The Influence of Illumination Color on the Subjective Visual Recognition of Biological Specimens

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ABSTRACT

Background Visual examination by the naked eye is integral to medical diagnosis and surgery. The illumination in conditioned color is widely used for visual inspection in the industry but has not been introduced to the biomedical context. The color that can enhance the visual recognition of individual tissues is still unknown. Therefore, we carried out a visual recognition experiment on biological specimens to determine the subjective preference for illumination color based on questionnaires.

Methods Twenty healthy subjects were asked to compare the visual recognizability of several rat tissues between the illuminations in test colors and white. The rats were anesthetized, and the femoral vein and abdominal cavity were exposed. Seven tissues were selected for a visual recognition test. Illumination was generated using a multi-color LED light. The subjects observed the tissues under the illuminations of white and one of the test colors alternately and reported which illumination is suitable for visual recognition using a questionnaire.

Results The analysis of the questionnaires showed that the blue test color was more effective than white illumination in the visual recognition of fine structures such as the branching of blood vessels and nerves, and red illumination disturbed the visual recognizability of the same tissues. On the other hand, the red but not the blue illumination improved the visual recognizability of the vein beneath the intact skin. As to the recognition of individual tissues in the abdominal cavity, the white illumination gave a better visual recognizability compared to every other test color.

Conclusion This study shows that the illumination color influences the visual recognition of biological specimens and the adequate color for the visual recognition of specific tissue parts is distinct among biological specimens. Using the lighting system to make fine adjustments to the illumination color may be useful in medical diagnosis and surgery.

Visual examination is integral to all classes of medical diagnosis and surgery. Knowing the color and structure of organs and tissues is essential in the evaluation of an operation area and the manipulation of tissues. Surgical lighting is important for visual examination, and the quality of lighting during an operation is influenced by factors such as illumination intensity, shadow control, color rendering index, and heat generation. Traditionally, white lighting with high luminance and color rendering index has been widely used because of its brightness and natural appearance.

The spectral characteristics of illumination are an important factor that influences visual examination. Visual inspection using the illumination in color conditioning is widely used for industrial purposes such as detection of surface imperfections on manufacturing materials and contaminants in food. However, color conditioning has not been adapted for clinical uses. Several simulation and instrumental studies have explored the optimal spectral distribution of illumination for the recognition of particular tissues.[1–3] For example, blue light enhanced the contrast between the skin on the wrist and the underlying blood vessels as evaluated using a spectroradiometer.[2] Although such techniques might be advanced via imaging techniques using camera and image analysis, which can be more sensitive to contrast or brightness than the naked eye, they usually require some time for calculation. Immediate inspection by the naked eye remains one of the primary tools in diagnosis and surgery. Colored illumination might be clinically useful in the naked eye examination of tissues by improving the recognition of fine structures such as blood vessels.

Because simulation and instrument-based results may not correlate with human visual perception, it may be necessary to determine the effect of the illumination color by subjective evaluation using real objects under real illumination. However, only a limited number of subjective experiments have been previously reported.[4, 5] Although Argyraki et al.[4] reported that white illumination is more suitable in the recognition of the human vein beneath the skin compared to the illumination in which the red component has been suppressed, Kurabuchi et al.[5] demonstrated that the recognition of blood vessels on the rat cecum was improved by an illumination containing less red component compared to white illumination. Because these studies claim the superiority of distinct illuminating colors using different tissues, it is still unknown whether each tissue has a specific color that can enhance its visual recognition or whether one color can improve the recognition of every tissue component. Therefore, we carried out visual recognition experiments to observe the subjective preference for the illumination color based on questionnaires using several rat tissues under the illumination of multiple colors. Because the previous subjective studies reported the effect of bluish illumination containing less red component,[4, 5] we focused on the effect of illumination colors along red-blue axis in this study for comparison.

EXPERIMENTAL PROCEDURES
Subjects
The subjects were 20 students who were enrolled at the Division of Neuroscience, School of Life Science, Faculty of Medicine, Tottori University in 2019–2020. None of the subjects self-reported color vision deficiency.

Visual recognition test
The subjects were asked to observe several rat tissues under the illumination of white and six test colors and to compare visual recognizability of the tissues between the test and the white illumination.

The rats were anesthetized using isoflurane (Pfizer, Tokyo, Japan) in O_2 and put on a heating pad set at 38°C. One of the femora was shaved and small incisions were made on the skin using a blade. On the other femur, the skin was incised to expose the femoral vein. In addition, the abdominal cavity was exposed. Seven tissues were used for the visual recognition test: the femoral vein beneath the intact skin, small incisions and bleedings on the skin, fine blood vessels around the exposed femoral vein, nerve branching around the exposed femoral vein, the whole structure of the abdominal cavity, fine blood vessels on the intestine, and the pattern of the hepatic lobules.

Illumination was generated using a multi-color LED light (Little Saia, Sumtech Innovations Co., Ltd., Okayama, Japan) equipped with four LEDs (red, green, blue, and white), the output intensity of which can be controlled independently. Six color tones were prepared by mixing the light of red, green, and blue LEDs and compared with the illumination by the white LED; color #1 chromaticity coordinate in the CIE 1931 color space (0.3771, 0.4426), #2 (0.2220, 0.1297), #3 (0.2199, 0.1694), #4 (0.1976, 0.1634), #5 (0.1714, 0.1564), #6 (0.1583, 0.1529), and white (0.3321, 0.3540) (Fig. 1A). The LED light was set at 50 cm above the animal. The intensity of illumination was 7,500–7,800 lux, and the intensities of the six test illuminations deviated less than 3% from that of the white illumination.

The subjects observed the tissue samples through a surgical microscope in which the built-in light was turned off except for the whole structure of the abdominal cavity which was observed by the naked eye. For each tissue, they illuminated it with white and one of the test colors alternately and reported the visual recognizability using a five-point scale, in which 1 or 5 indicates that white or the test color is superior for visual recognition to the other, 3 indicates that the two colors are comparable to each other, and 2 and 4 indicate a moderate difference between the two colors.

Data analysis
The visual recognizability of tissue samples was evaluated for each test color by calculating the preference index as follows:

Preference Index (PI) $= [(n_5-n_1) + (n_4-n_2)/2]/n_T,$

where n_i is the number of subjects who reported score i and n_T is the total number of subjects which is 20 in the present study. PI ranges between 1 and −1 at which all subjects reported score 5 and 1, respectively. PI value 0 indicates that the test color is comparable to white in visual recognizability.

We determined statistically whether individual PIs significantly deviated from zero using bootstrap test. Briefly, the scores of all of the subjects for one tissue and one test color ($n = 20$) were shifted so that the recalculated PI is zero. We took 20 scores randomly from these shifted scores allowing repetition and calculated their PI. We repeated the re-sampling and PI calculation 10,000 times to simulate the distribution of PI with its mean at zero. Finally, we calculated the probability that a PI value in the simulated PIs is more extreme than the original PI value. When the probability was less than
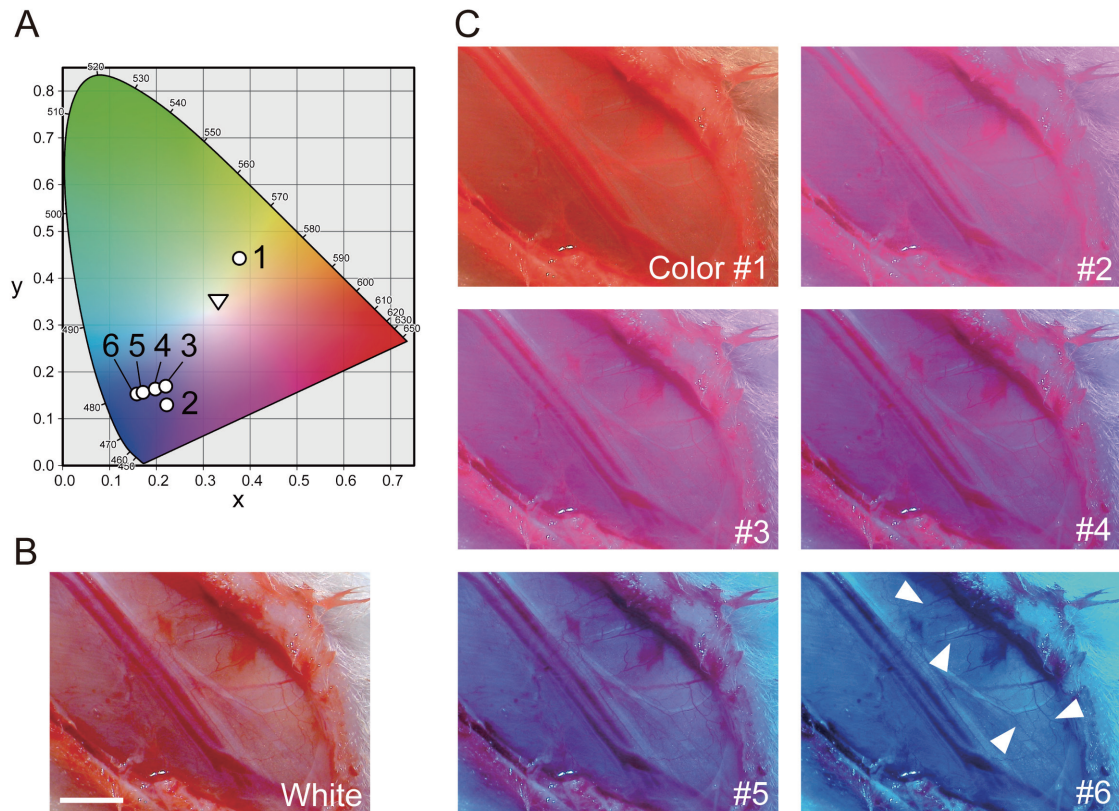
Fig. 1. Examples of blood vessel images under the illuminations used in this study. **A**: The illumination colors used in the present experiments are plotted on the CIE 1931 color space chromaticity diagram. Triangle represents the illumination by white LED, and open circles represent six conditions of mixed illumination by RGB LEDs. **B, C**: Images of a femoral vein and fine vessels under the white illumination (**B**) and the six colored illuminations (**C**). The number of the test color in each panel corresponds to the number in A. Arrowheads in panel #6 indicate representative fine vessels. Scale in B, 2 mm.

0.05, we regarded the original PI as having significantly deviated from zero.

Ethical considerations

The subjects were recruited through an advertisement. They received a written overview of the study and explanations that the data gathered in the study would be analyzed so that individuals could not be identified and that they would not suffer any disadvantage when they do not participate in the study. Then informed consent was obtained from all of the subjects. This study was approved by the Ethics Review Committee of Tottori University Faculty of Medicine (approval number: 19A087).

Adult male Sprague-Dawley rats were obtained from Japan SLC Inc. (Hamamatsu, Japan). They were raised under controlled laboratory conditions (temperature, 21–24°C) with free access to food and water under a 12-h light/dark cycle (light onset at 07:00 AM). The nesting material in each cage was replaced twice a week. The experimental procedures were approved by the Animal Care Committee of Tottori University (approval number: 18-Y-44).

RESULTS

In the present study, the subjects were instructed to compare the visual recognizability of several rat tissues between white and other colored illuminations. The test colors were four blue colors in which the intensity of the red component was reduced (colors #3–6 in Fig. 1A), purple color in which the green intensity was reduced (#2), and red–yellow in which the blue intensity was reduced (#1). Examples of blood vessel images under each illumination are displayed in Figs. 1B and C. Fine branches of blood vessels are observed around the large femoral vein as exemplified by arrowheads in Fig. 1C-#6. These structures are more easily recognized in colors #5 and 6 compared with the white illumination (Fig. 1B). On the other hand, the illumination in color #2 clearly disturbed the visual recognizability of the structure.

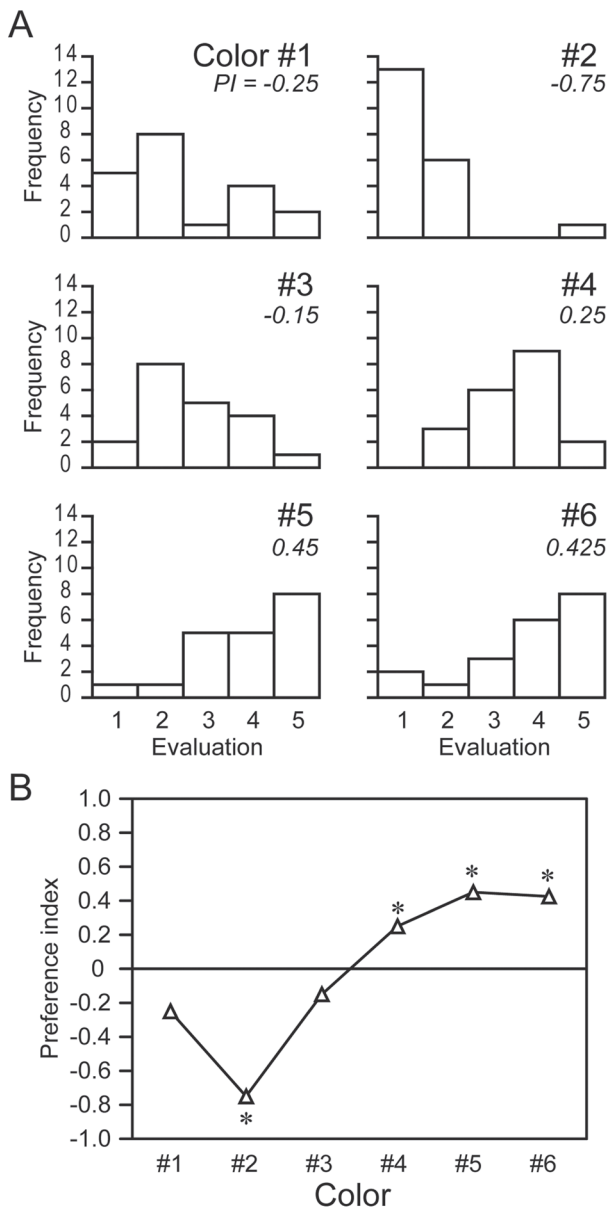The responses of all of the subjects are summarized

Fig. 2. Evaluation of visual recognizability of blood vessels under colored illuminations. **A**: Frequency histograms of evaluation scores of all subjects. The illumination color is described at the top-right of each histogram together with the PI value of the histogram. **B**: PIs of each color illumination. Asterisks represent a significant deviation from zero. *$P < 0.05$.

color #2. The skewness of each histogram was evaluated by calculating the preference index (PI). The PI values are described in each histogram of Fig. 2A and summarized in Fig. 2B. The statistical analysis showed that the PIs are significantly deviated from 0 for 1 in colors #4–6 and for −1 in color #2. Therefore, it is possible to state that blue illumination as colors #4–6 improves the visual recognition of fine blood vessels whereas purple illumination as color #2 disturbs it.

We calculated PIs for each color in seven tissue samples as summarized in Fig. 3. As observed in blood vessels (tissue C in Fig. 3, same data as Fig. 2B), blue test colors (#5 and 6) yielded better visual recognizability than the white illumination, whereas red (#1) and purple (#2) disturbed visual recognizability in the following tissues: nerve branching around the exposed femoral vein (Tissue D in Fig. 3), fine blood vessels on the intestine (Tissue F), and the pattern of the hepatic lobules (Tissue G). The opposite tendency was observed in the femoral vein beneath the intact skin (Tissue A) because red (#1) and pale blue (#3) colors improved but blue colors (#5 and 6) disturbed visual recognizability. Small amounts of bleeding on the skin surface (Tissue B) could be identified significantly better than the white under pale blue and blue illuminations (#3, 4, and 5). As to the whole structure of the abdominal cavity for which the subjects were asked to discriminate individual organs (Tissue E), four test colors (#1, 2, 3, and 6) significantly reduced PI; thus, the white illumination gave the best visual recognizability. These results demonstrate that color illumination can improve the visual recognizability of several tissues and the appropriate color would be distinct among tissues.

DISCUSSION

In the present study, the illumination color influenced the visual recognition of biological specimens. For example, the blue illumination improved the visual recognition of fine blood vessels, whereas the purple illumination disturbed it (Fig. 2). As to the femoral vein beneath the intact skin, however, the blue illumination disturbed visual recognition, whereas the red illumination improved it (Fig. 3, Tissue A). These results are consistent with the previous findings that the illumination in which the red component has been suppressed is more suitable in the recognition of blood vessels on the rat cecum[5] but less suitable in the recognition of the human vein beneath the skin compared to white illumination.[4] Because those previous studies compared a single illumination color with white using single-tissue samples, it was not clear why the bluish illumination exerted the opposite effects on the visual recognition
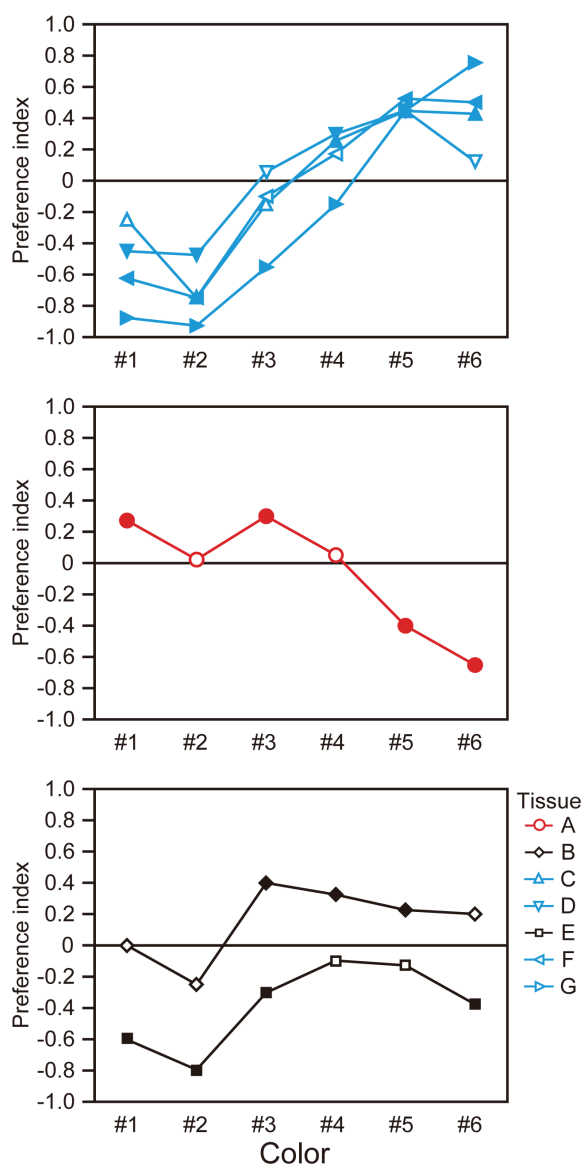
as histograms in Fig. 2A. Under the illumination of color #5, 65% (13 subjects) reported better recognizability in the test color (evaluations 4 and 5), and the histogram is clearly skewed for the test color. The histogram for color #6 also shows a skew for the test color (70% of the subjects in evaluations 4 and 5). On the other hand, most of the subjects (19/20) preferred the white illumination for the detection of fine blood vessels when compared with

Fig. 3. Suitable illumination color for visual recognition is different among tissue samples. PIs for individual illumination colors are shown for seven tissue samples. **A**: the femoral vein beneath the intact skin, **B**: small incisions and bleedings on the skin, **C**: fine blood vessels around the exposed femoral vein, **D**: nerve branching around the exposed femoral vein, **E**: the whole structure of the abdominal cavity, **F**: fine blood vessels on the intestine, and **G**: the pattern of the hepatic lobules. The illumination color is described at the bottom of each panel. Filled and open symbols represent significant ($P < 0.05$) and not-significant deviations from 0, respectively. PIs for each tissue are shown in three groups. Top (blue): PIs are less than 0 for colors #1 or 2 and better in #5 or 6, middle (red): opposite tendency to those in the top panel, and bottom (black): others. The data for tissue C in the top panel are the same as those in Fig. 2B.

of biological specimens. Using multiple illumination colors and tissues, the current study found that biological specimens have a characteristic illumination color that is adequate for the visual recognition of individual tissues. This finding raises the question why visual recognizability of blood vessels was affected in the opposite way by bluish illumination. When blood vessels are observed through the skin, short wavelength light is absorbed effectively and scatters easily in the skin, thus hardly reaching blood vessels.[6] Therefore, long wavelength light as red mainly contributes to the visual discrimination between blood vessels and the surrounding tissues. On the other hand, when blood vessels are exposed, blue illumination can effectively differentiate blood vessels and the surrounding tissues because the difference of light absorption between blood vessels and the surrounding tissues is larger with short wavelength rather than long wavelength.[5]

Although a particular test color yielded better visual recognition compared to white in six out of the seven biological specimens, tissue discrimination in the abdominal cavity was more evident under white illumination compared to every test color. This might reflect the difference of visual information used in each recognition task. Recognition of a fine tissue (blood vessels, for example) from background tissues would require visual contrast between the two tissues. The contrast between the target and background tissues can be enhanced by modifying the illumination spectrum as reported in simulation and instrumental studies.[1–3] On the other hand, visual recognition of organs in the abdominal cavity would require additional visual information such as the difference in color and texture, which is provided by the illumination of a wide range of spectral characteristics as white.

In the present study, we evaluated how six colors (prepared by increasing or decreasing the red or blue component of an LED light system) influence the visual recognition of biological specimens. We especially focused on the effect of the blue component (see colors #3–6 in Fig. 1A). Therefore, the present results do not specify the most effective illumination color among all visible colors. As such, a comprehensive exploration as a follow-up study is necessary to determine the best illumination color for individual tissues.

Although the group data of all of the subjects indicated significant improvement or obstruction of visual recognition under particular illumination, individual subjects did not necessarily show the same tendency as the whole subject group. For example, in the recognition of fine blood vessels (Fig. 2A), color #6 significantly improved visual recognition, but 3 among the 20 subjects

reported better recognition under the white illumination (evaluations 1 and 2). Visual recognition tasks utilize various visual information such as color, contrast, and texture. Individual subjects might have given greater importance to one property than others to reach a distinct evaluation. Alternatively, individual differences in color perception might have influenced the evaluation of the illumination color. For example, the response spectrum of human cone cells in the retina varies even among individuals with normal color vision.[7] In the present study, we confirmed the absence of color vision deficiency by self-report at the time of enrollment and did not perform color vision test. Thus, we can not rule out the possibility that color vision deficiency in any of the subjects might have affected the evaluation of the illumination color.

Although the "extended vision" by modern imaging techniques using camera and image processing has produced significant advances in surgical technology, real-time inspection by the naked eye remains one of the primary tools in surgery and can be improved by appropriate illumination, as demonstrated in the present study. Considering that distinct illumination colors favor the discrimination/identification of various tissues, a surgical lighting system should be able to make fine adjustments in illumination color and readily switch colors between white and other task-specific colors.

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