical matches, where a grating would look the same to both eyes. Lower curve shows hypothetical contrast matches predicted from the threshold data of Figure 1a, without any neural boost. Upper curve shows actual contrast matches. These suggest a neural boost of roughly a factor of 3 for contrast matching relative to threshold.

B. Matches to 0.3 reference contrast in the cataract eye. The suggested boost here is similar despite the use of a reference contrast closer to threshold.

**Figure 3: Pre-operative binocular suprathreshold contrast matches at 0.5 cpd.** Upper line of unit slope would indicate veridical matches, in which a given grating looks the same contrast to both eyes. Lower line of unit slope would indicate matching of equal multiples of threshold contrast. The threshold contrast is about 0.8 log units higher in the cataract eye, and that inequality is preserved along this line. Actual matches (solid curves) show a transition as physical contrast increased. A near-threshold log contrast of -1.0 shown to the cataract eye was matched by a log contrast of -1.8 in the fellow eye, as expected from the difference in thresholds. However, contrasts of 0.2 (-.7 log contrast) or more shown to the cataract eye were matched with only a 0.25 log contrast reduction in the fellow eye. This suggests a compensatory neural boost of 0.55 log units in suprathreshold contrast matching. Results for 2 sessions are plotted separately as open and filled circles.

## Figure 4. Post-operative dichoptic contrast matches at 0.5 cpd.

Upper line of unit slope: equal physical contrasts. Lower line: equal multiples of threshold. Dashed curve and error bars: contrast matches. Contrasts shown to the post-operative eye were matched not to similar multiples of threshold, but to similar or significantly higher contrasts in the fellow eye. This suggests that the suprathreshold boost enjoyed by the operated eye was sufficient, or even excessive, to compensate for the slight remaining post-operative optical loss.

## Figure 5: Post-operative dichoptic contrast matches to 0.2 contrast in the cataract eye.

Horizontal line at  $\log y = -0.7$  would represent matching of physical contrast. Lower curve

[Insert Running title of <72 characters]

shows hypothetical matches, predicted from threshold curves in Figure 1b with no neural boost. Upper curve shows actual matches. Contrast matches were almost veridical from 0.25 to 2.5 cpd, but fell back toward the threshold prediction at 5 cpd. Vertical gap between the upper and lower curves shows the presumed neural boost, which was approximately 0.4 log units over the range from 0.25 to 2.5 cpd.

**Figure 6: Pre-operative contrast discrimination by each eye**

Forced-choice contrast discrimination threshold for left eye (filled circles) and right eye (open circles), with Weber line. **Performance is about the same in both eyes.**

Figure 1.

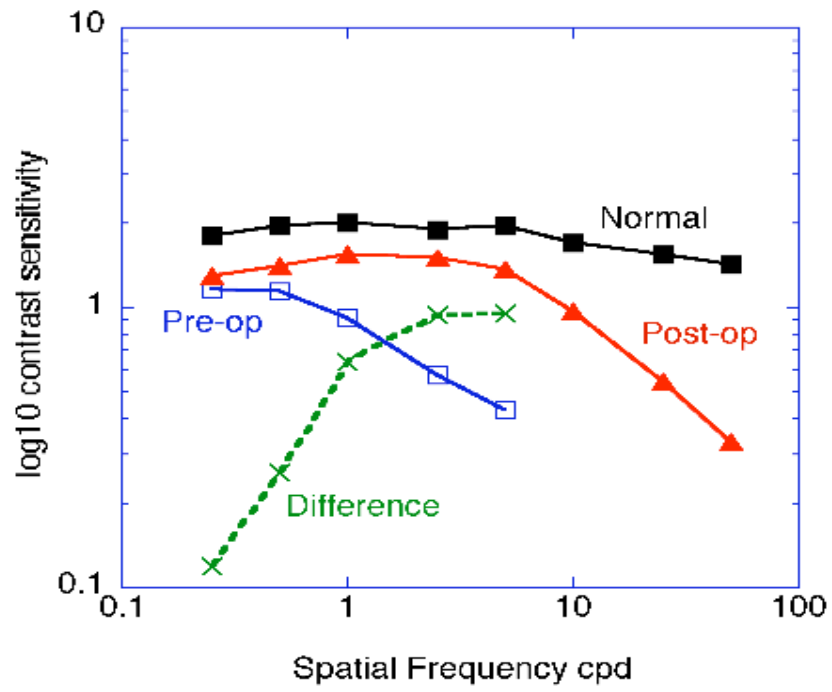
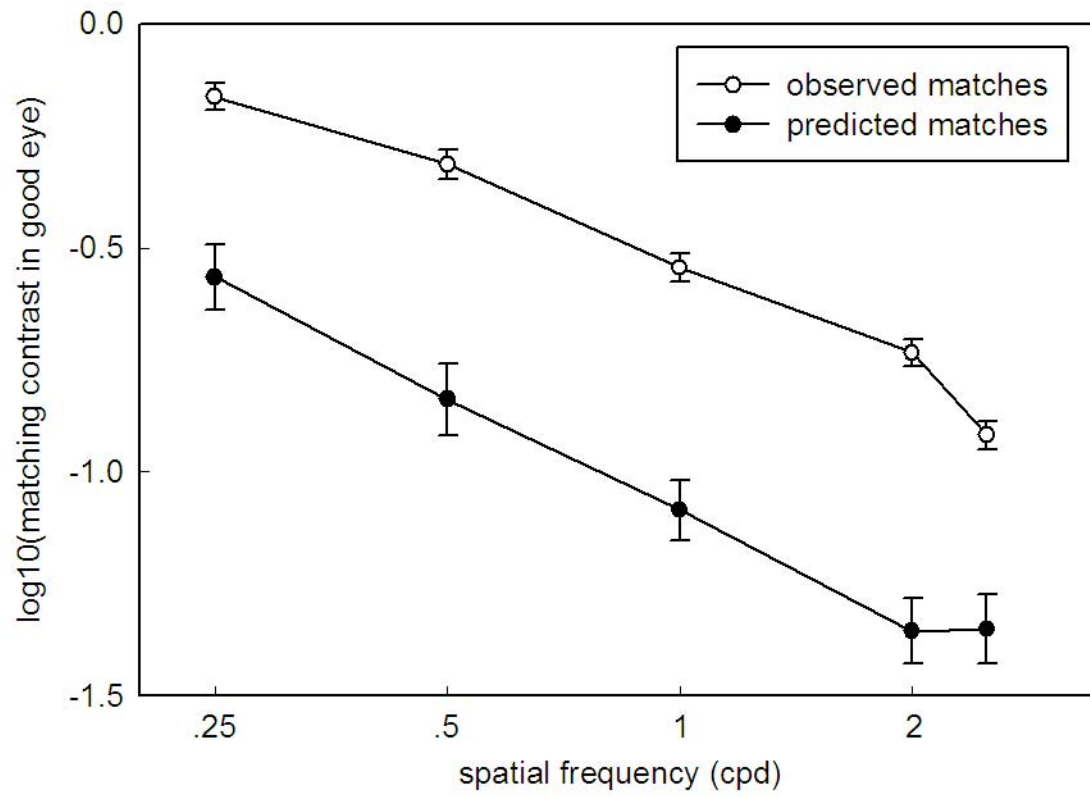
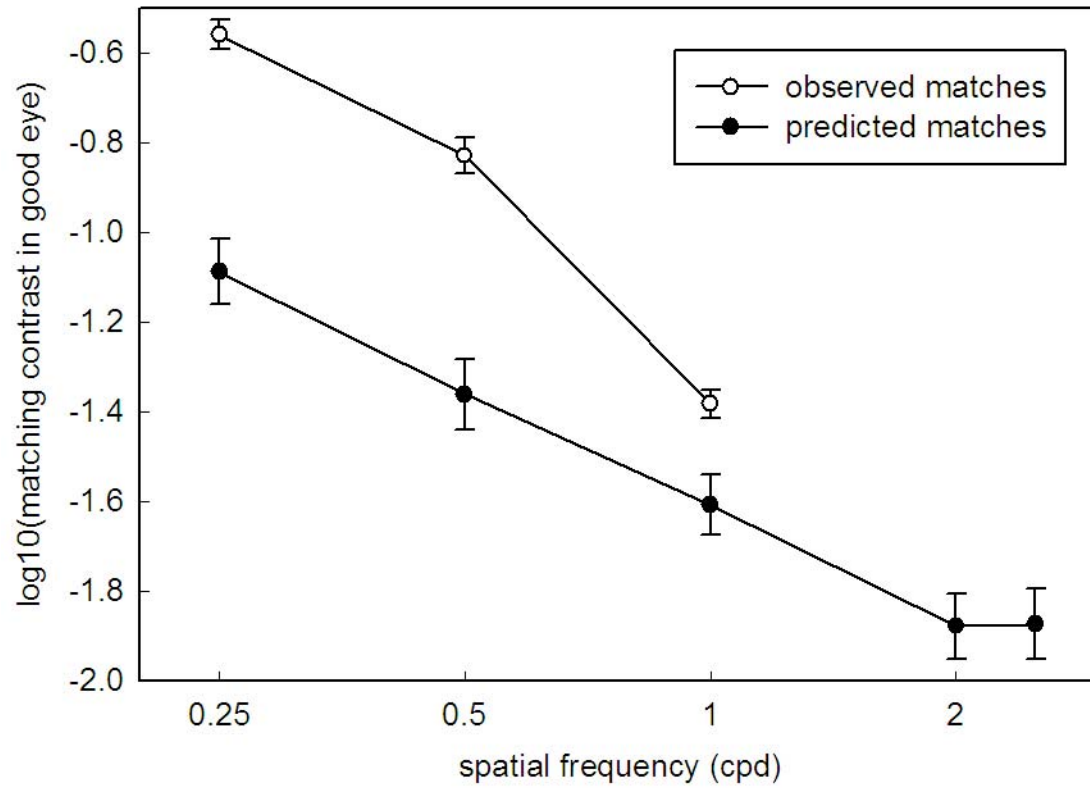


Figure 2A.



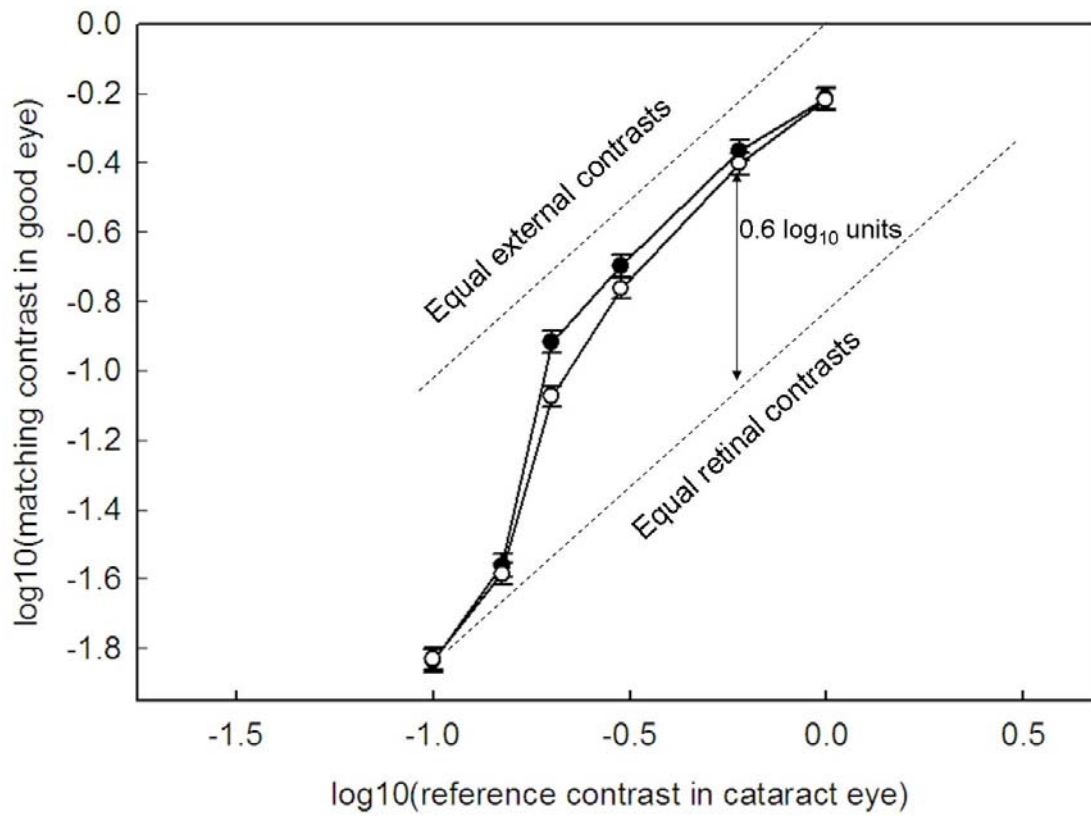
[Insert Running title of <72 characters]

Figure 2B.



[Insert Running title of <72 characters]

Figure 3.



**Figure 4.**

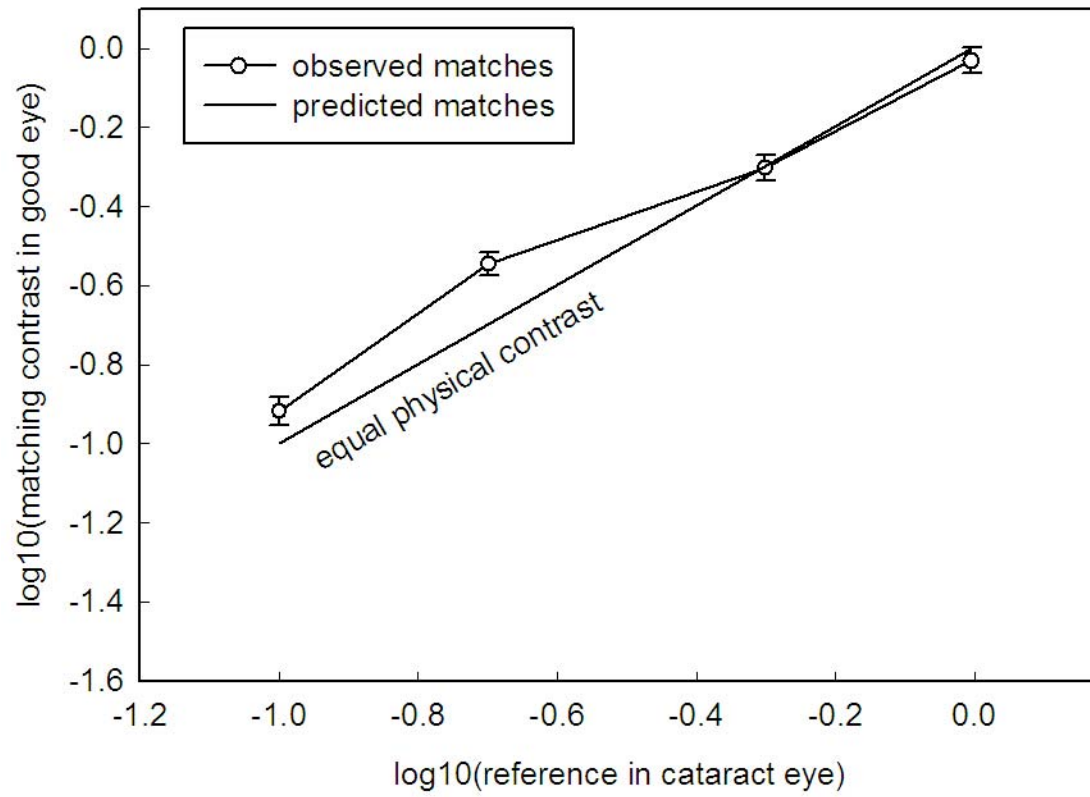


Figure 5.

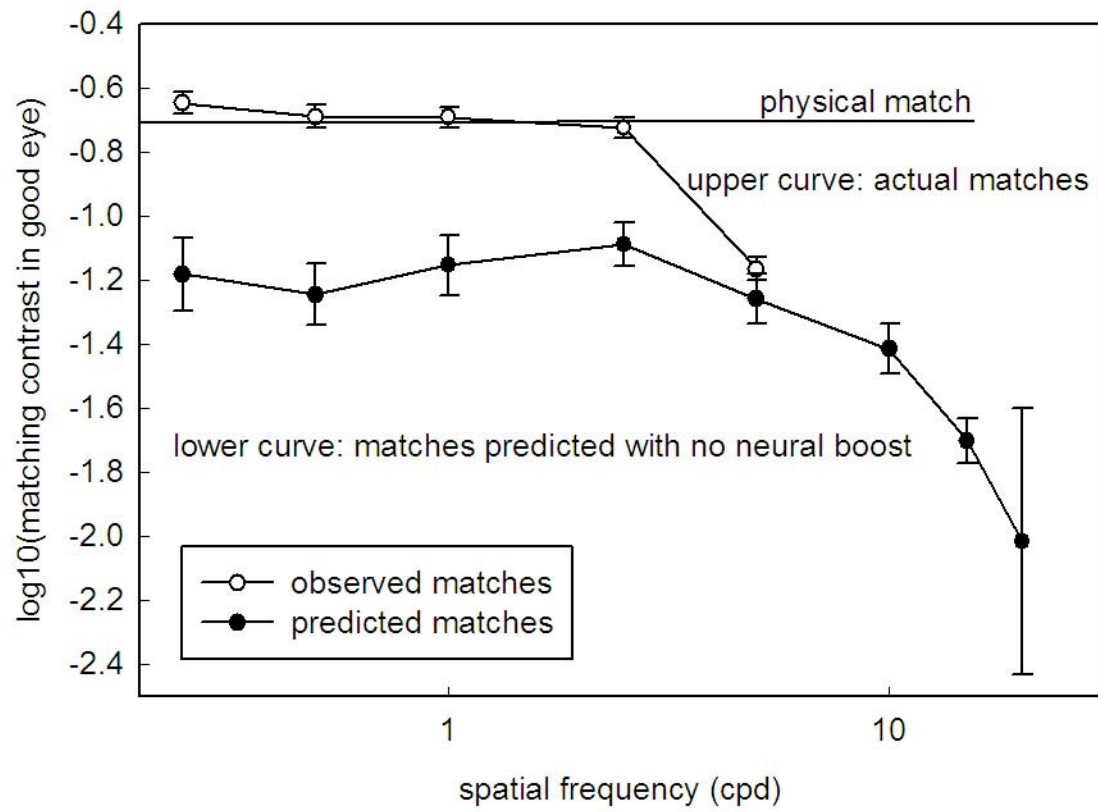
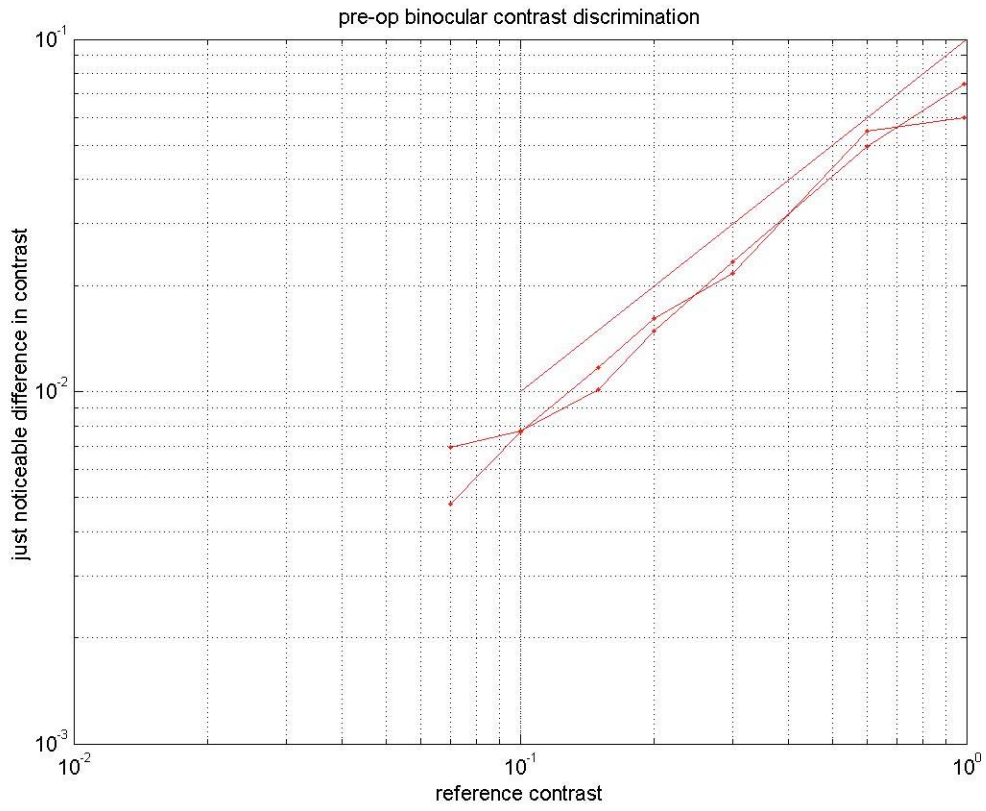


Figure 6.



[Insert Running title of <72 characters]