How to display data by color schemes compatible with red-green color perception deficiencies

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Abstract: Visualization of data concerns most scientists. The use of color is required in order to display multidimensional information. In addition, color encoding a univariate image can improve the interpretation significantly. However up to 10% of the adult male population are affected by a red-green color perception deficiency which hampers the correct interpretation and appreciation of color encoded information. This work attempts to give guidelines on how to display a given dataset in a balanced manner. Three novel color maps are proposed providing readers with normal color perception a maximum of color contrast while being a good compromise for readers with color perception deficiencies.

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1. Introduction

The display or visualization of data is an important task concerning almost all scientists. This task translates to using color maps to relate a given data-value to a predefined color value (any shade of color or black & white). Two cases of images should be distinguished: firstly, those displaying univariate data (one dimensional) such as intensity images and secondly those displaying multivariate data (multi-dimensional), such as multi-channel fluorescence images.

Several groups have addressed and proposed apparently quite adequate ways to display data using colors and colorscales. Bergman et al. described rules on how to select color maps based on the problem to be displayed [1], Levkowitz focused on the human perception of color maps [2,3], whereas Healey improved color maps for a most effective contrast [4] and Rogowitz et al. investigated the potentially misleading representation by a rainbow-like color map [5]. This is by far not a full appreciation of all related contributions, a more detailed overview can be found in two reviews by Silva et al. [6, 7] which attempt to survey the research and investigations related to color and visualization.

Besides all these efforts, still two very common difficulties persist, firstly only very few color maps have been designed for printing and secondly too few if not none have addressed perception problems due to color vision deficiencies.

When using standard printers, the range of available colors is typically reduced to the CMYK-colorspace, which is based on a subtractive color model, which in turn is based on the fact that paper can only be darkened with the addition of ink (addition of 100% of each color component will result in black). This colorspace is fundamentally less broad than the RGB-colorspace (used for screens and projectors) which is an additive color model (addition of 100% of each component results in white). As a result, colors that require a mixture of CMYK-components (such as green (Cyan+Yellow), orange, but also the basic components of

the RGB-colorspace such as blue and red) are difficult to be reproduced in printing (CMYK) with the same color-intensity and brightness as on screen (RGB). It appears, that many of the commonly employed color maps neglect this aspect and are designed only to "look fancy on the screen" for persons with normal color perception.

Color perception deficiency is a very common problem which affects 8 to 10% of caucasian men [8] and 0.4 to 0.5% of women. As discussed by Light et al. [9], any color map employed to display scientific data should ideally be designed in such a way that persons with a color perception problem are able to view and perceive the true data and not be misled by incomplete color perception. The most commonly encountered deficiency is a red-green deficiency (Deuteranomaly and Protanomaly) which affects between 8 and 10% of adult men [8]. Brettel et al. have investigated how to visualize this reduced color perception [10]. For our investigation we have used Vischeck [11], an imageJ [12] plugin, to simulate the perception with red-green color perception deficiencies.

In this work, we attempt to give a guideline as to how to display your data. In particular we propose three novel color maps: "isolum" and "ametrine" color maps to display two channels of information: one encoded in color and one encoded in brightness and "Morgenstemning" a color map for visualizing univariate images with optimized contrast. Table 1 shows an overview of the methods proposed in this publication and serves as a guide for the preparation of images.

As a side note it should be mentioned that in addition to red-green color perception deficiencies (deuteranoptic and protanoptic color perception) there exists also a very rare blue-yellow color perception deficiency (Tritanomaly) affecting some 0.001-0.2% of people [13, 14]. Taking into account all different anomalies for the display of data would limit the display to black and white images, however it is obvious that completely omitting colors does not allow any multi-dimensional display of information which is a too severe limitation. There is no "cure for all problems", we have therefore opted for a compromise which neglects the rare form of Tritanomaly.

2. Displaying one channel (univariate data)

Visualizing univariate data (one-dimensional), an image can be displayed by mapping the data-values onto a linear-gradient between black and white. Such a "color"-map provides in addition maximum compatibility with a black and white printer. However most scientists explore data with a color monitor or by printing the data on a color printer. This additional degree of freedom allows pseudo-coloring of the dataset, yielding strongly enhanced contrast as compared to the black & white gradient visualization. The human eye can distinguish up to 10 million colors [15], and on the other hand, it can distinguish shades of gray among only 60-90 just-noticeable-differences (JNDs) [3], the advantage of color encoding is obvious.

The color map "hot" (Fig. 1(a)) is commonly used in science attempting this pseudo-coloring with the use of red and yellow color control points in addition to black and white while keeping a linearly increasing luminance for compatibility with gray-scale printing. However persons with a red-green color perception deficiency can only distinguish between red and yellow by differences in brightness and saturation and not by color. Hence they will only partially benefit from the increased contrast provided by the use of red and yellow.

Another commonly used color map "fire", adds blue to these previous colors (Fig. 1(b)). However at least the implementation within imageJ [12] suffers from a poor black and white compatibility, leading to distortions of image perception when printing the color encoded image on a black and white printer. As seen in Fig. 1(d), the gray conversion does not increase monotonically.

We propose "Morgenstemning" as a novel color map, this is based on the same idea of using blue, red and yellow in addition to black and white. We have carefully choosen the color control

Table 1. Guideline how to display your image data.

Input	Typical applications	How to display it?	Example
One channel	Fluorescence, OCT, MRI, Holography, calculations, simulations	color map *Morgenstemning* for improved contrast and printing compatibility (b&w or color)	Fig. 2(b)
Two channels (unlinked)	Two channel fluorescence, combination of images (i.e. same sample measured with different techniques or at different wavelengths)	*orange-blue* or *magenta-green* channels (complementary colors)	Fig. 3(b), Fig. 3(c)
Two channels (linked)	Fluorescence lifetime (ch1: intensity, ch2: lifetime), phase imaging (ch1: intensity, ch2: phase), depth encoding (ch1: Intensity, ch2: z-position)	ch1: *transparency map* and ch2: color map *isolum* by carefully choosing the limits to center on the information to be visible.	Fig. 5(b), Fig. 6(b)
		If your main information content is within ch2 and ch1 is only used to switch ch2 on/off: try *ametrine* for improved contrast of ch2	Fig. 7(b)
Three channels (ch1&2 linked, ch3 unlinked with ch1&2)	Fluorescence lifetime combined with phase contrast image (ch1: intensity, ch2: lifetime, ch3: phase-contrast)	*No unambiguous solution*. Try ch3 in *gray-scale* overlaid with ch1&2 prepared as for two linked channels, check output using color perception deficiency simulations	Fig. 7(c)
Three or more channels (unlinked)	Multi-channel fluorescence	*No solution* that is compatible with color perception deficiencies (only two dimensions of color available). Combine at best, check output using color perception deficiency simulations and provide individual channels as supplementary information (download). Or make multiple combinations of two channels to show their respective correlation.	

points such that persons with a red-green color perception deficiency will benefit to a maximum from the addition of color while maintaining a maximum contrast for persons with normal color perception. In order to ensure compatibility with color printing, the control points have been selected to ensure a CMYK-conversion without distortions.

In addition, special care has been taken of the linear luminance increase that provides black and white conversion compatibility. There exist several conversion algorithms between color and grayscale. A commonly used conversion is based on the spectral response of the human eye. It has been standardized by the NTSC television standard as the conversion to luminance [16] as:

$$\text{Gray} = 0.2989 \, \text{Red} + 0.5870 \, \text{Green} + 0.1140 \, \text{Blue} \qquad (1)$$
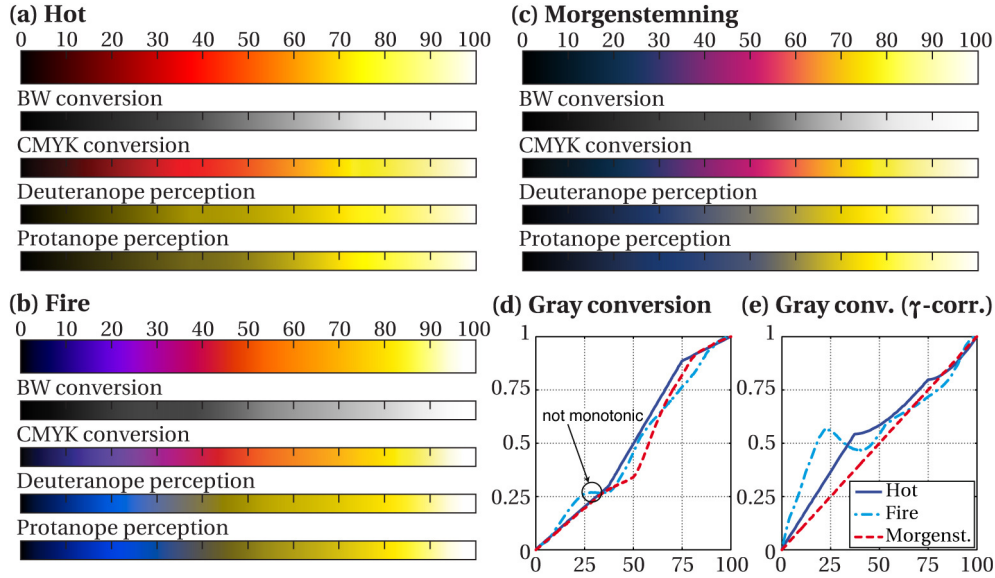
Fig. 1. Color maps for the display of univariate data and their corresponding conversion to black&white, CMYK color space (as used by color printers) as well as perception simulations for deuteranope and protanope perception. (a) Hot, (b) Fire and (c) the novel Morgenstemning. (d) Conversion to gray-scale of these color maps according to Eq. 1 and (e) according to Eq. 2. The CMYK conversion is best seen on screen.

However not all printers and software applications apply this formula. Some simply take the mean value between the three different color components and some take a gamma-correction into account prior to the conversion. After comparing the different conversion algorithms, we found most consistent results for the interpolation of the color control points when ensuring the linear luminance increase in a gamma-corrected RGB space such as proposed by Kindlmann et al. [17]. Hence our gray-value has been calculated as follows:

$$\text{Gray} = (\text{Red}^\gamma + \text{Green}^\gamma + \text{Blue}^\gamma)^{1/\gamma} \tag{2}$$

where γ is the gamma-correction factor of the device where the color map is shown (in the shown maps $\gamma = 1.8$).

Figure 1(c) shows the resulting color map, Fig. 1(d) shows the result of the gray conversion according to Eq. 1 for all the three shown color maps and Fig. 1(e) shows the gray-conversion in the gamma-corrected RGB space that has been used for the linear interpolation between the control points.

Finally Fig. 2 shows an example using the different ways of displaying univariate data. The image shows islets of Langerhans in a fixed pancreas measured with extended focus optical coherence microscopy (xfOCM) [18]. Morgenstemning provides an enhanced contrast compared to the gray-scale image and, as can be seen from the simulations of deuteranope and protanope perception, it provides optimal contrast also for color perception deficient viewing.

3. Displaying two unlinked channels (multivariate data)

Multi-dimensional data requires the use of color in order to overlay several channels of information within the same image. The case where the two channels are "unlinked" (such as two different wavelength channels of a fluorescently labeled sample containing two different labels)
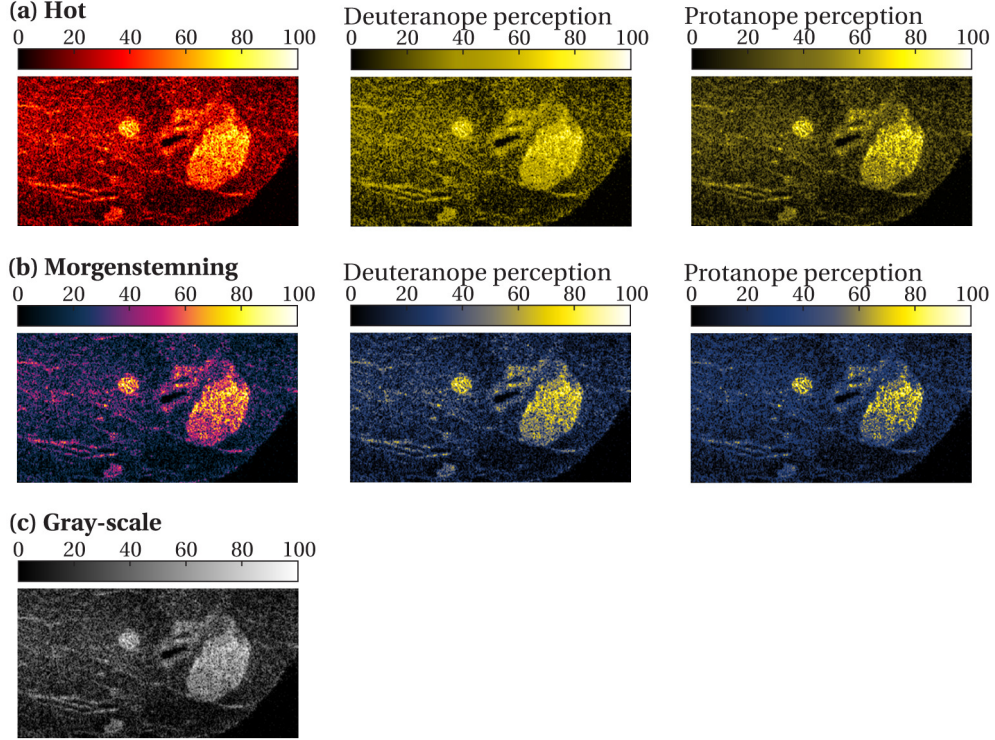
Fig. 2. Example of univariate data shown with different color maps. Image showing islets of Langerhans in a fixed pancreas measured with extended focus optical coherence microscopy (xfOCM) [18]. (a) Display using "hot" as a color map, (b) Morgenstemning with their corresponding simulated appearance for persons with color perception deficiency. (c) a normal gray-scale map for comparison.

can easily be performed by assigning different colors to each channel and encoding the respective data values as different brightness values of the respective color. Most scientists use red and green for the respective colors because the overlapping color yellow is easily distinguishable and facilitates the discrimination on colocalization.

$$
\begin{bmatrix} R_{\text{red-green}} \\ G_{\text{red-green}} \\ B_{\text{red-green}} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} Ch_1 \\ Ch_2 \end{bmatrix} \tag{3}
$$

However persons with red green color perception deficiencies are almost unable to distinguish between these three colors (Fig. 3(a)). Although regular comments on journal websites [19, 20] point to this issue, most scientists are are not aware of it. A simple solution to this problem has been proposed by Okabe et al. [14]

$$
\begin{bmatrix} R_{\text{magenta-green}} \\ G_{\text{magenta-green}} \\ B_{\text{magenta-green}} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 1 & 0 \end{bmatrix} \begin{bmatrix} Ch_1 \\ Ch_2 \end{bmatrix} \tag{4}
$$

The use of magenta and green allows for an optimal distinction between the two channels while keeping colocalization studies possible.
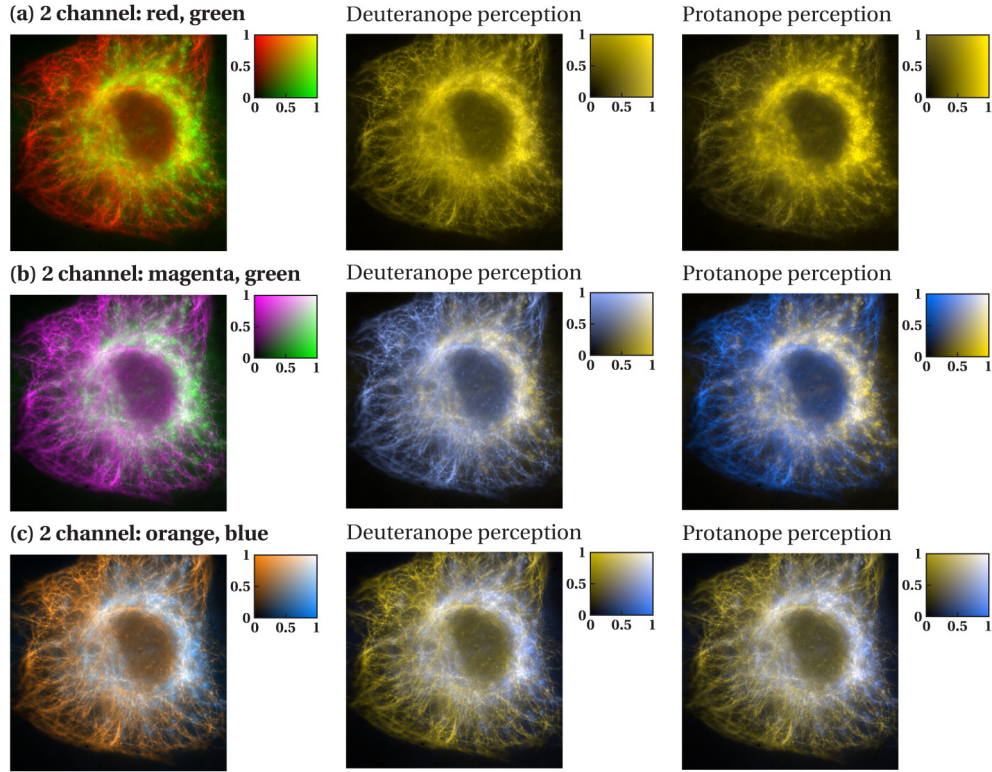
Fig. 3. Image with two color coded fluorescent channels. HeLa cells were labeled using conventional immunocytochemistry approaches. The microtubuli were marked indirectly with Alexa 568 (Channel 1) and, while a protein present in the mitochondria was labeled with Alexa 488 (Channel 2). Overlay of the channels with (a) ch1: red, ch2: green, (b) ch1: magenta, ch2: green and (c) ch1: orange, ch2: blue. The images on the right side, show simulations of how these images appear to persons with color perception deficiencies.

As an alternative to their approach, we propose the use of the two complementary colors blue and orange.

$$\begin{bmatrix} R_{\text{orange-blue}} \\ G_{\text{orange-blue}} \\ B_{\text{orange-blue}} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0.5 & 0.5 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} Ch_1 \\ Ch_2 \end{bmatrix} \tag{5}$$

Just as for the magenta-green solution by Okabe et al., overlapping areas will show up as white-gray because of the complementarity of the chosen colors.

Figure 3 shows an example of two channels combined in a single color image. In all three images, persons with normal perception can distinguish between the two channels and identify areas of colocalization easily. However persons with deuteranoptic or protanoptic perception, can only distinguish between the channels in the case of magenta-green and orange-blue combinations. In order to improve, the red-green combination has to be avoided, however the choice between orange-blue or magenta-green is mainly a question of personal taste.

4. Displaying two linked channels (multivariate data)

The case where the two channels are "linked" is more difficult to resolve. A typical example for such a case is a fluorescence lifetime image, where each pixel carries a fluorescence in-

tensity value and a fluorescence lifetime value. The lifetime value is only meaningful where fluorescence emission was detected. Other typical examples include depth encoding within an intensity image or any imaging method leading to an intensity value and an additional functional value (such as molecular brightness, fluorescence on-time ratio, etc...).

A suitable solution to this problem consists of encoding the functional channel (ch2) as a shade of color and the intensity (ch1) as the brightness value of that color (transparency map). It seems hence natural to use a HSV-color model, where one can simply use the *hue*-value (H) for channel 1, and the *value* (V, also called *brightness* B) for encoding channel 2. However the HSV-color model bears an inherent ambiguity since the color for the maximum value (red) corresponds to the minimum value as well. In addition, the colors are not isoluminescent, leading to additional distortions. The default color map within Matlab, called "Jet" removes the ambiguity between the maximum and minimum value. However the brightness decreases strongly towards the borders, which does not make it a suitable color map for encoding the first channel in color when combining with a second channel encoded as brightness value.

It has been quiet common [21,22] to use a color map that mimics the visible spectrum instead (color map "rainbow"). The perception of color depends on the context in which the color is used as well as on the intrinsic human perception [23]. Kindlmann et al. [17] have optimized the rainbow like color map for true isoluminescence using specially designed surveys. In order to achieve this, he has choosen a medium-low value of mean brightness. However in this specific case, we want to mask the displayed color with an additional intensity channel. Hence it is not only important that the colors are isoluminscent, they should also be bright. Figure 4 shows an overview of these different color maps.

We propose "Isolum" (Fig. 4(g)) as a novel isoluminescent color map for the display of "linked" multivariate data. Persons with a red-green color perception deficiency, will perceive this color map as a linear ramp between blue and "yellow", which corresponds to the limited two degrees of freedom of their color perception. However in order to employ the richness of colors available to the majority of people, the color map is not limited to these colors, but has been enriched with a red control point that has been carefully adapted in order to avoid a shade of color that would result in a nonlinear color-ramp for persons with red-green color deficiencies.

In addition all the control points have been chosen such that a conversion onto the CMYK colorspace neither distorts the isoluminescence nor color rendering, providing hence maximum compatibility for printing on a color printer. As for the color map "Morgenstemning", we have taken special care regarding the isoluminescence during the interpolation between the control points by using equation 2 to ensure isoluminescence (constant gray-level) over the full range of colors of the color map.

Figure 5 shows an example using this color map on an image of the vasculature of a mouse brain acquired using phase variance analysis on an xfOCM setup [24,25]. The functional channel contains the depth position and the intensity channel consists of the OCM A-scan signal. The image is shown once with a rainbow color encoding and once with an isolum encoding. The arrows 1 and 2 indicate a curved vessel and arrow 3 indicates a vessel crossing underneath this first vessel. In order to further indicate the position of these vessels, we added the xz-cut through the respective area. Persons with normal color perception will have no difficulty in identifying the position of these three vessels on either of these two proposed color encodings. However for a person with a red-green color perception deficiency, under the rainbow color-encoding it appears as if the three vessels are actually connected and form a Y-shape! Only when using the new isolum encoding are they able to identify correctly that the three vessels are not all connected.

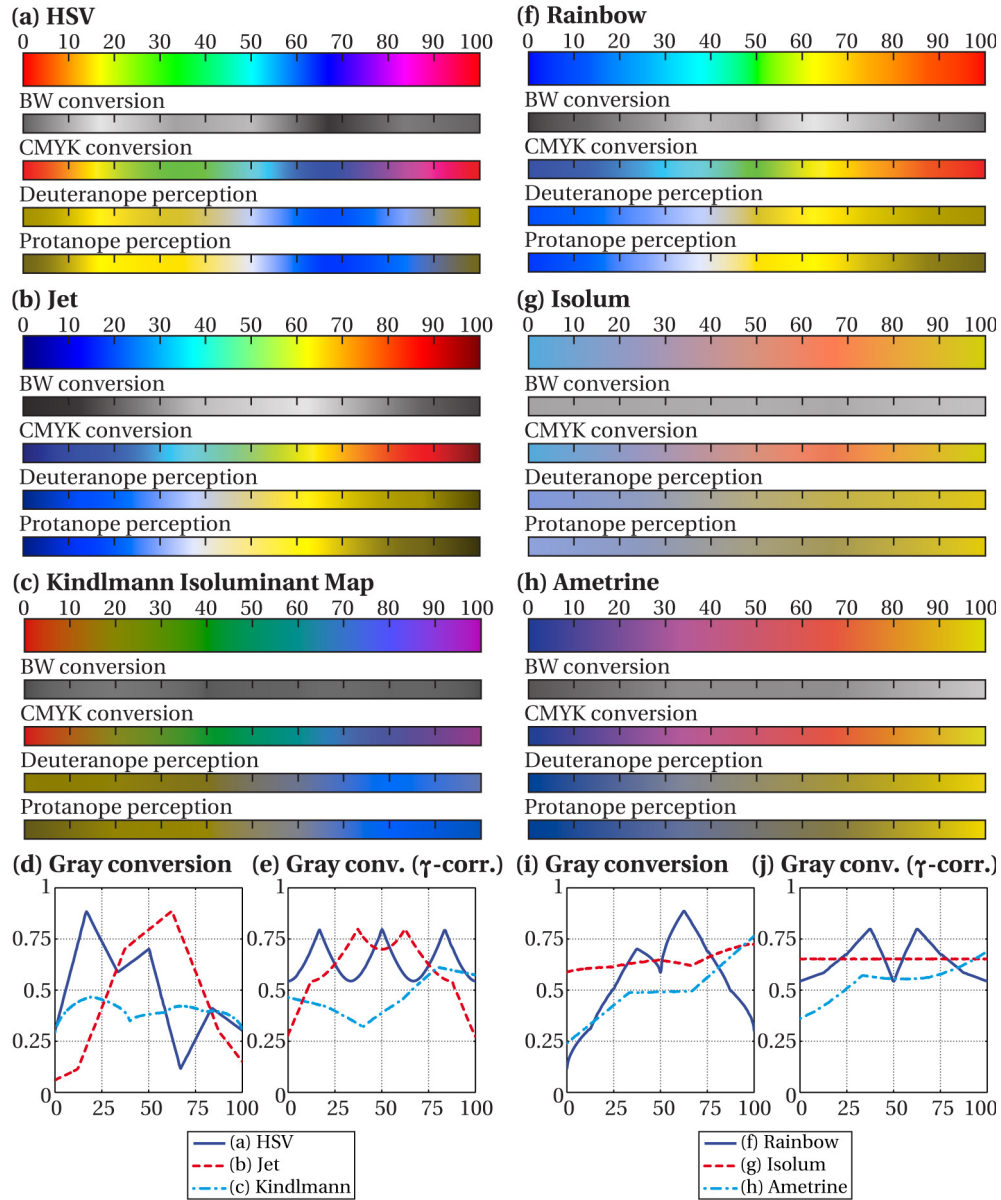Figure 6 shows a second example using the "isolum" color map for an image of Alexa647-

(a) HSV

0 10 20 30 40 50 60 70 80 90 100

BW conversion

CMYK conversion

Deuteranope perception

Protanope perception

(b) Jet

0 10 20 30 40 50 60 70 80 90 100

BW conversion

CMYK conversion

Deuteranope perception

Protanope perception

(c) Kindlmann Isoluminant Map

0 10 20 30 40 50 60 70 80 90 100

BW conversion

CMYK conversion

Deuteranope perception

Protanope perception

(f) Rainbow

0 10 20 30 40 50 60 70 80 90 100

BW conversion

CMYK conversion

Deuteranope perception

Protanope perception

(g) Isolum

0 10 20 30 40 50 60 70 80 90 100

BW conversion

CMYK conversion

Deuteranope perception

Protanope perception

(h) Ametrine

0 10 20 30 40 50 60 70 80 90 100

BW conversion

CMYK conversion

Deuteranope perception

Protanope perception

(d) Gray conversion

(e) Gray conv. (γ-corr.)

(i) Gray conversion

(j) Gray conv. (γ-corr.)

— (a) HSV
--- (b) Jet
-·- (c) Kindlmann

— (f) Rainbow
--- (g) Isolum
-·- (h) Ametrine

Fig. 4. Color maps for the display of two linked channels and their corresponding conversion to black&white, CMYK color space (as used by color printers) as well as perception simulations for deuteranope and protanope perception. (a) HSV, (b) Jet, (c) Kindlmann Isoluminant Map [17], (d) Conversion to gray-scale of color maps (a-c) according to Eq. 1 and (e) according to Eq. 2, (f) Rainbow, (g) Isolum, (h) Ametrine, (i, j) conversion to gray-scale of color maps (f-h). The CMYK conversion is best seen on screen.
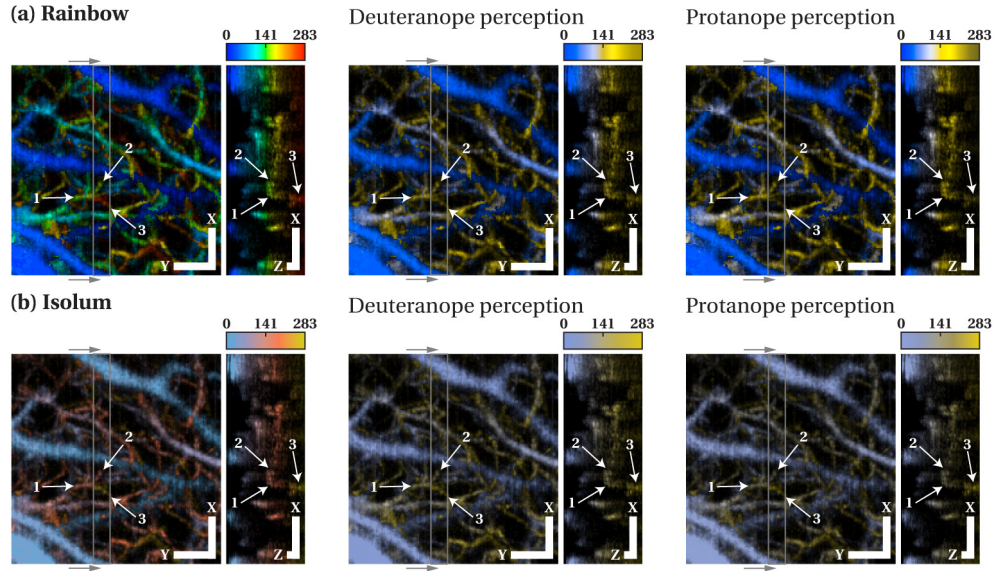
Fig. 5. Example of data of two linked channels shown with two different color maps. Imaging cerebral vasculature inside a mouse brain using phase variance analysis on an xfOCM setup [24, 25]. The images show maximum intensity projections along z and y respectively with a color-encoded z-position for a (a) rainbow-like color map and (b) isolum color map with their corresponding simulated appearance for persons with red-green color perception deficiency. Scalebars: 50 μm. The Fig. is best seen on screen.

labelled microtubules inside HeLa cells. The super-resolved imaging technique bSOFI displays the molecular brightness in addition to the intensity image [26]. Under normal color perception, both color encodings allow the different areas of molecular brigthness to be distinguished. However in particular the two zones indicated by a white arrow cannot be distinguished by a person with red-green color perception deficiency when the image is encoded with the color map rainbow. However on the image with the isolum color encoding they perceive correctly these two zones which do not show the same molecular brightness.

4.1. Ametrine, an alternative with slightly improved color-contrast

In the previous examples we have used a strictly isoluminescent color map for color encoding. In order to increase the contrast of the functional channel slightly, we have altered slightly the isoluminescence. The resulting color map "Ametrine" offers a slightly increased contrast at the cost of being slightly unbalanced in luminescence. This trade-off can be interesting in particular for cases where the main information to be shown is within the "functional" channel to be encoded as color, while the "intensity" channel is merely used as a binary-like mask (in order to switch the display of the color channel on/off).

Figure 7 shows an example of color encoding a Triplet Lifetime Image with this color map. The image shows adhering CHO-cells that have been incubated with TMR-labeled hyperbranched poly-Lysine [27, 28]. A person with normal color perception can distinguish on the rainbow-color encoding as well as on the ametrine encoding that the cells show a cell polarization (indicated by the arrows) and that there appear to be granular like structures in the interior of the cell. However to persons with deuteranoptic or protanoptic color perception, the images with the rainbow-color encoding appear as if the cell polarization happens in a large
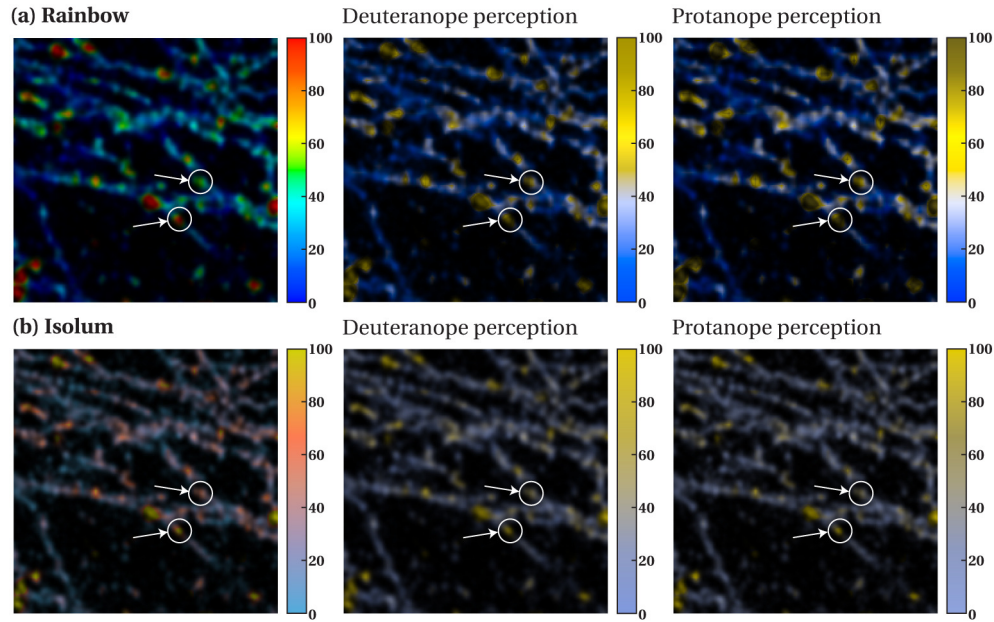
Fig. 6. Color-coded molecular brightness (in arbitrary units) of HeLa cells with Alexa647-labelled microtubules overlaid with the 5th order balanced cumulant measured by bSOFI [26]. Color encoding by a (a) rainbow-like color map and (b) isolum color map. The Fig. is best seen on screen.

"diffuse" area (about the size of one third of the cell). Ametrine-encoding overcomes this issue, and persons with color perception deficiencies can distinguish the granular-like structure of the cell polarization-phenomena.

5. Displaying three channels (ch1&ch2 linked and unlinked with ch3)

Adding an additional third channel (for example fluorescence overlaid with a phase contrast image) to the image in Fig. 7 we should first notice that the accessible spectrum for those with a red-green color perception deficiency is restricted to two colors. Therefore there is no unique way to avoid an ambiguity upon adding a third channel.

Nevertheless, it might be a suitable compromise to encode this third channel as a gray-scale image and to overlay it with the previous color encoded channel 2 modulated in luminescence by channel 1.

Figure 7(c) shows an example of such a color encoding. For persons with normal color perception, this image does not present any ambiguity: channel 1 and 2 appears colored and the information content of channel 3 appears in gray-scale. However for a person with color perception deficiency there is an ambiguity between triplet lifetimes around 4 μs (resulting in a "gray" color instead of blue or yellow) and pixels of the phase-contrast image with this same gray-value. The ambigous shade of color has been marked with an A-arrow within the images with the simulated deficient color perception. This issue is a necessary consequence of the limited available colors for the color deficient perception. Therefore any "gradient" between the available colors will cross a grey-like value.

In order to cope with this problem, it is important to verify the final image with the help of color perception deficiency simulations and check if there is any severe ambiguity or if the compromise is acceptable.
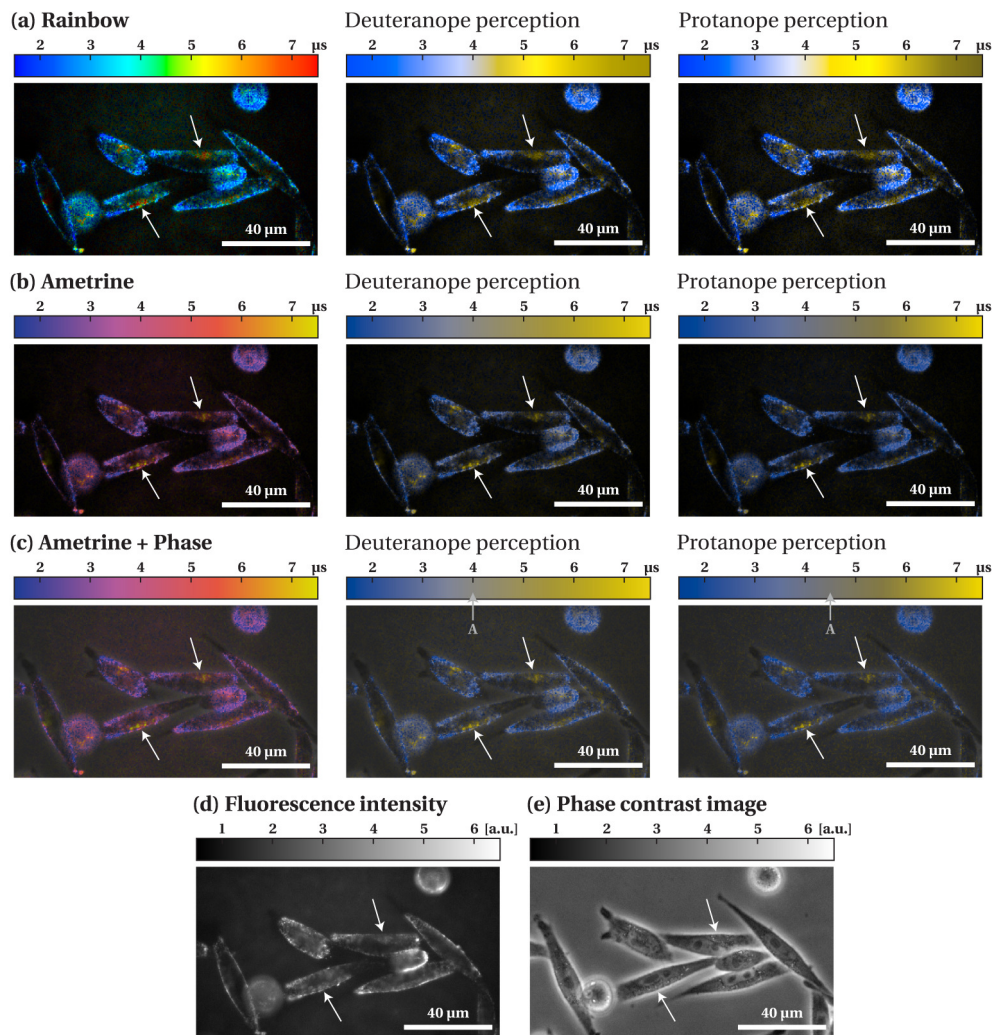
Fig. 7. Color-coded triplet lifetime image of adherent CHO-cells with TMR-labeled hyperbranched poly-Lysine [27, 28]. Color encoding by a (a) rainbow-like color map and (b) ametrine color map and (c) overlay of (b) with the phase-contrast channel. The cells have been incubated for 15 min with TMR-labeled hyperbranched poly-Lysine HBPL 80kDa. The image has been taken 43 min after washing the cells with ProCHO5 at 37° C. The "A" arrow marks the triplet lifetimes leading to a grey-color which for color deficient perception results in an ambiguity compared to the grey-scale encoded phase-contrast image. The Fig. is best seen on screen.

In the image shown in Fig. 7(c), the main information content is the granular-like cell-polarization. This remains perceptible even under deuteranoptic and protanoptic perception and hence the combination of three channels appears as a an acceptable compromise.

6. Displaying three unlinked channels

As mentioned in the previous section, there is no solution in combining three channels of information without ambiguity for persons with red-green color perception deficiency that have only

two dimensions of color available. As proposed by Okabe et al. [14], the reader is encouraged to display the channels individually, provide them as a supplementary download file, or to show multiple images of two channels in order to show the correlation or colocalization between two channels at a time.

7. Conclusion

Red-green color perception deficiency is a common physical deficiency of human color perception and affects up to 10% of the adult male population.

In this publication, we have shown guidelines for displaying different types of image data compatible with red-green color perception deficiencies.

We have demonstrated Morgenstemning as a novel color map for the display of univariate data including a maximum of color while staying strictly monotonic in luminescence for maximum compatibility with black & white printing.

In addition we have explained how two unlinked channels can be combined within a single image with no information loss for persons with red-green color perception deficiency.

Last but not least we propose two new color maps "Isolum" and "Ametrine" which allow the display of two linked channels: a functional channel (color encoded) and an intensity channel (luminescence/brightness encoded). We have compared these new color maps to the classically employed "rainbow"-color map and demonstrated examples where our new maps allow viewing without ambiguity even by persons with red-green color perception deficiencies.

The color map has already been proven useful in multiple publications [26, 28, 29].

Supporting information

The different color maps are available free of charge. An archive containing the proposed color maps "Morgenstemning", "isolum" as well as "ametrine" in various file-formats (Matlab, ImageJ, comma-separated values) can be downloaded via the Internet at http://lob.epfl.ch/ under "Research/Color maps".

The name "Morgenstemning" is the Norwegian word for "morning mood" and the name of a movement from the "Peer Gynt" suite, a composition by Edvard Grieg. "Ametrine" is the name of a naturally occurring variety of quartz containing zones of purple and yellow or orange.