

# Experiment on the Relation between Color Discriminability and Genetic Polymorphism in the L Cone Using Four Color Primary Display Device

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## Abstract

*In this study, the correlation between the color discrimination ability and the genetic polymorphism is investigated. It is known that the variation in spectral sensitivity of the L cone is common among normal color vision subjects. It is due to the genetic polymorphism in cone pigments (opsins). The 180th amino acid residue of the L cone opsin is frequently replaced from serine to alanine. It is also known that due to the replacement the wavelength of the L cone peak sensitivity shifts about 6nm to the short wavelength direction. Assuming that the neural processing in the neurons and the brain is the same for both the standard observer and the observer whose spectral sensitivity of the L cone opsin shifts by 6nm (shift observer), we designed color pairs so that the color difference between the pairs looks larger to the standard observer than to the shift observer. To extend the color difference only for one of the two observers, the four primary color display 'Quattron' developed by SHARP Corporation was used. The experimental results, surprisingly, showed that the subjects whose 180th amino acid residue of the L opsin is alanine could better discriminate the pairs of colors that were designed to be discriminated by the standard observer. This result may mean that the neural processing is dependent on the polymorphism, and the human color discriminability variation cannot be explained simply by the cone spectral sensitivity shift.*

## Introduction

Human color vision is based on the three types of cones, whose spectral sensitivities are different from each other. It is known that there is some variation in the sensitivities among normal color vision subjects. The variation is accounted for by the genetic polymorphism in cone pigments (opsins). Though the opsins in the L cone and the M cone are structurally similar to each other, fifteen amino acid residues, which are coded by the genetic information (DNA), are different [1]. The peak sensitivity of the L cone is 560nm and that of the M cone is 530nm. The difference is caused mainly by the difference in the 180th, 277th and 285th amino acid residues. Among them, the latter two amino acid residues normally do not change, and they discriminate between the L cones and the M cones. However, the variation in the 180th amino acid residue is common [2].

The 180th amino acid residue of the L cone opsin is normally serine. However, it is often replaced by alanine, and the absorption spectra analysis revealed that the spectral sensitivity of the L cone shifts to the short wavelength direction by 6nm [3]. It was also confirmed by the psycho-physical color matching

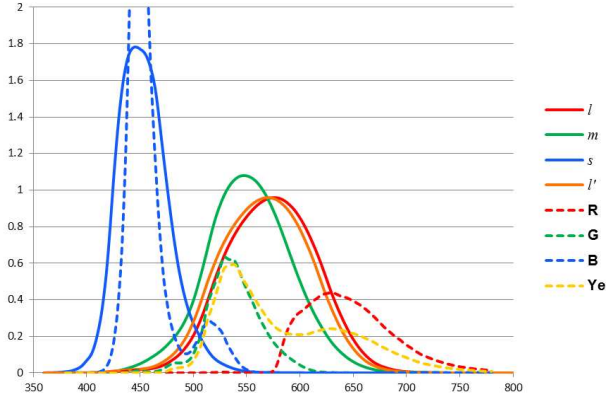
experiment that the color appearance varies depending on this phenomenon [4]. When we call the subjects, who have a serine as the 180th amino acid residue, 'Observers S', and the subjects who have an alanine there 'Observers A', it is reported that about 16 percent of Japanese males are Observers A [2]. The purpose of this study is to make clear the relation between the color discrimination ability of two types of observers and the genetic polymorphism, by the observation experiment and the genetic (DNA) analysis.

The human color perception is obtained not only by the cone spectral sensitivity difference but also by the neural processing. This study was conducted on the assumption that the neural processing is the same for both Observers S and Observers A. It was deduced that, for certain designed color pairs, Observers S be more sensitive to the color differences than Observers A. However, contrary to our expectation, the subjective experiment showed that Observers A were more sensitive. It is very interesting result, and the further study on the human color perception mechanism is necessary to explain it.

The four primary color display 'Quattron' developed by SHARP Corporation was used for the subjective experiment. As this LCD display has Ye(llow) as a primary color, in addition to the normal R(ed), G(reen) and B(lue) primary colors, it is well known as the wide gamut display [5]. Though its essential advantages are the wide gamut, the low power consumption, the enhanced sharpness, etc., this study made use of the property that it can generate metamers. The metamers for Observers A are not exactly the metamers for Observers S. Using this property, the color difference sensed by Observers S can be enlarged without changing that sensed by Observers A. In addition, the specially provided computer interface allows 10 bits assignment for each primary color intensity. It makes the displayed colors accurate.

## The influence expected by the cone sensitivity shift

Human color perception is considered to occur as a result of complicated processing of the neurons and the brain on the signals obtained by the cones in the retina. The simplest model for predicting the color appearance of the observer who has the cones with shifted spectral sensitivities ('shift observer' is used in the following paragraphs) is that only the sensitivity of L cone ( $\bar{l}(\lambda)$ ) is shifted to ( $\bar{l}'(\lambda)$ ) (Figure 1 -- solid lines), but the following neural processing is not changed.



**Figure 1.** Cone sensitivities (solid lines) with the shifted L cone sensitivity  $\bar{l}'(\lambda)$ , and primary colors' spectral intensity (dotted lines) of Quattron.

As our main concern is the color discrimination, the perception color space is assumed to be the CIE  $L^*a^*b^*$  uniform color space, where the color differences are approximately expressed by the Euclidean distance. The conversion from the  $L$ ,  $M$  and  $S$  tristimulus values to the CIE-1931 XYZ tristimulus values is well known, and the conversion from the  $X$ ,  $Y$  and  $Z$  values to the  $L^*$ ,  $a^*$  and  $b^*$  values is also well known. Hence, the perceived color difference for the shift observer may be modeled using these conversion equations. In this study, the Hunt-Pointer-Estevéz cone sensitivities are used for the calculation. The conversion from  $L$ ,  $M$ ,  $S$  to  $X$ ,  $Y$ ,  $Z$  is represented as (1), and the conversion from  $X$ ,  $Y$ ,  $Z$  to  $L^*$ ,  $a^*$ ,  $b^*$  is (except for very dark colors) represented as (2) [6].

$$\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} = \begin{pmatrix} 1.91019 & -1.11214 & 0.20195 \\ 0.37095 & 0.62905 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} L \\ M \\ S \end{pmatrix} \quad (1)$$

$$\begin{cases} L^* = 116(Y/Y_n)^{1/3} - 16 \\ a^* = 500[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}] \\ b^* = 200[(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}] \end{cases} \quad (2)$$

where  $X_n$ ,  $Y_n$  and  $Z_n$  are  $X$ ,  $Y$  and  $Z$  values for the reference white.

As a color is presented on the four primary color display system Quattron developed by SHARP Corporation, the presented color  $C(\lambda)$  is expressed as (3).

$$C(\lambda) = R\mathbf{R}(\lambda) + G\mathbf{G}(\lambda) + B\mathbf{B}(\lambda) + Y_e\mathbf{Y}_e(\lambda) \quad (3)$$

where  $\mathbf{R}(\lambda)$ ,  $\mathbf{G}(\lambda)$ ,  $\mathbf{B}(\lambda)$  and  $\mathbf{Y}_e(\lambda)$  are the primary colors (Fig.1 -- dotted lines), and  $R$ ,  $G$ ,  $B$  and  $Y_e$  are given signals (the gamma characteristics are ignored). The measured chromaticity values of the primary colors were,  $(x_R, y_R) = (0.644, 0.334)$ ,  $(x_G, y_G) = (0.270, 0.662)$ ,  $(x_B, y_B) = (0.151, 0.057)$  and  $(x_{Y_e}, y_{Y_e}) = (0.398, 0.573)$ , and that of white ( $R=G=B=Y_e=1$ ) was  $(x_w, y_w) = (0.264, 0.359)$ . The luminance of the white was 287  $\text{cd/m}^2$ . Denoting the  $L$ ,  $M$  and  $S$  cone sensitivities as  $\bar{l}(\lambda)$ ,  $\bar{m}(\lambda)$  and  $\bar{s}(\lambda)$ , the tristimulus values  $L$ ,  $M$  and  $S$  are calculated as (4).

$$\begin{pmatrix} L \\ M \\ S \end{pmatrix} = \begin{pmatrix} L_R & L_G & L_B & L_{Y_e} \\ M_R & M_G & M_B & M_{Y_e} \\ S_R & S_G & S_B & S_{Y_e} \end{pmatrix} \begin{pmatrix} R \\ G \\ B \\ Y_e \end{pmatrix} \quad (4)$$

where  $L_R = \int \mathbf{R}(\lambda)\bar{l}(\lambda)d\lambda$ ,  $M_R = \int \mathbf{R}(\lambda)\bar{m}(\lambda)d\lambda$ ,

$S_R = \int \mathbf{R}(\lambda)\bar{s}(\lambda)d\lambda$ , etc.

Because Observers S or Observers A have their own  $\bar{l}(\lambda)$ , putting them as  $\bar{l}_s(\lambda)$  and  $\bar{l}_A(\lambda)$ , the coefficients in (4) are different only in terms relating to  $L$ , the tristimulus values for the two groups of observers are  $(L_s, M, S)$  and  $(L_A, M, S)$ . Applying (1) and (2) to them, the perceived colors are obtained as  $(L_s^*, a_s^*, b_s^*)$  and  $(L_A^*, a_A^*, b_A^*)$ . As the color difference between two colors perceived by human vision is approximated by  $\Delta E_{ab}$  (5) in the  $L^*a^*b^*$  space, the color differences for both Observers S and Observers A can be calculated in the same way.

$$\Delta E_{ab} = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad (5)$$

In normal color display, each set of signal values ( $R$ ,  $G$ ,  $B$ ) corresponds to a set of values ( $L^*$ ,  $a^*$ ,  $b^*$ ) of the displayed color. As multiple sets of signal values ( $R$ ,  $G$ ,  $B$ ,  $Y_e$ ) correspond to a set of values ( $L^*$ ,  $a^*$ ,  $b^*$ ) in the case of the four primary color display, metamers with various sets of ( $R$ ,  $G$ ,  $B$ ,  $Y_e$ ) can be displayed for an observer. However, for example, two metamers for Observers S may have some color difference for Observers A. Utilizing this property, color difference can be extended only for Observers A.

To know how much the color difference for the other observer can be extended, metamers for an observer were searched for every set of ( $R$ ,  $G$ ,  $B$ ,  $Y_e$ ) values. Let us hypothesize that Observers S are the standard observers, and have the normal L cone whose spectral sensitivity can be converted from CIE-1931 XYZ color matching functions by the inverse function of (1), and Observers A are the shift observers. By the above mentioned metamer search, it was found that the maximum color difference for the shift observer that is given by the metamers for the standard observer,  $\max(\Delta E_{ab})_{\text{shift}}$ , is 0.160, while that for the standard observer that is given by the metamers for the shift observer,  $\max(\Delta E_{ab})_{\text{standard}}$ , is 0.234. It was also found that the color difference in the  $L^*a^*b^*$  space is almost in  $a^*$  direction, and the difference in  $L^*$  and  $b^*$  directions may be ignored. Using this property, the following procedure was adopted to generate the color pairs for the color discrimination observation experiment.

- A pair of colors which have small color difference in  $a^*$  direction for the shift observer is defined. Let us put the colors  $C_1$  and  $C_2$ .
- The metamers of  $C_1$  and  $C_2$  for the shift observer are searched, so that the color difference between the metamer of  $C_1$  and the metamer of  $C_2$  for the shift observer be largest for the standard observer. These are the target colors.
- Though the linear values for  $R$ ,  $G$ ,  $B$  and  $Y_e$  can be obtained by calculation, the display has own gamma characteristics. The real  $R$ ,  $G$ ,  $B$  and  $Y_e$  values are determined by the measurement and adjustment procedure. The measurement was made using a KONICA-MINOLTA CS-2000 spectroradiometer. The four values are fixed, when the displayed colors for a pair have the colors that are very near the target colors for the standard observer and the shift observer.

This procedure generates the color pairs whose color difference may be sensed by the standard observer, but may not be sensed by the shift observer. It was expected that the result conforms the result of the genetic analysis.

**Table 1. The mean of measured  $L^*$ ,  $a^*$  and  $b^*$  values of the first color of the color pairs, and the mean and the standard deviation of the measured color difference  $\Delta E_{ab}$  between the first and second colors in the color pairs.**

		Gray	Sky Blue 1	Yellow	Yellow Green	Skin	Sky Blue 2
$L^*(Std)$	Mean	75.259	76.825	71.527	77.512	74.351	76.752
$a^*(Std)$	Mean	1.842	9.057	-35.309	-49.868	-13.416	9.186
$b^*(Std)$	Mean	-10.762	-24.881	54.502	62.889	37.819	-24.993
$\Delta E_{ab}(Std)$	Mean	1.042	1.066	0.742	1.079	0.260	0.418
	Std Dev	0.036	0.040	0.045	0.041	0.019	0.034
$L^*(Shft)$	Mean	75.365	77.041	71.547	77.648	74.207	76.965
$a^*(Shft)$	Mean	3.608	12.294	-33.640	-44.898	-15.766	12.365
$b^*(Shft)$	Mean	-10.578	-24.509	54.537	63.124	37.590	-24.626
$\Delta E_{ab}(Shft)$	Mean	0.697	0.647	0.372	0.629	0.255	0.412
	Std Dev	0.030	0.031	0.033	0.029	0.015	0.031

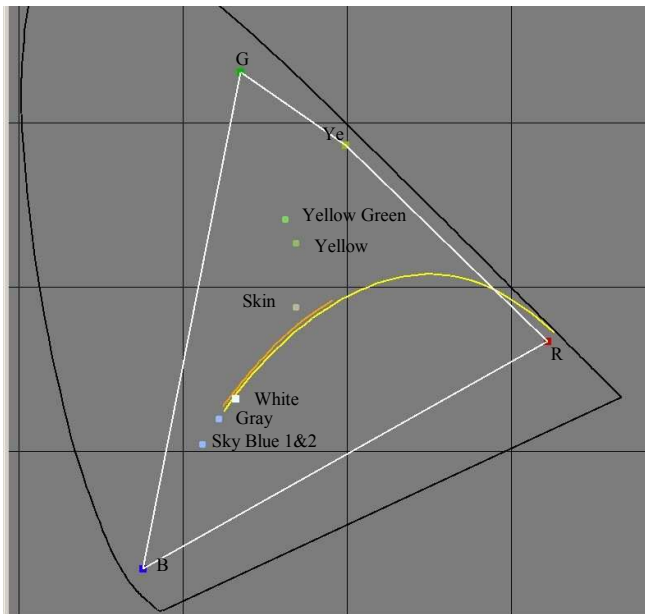
## Experiments

### Preparation for the observation experiment

Color pairs were generated for the color discrimination experiment described in the previous chapter. We were careful so that such three kinds of color pairs are generated:

- Color pairs whose color difference should be discriminated by both the standard and the shift observers.
- Color pairs whose color difference should be discriminated by the standard observers but should not be discriminated by the shift observers.
- Color pairs whose color difference should be discriminated by neither the standard nor the shift observers.

The generated six color pairs are shown in Table 1. Their chromaticities for the standard observer are shown in the chromaticity diagram (Fig.2). The measurements were carried out more than two hours after the power of the display device and the spectro-photometer had been switched on. In Table 1, their color is represented using  $L^*$ ,  $a^*$  and  $b^*$  values. As the correlated color

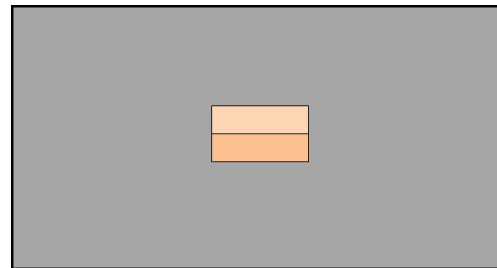


**Figure 2.** Chromaticities of the color pairs used for the experiment with those of the primary colors of the display. White line shows the gamut of the display. Yellow and orange curves show the chromaticity of the black body radiation and the CIE daylight, respectively.

temperature of the reference white for this display is about 14,000K and bluish, the use of the CIELAB color space is not necessarily adequate. These values are used only for a guide to generate the color pairs.

### Observation conditions

The observation experiment takes place in a dark room. Figure 3 shows the design of the color presentation on the 46 inch Quattron display. At the center of the panel, a color pair is placed in a rectangular window. The color of its surround is gray (20% brightness of the reference white). The window size is 22.2cm (Hor.)  $\times$  11.7cm (Ver.), which is  $\pm 5.3$ deg (Hor.)  $\times$   $\pm 2.8$ deg (Ver.) in angles. The observation distance is 120cm. The color pairs are separated with black 1 pixel line.



**Figure 3.** The design of color presentation on the Quattron display.

Though the number of color pairs is six, the patterns for the presentation were made in three ways as below.

- The first and second colors in a pair are assigned to the upper and the lower rectangles, respectively.
- The first and second colors in a pair are assigned to the lower and the upper rectangles, respectively.
- Only the first color is assigned to both rectangles.

In addition, each color pair with each pattern was presented three times to check the consistency. Thus  $6 \times 3 \times 3 = 54$  observations are requested of one subject. The order of 54 presentations is completely random.

The 20% gray and the randomly ordered color pairs are alternately switched. Subjects are instructed to answer which of the upper or the lower rectangle looks 'redder', or both look the same, after waiting till the visual effect of the switching diminishes, since the direction of the color difference is mainly in  $a^*$  direction (red-cyan direction).

Subjects were ten males with normal color vision. Their ages were between 23 and 64. The normality was verified with

“Standard Pseudoisochromatic Plates [7]”. We adopted only male subjects, since the opsin related genes for the L and M cones are on the X chromosome. The DNA analysis is simple for males who have only one X chromosome. In the case of females, as genetic information from two X chromosomes are expressed as opsins, more complicated analysis is required.

### Genetic analysis

The 180th, 277th and 285th amino acid residues of both L and M opsins are the major determinants of the spectral sensitivity. The DNA sequences coding these amino acid residues of both L and M opsins of the subjects were determined. Genomic DNAs were prepared from saliva. The primer pairs specific to OPN1LW (Gene ID:5956) and OPN1MW (Gene ID:2652), and those specific to each exon 3 and exon 5 were designed. The nested PCR was carried out on a PCR Thermal Cycler Dice (TaKaRa, Japan) using Prime STAR-HS DNA Polymerase (TaKaRa, Japan) for 30 cycles according to the manufacturer’s instructions. BigDye Terminator v3.1 Cycle Sequencing Kit was used for the sequence reaction and the DNA sequences were determined with the 3500 Genetic Analyzer (Applied Biosystems, USA).

### Results

The experimental results of the above mentioned observation experiment and the genetic analysis are shown in Table 2. 1~12 are the numbers of the subjects; subjects #5 and #8 were rejected by the normality verification. ‘Yes’ means that the subject could stably discriminate the color difference of the color pair, while ‘No’ means that he could not discriminate it, and both colors looked the same for him. The row ‘Number of Discriminable Color Pairs (NDCP)’ shows the number of color pairs whose color differences are noticed by the subject.

The result of the genetic analysis is simultaneously shown in the table. The 180th, 277th and 285th amino acid residue of the L cone opsin and the M cone opsin obtained from each subject are shown. ‘Ser’, ‘Ala’, ‘Phe’, ‘Thr’ and ‘Tyr’ are serine, alanine, phenylalanine, threonine and tyrosine, respectively. ‘Ala-1/3Thr’ means that the signal for alanine is dominant, but the signal for threonine appears with 1/3 magnitude.

### Discussion

According to the above experimental result, the number of discriminable color pairs (NDCP) is distributed from 1 to 4. It clearly corresponds to the genetic analysis result. The relation between the 180th amino acid residue of the L cone opsin and NDCP is shown in Fig.4. Subjects whose NDCP is 1 or 2 have serine. The subjects whose NDCP is 3 or 4 have alanine except for the subject #7. The difference of NDCP arises from the color pairs ‘Yellow’ and ‘Yellow Green’, and these color pairs are discriminated by the subjects who have alanine (Ala180) rather than those who have serine (Ser180). (It is also interesting that all subjects who have Ala180 in the L cone opsin, and Thr285 in the M cone opsin, in addition to alanine, could discriminate four color pairs. We will not discuss this point, since the issue about the second signal looks too complicated.)

What is most surprising and interesting is that the obtained result is contrary to what we expected. As is described before, we designed the observation experiment so that the color difference of some color pairs are too small for the shift observer to discriminate it, while it may be discriminated by the standard observer. The sensitivity of L cone of the shift observer is shifted by 6nm from that of the standard observer to the shorter wavelength direction, assuming that the standard observers are the Observers S and the shift observers are the Observers A. If the neural processing deals with the input signal from cones in the same manner for both observers, NDCP for the Observers S should be larger than that for the Observers A.

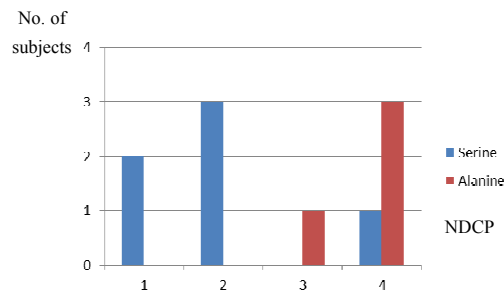


Figure 4. The relation between NDCP and the 180th amino acid residue of the L cone opsin.

Table 2 The result of observation experiment and DNA analysis.

Subject No.	1	2	3	4	6	7	9	10	11	12
Gray	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Sky Blue 1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Yellow	Yes	Yes	No	Yes	No	Yes	Yes	No	No	No
Yellow Green	Yes	No	No	Yes	No	Yes	Yes	No	No	No
Skin	No	No	No	No	No	No	No	No	No	No
Sky Blue 2	No	No	No	No	No	No	No	No	No	No
Number of Discriminable Color Pairs (NDCP)	4	3	2	4	2	4	4	1	1	2
L cone opsin	180 <sup>th</sup> amino acid residue	Ala	Ala	Ser	Ala	Ser	Ser	Ala	Ser	Ser
	277 <sup>th</sup> amino acid residue	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr
	285 <sup>th</sup> amino acid residue	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr
M cone opsin	180 <sup>th</sup> amino acid residue	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ser
	277 <sup>th</sup> amino acid residue	Phe·Tyr	Phe	Phe	Phe	Phe	Phe	Phe·1/2Tyr	Phe	Phe
	285 <sup>th</sup> amino acid residue	Ala·Thr	Ala	Ala	Ala·1/3Thr	Ala	Ala	Ala·Thr	Ala	Ala

Though the reason for this phenomenon is not known, possible explanations are as follows.

- i. The assumption in the second chapter, that the processing in the neurons or in the brain is the same for everyone, is wrong. The neural processing may develop so that the brain can discriminate colors adapting to the cone sensitivities. At least for the color region Yellow ~ Yellow Green, where the clear difference of NDCP appears, the processing has such characteristics that the nearer the spectral sensitivities of the L and M cones, the better the color discrimination.
- ii. In [3] and [4], it was said that the variation of the 180th amino acid residue in the L cone opsin from serine to alanine causes the absorption peak shift to the shorter wavelength direction. However, it is not said that the spectral absorbance curve shape does not change. The calculated color difference may change, if the shape of the spectral sensitivity curve is severely deformed. Such deformation, however, is unrealistic.

Because the experimental result was contrary to what we expected, we re-calculated the color difference in several alternative conditions.

- a. The standard observer (CIE-1931) had been established before the genetic polymorphism was found. A part of the subjects for the determination of the standard observer should have had alanine as the 180th amino acid residue in the L cone opsin. Hence, as the other extreme, we re-calculated the color difference, hypothesizing the subjects with Ala180 as the standard observers. In this case, the subjects with Ser180 shall have the spectral sensitivity which is 6nm shifted to the long wavelength direction.
- b. As described above, the white chromaticity is very bluish. Assuming that the human vision cannot adapt to such bluish white, the color difference was re-calculated using the D65 illuminant chromaticity as the reference white.
- c. The CIE  $L^*a^*b^*$  uniform color space was defined to evaluate large color differences. The CIE  $L^*u^*v^*$  uniform color space may be better to evaluate such color difference perceptibility. We also re-calculated the color difference using the CIE  $L^*u^*v^*$  color space.

However, the above three conditions did not change the situation. In these re-calculations, the L opsin with the longer spectral sensitivity peak (Ser180) always showed larger color difference between the colors pairs as designed.

## Conclusions

It is known that the polymorphism exists in the normal color observers' L cone opsin, and the variation in color appearance occurs due to the polymorphism. We conducted an experiment to know the relation between the genetic type and the color discriminability. A four primary color display Quattron was used to generate color pairs whose color difference is sensible for the people in a genetic type and is insensible for the people in other genetic type, assuming that the neural processing is independent of the polymorphism.

The experimental result clearly showed the correlation between the polymorphism and the color discriminability. However, the correlation is contradictory to the above assumed independence of the neural processing from the polymorphism. This means that the neural processing is dependent on the polymorphism. Though this result is very interesting, it means that the human color discriminability is too complicated to be estimated simply by the cone spectral sensitivity shift.

However, the experiment in this study is preliminary in the sense that both the number of colors used for the observation experiment and the number of subjects are small. Especially, the range of the color pairs should be extended to other hue or saturation, in the future study.

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## Author Biography

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