Seeing Colours

Image Processing & Analysis for Life Scientists

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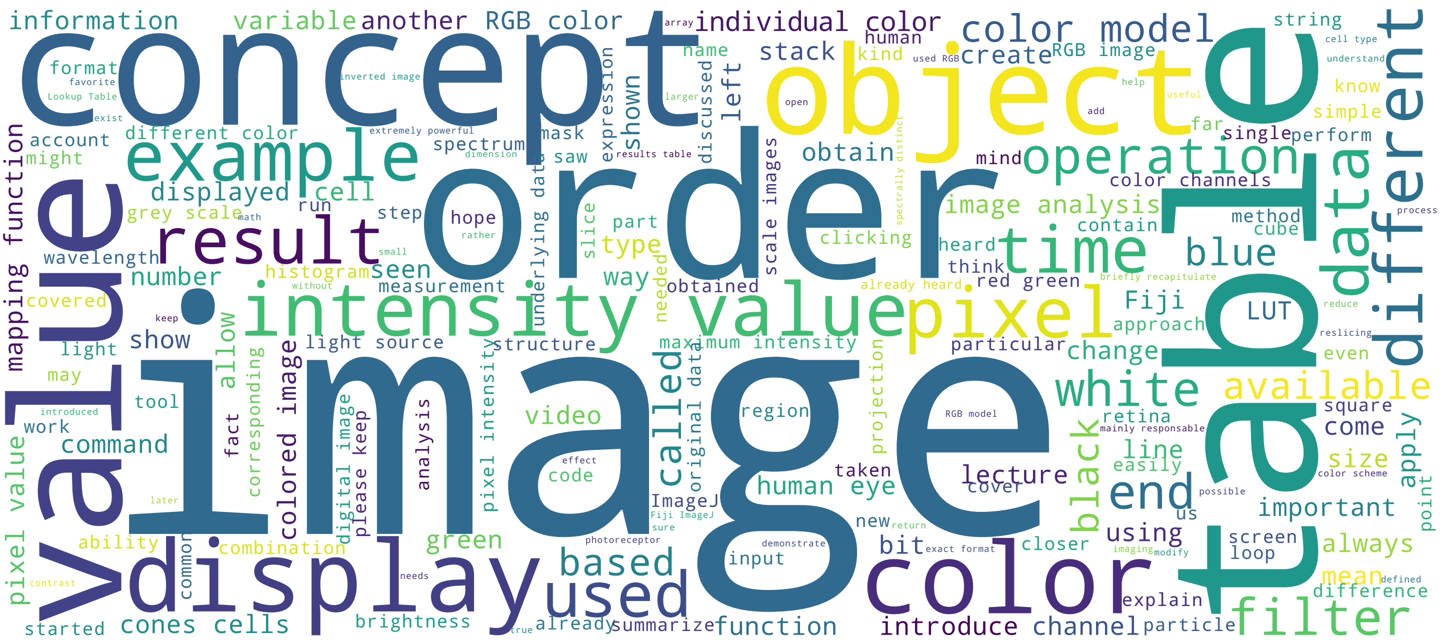

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Outlook

- Seeing Colours
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- Look up Tables (LUT)
- LUTs in Fiji/ImageJ

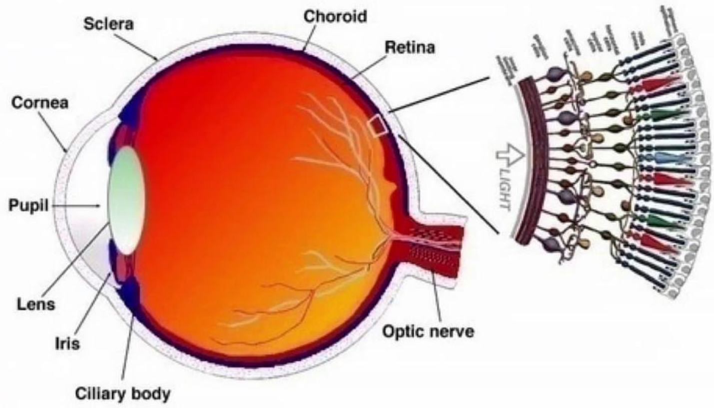
Welcome to this lecture in which we will discuss color images and how they are used in life sciences. We will briefly recapitulate the human eye and its capability to see colors. Then we'll introduce the most common color scheme for images called RGB. And we'll explain the concept of look up tables towards the end of the lecture.

Notes

Summary

0m 05s

The Human Eye



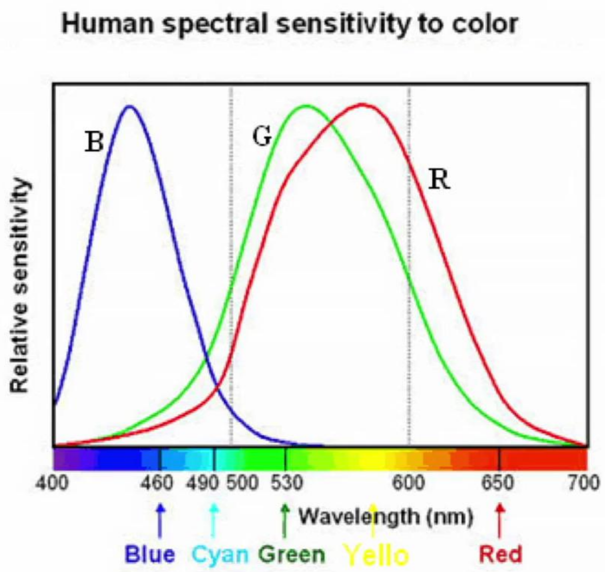
Up to now, we have used black and white, also termed greyscale images. Now we'll add an additional layer of complexity and color them up. Before doing so, we should briefly recapitulate the mechanism in the human eye which enables us to see colors. Most of you may have heard this already. But it's important to understand the widespreadly used RGB color model. And we will briefly look into it. The retina is the part of the eye which contains the photoreceptor cells. The image formed by the lens is projected onto this layer of cells. The retina of vertebrates has 2 types of photoreceptors. The so called rods cells are mainly responsable for situations where we have to deal with low light levels. You might have already heard the proverb "all cats are grey by night" So these cells have a much higher sensitivity than the cones cells that deliver black and whites images. For seeing colors, other photoreceptors are needed, the so-called cones cells. These cells exist in 3 spectrally different variants. The spectrum of the rod cells covers the range from 400 to 800 nm. Which is therefore also known as the visible range.

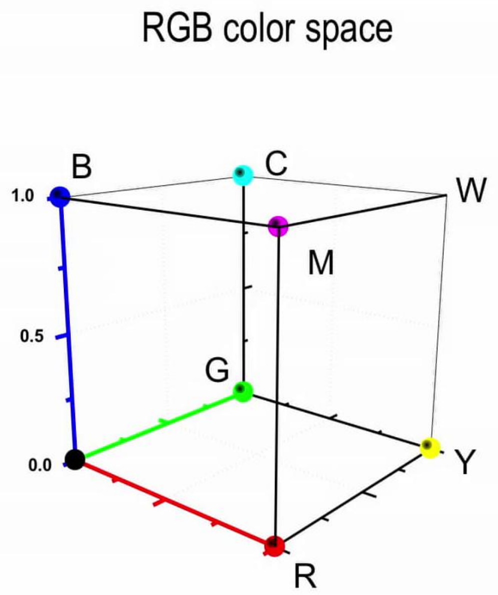
Notes

Summary

0m 27s

Seeing Colors





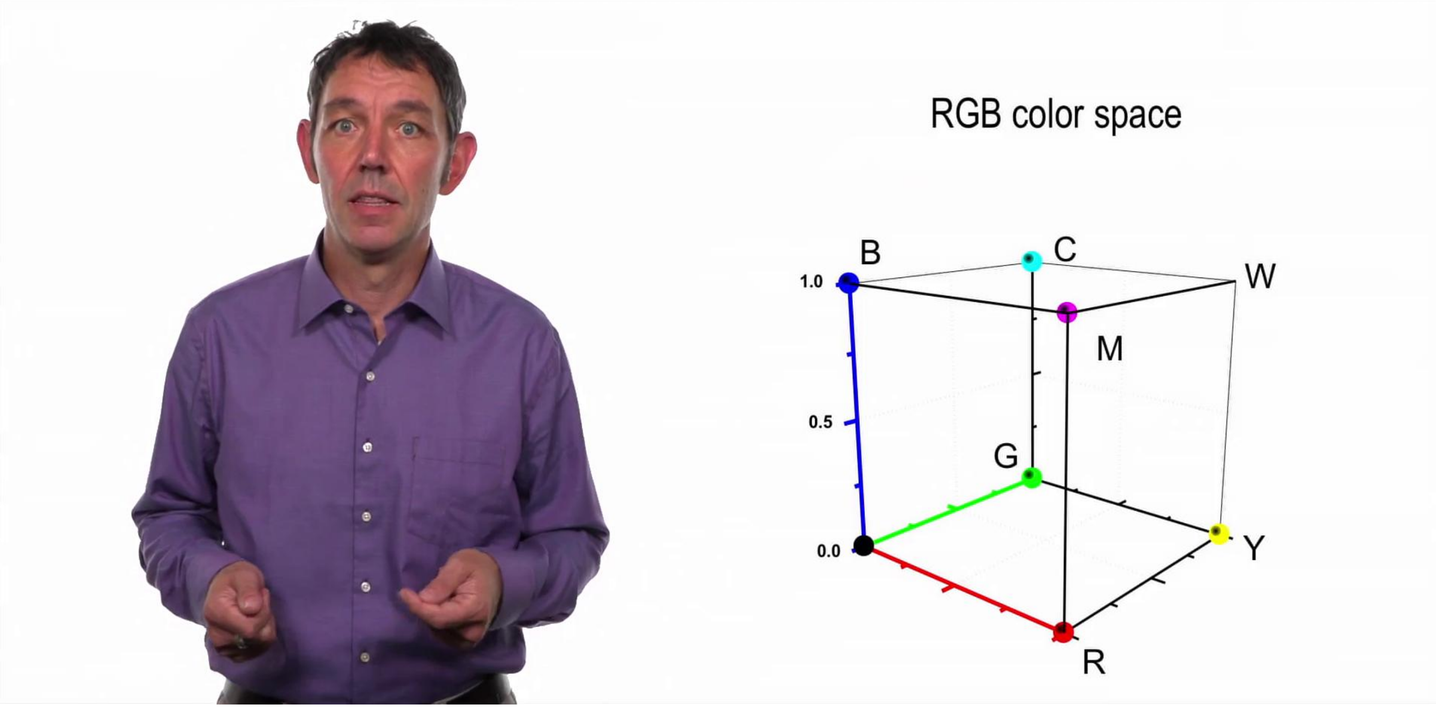
The relative sensitivity of the 3 different cones cells is plotted versus the wavelength. Our ability to see colors stems from the fact that a given wavelength is stimulating each cell type to a certain extent. The combination of these 3 different stimulation levels is responsible for the color we're recognizing. Looking at the spectrum properties of the 3 types of cones cells, again we can see that it is mainly responsable for detecting short wavelength. It covers the blue part of the spectrum. The other cells have the maximum sensitivity at longer wavelengths, are covering the green and red parts of the spectrum. So we can conclude that the ability to see different colors is based on 3 spectrally distinct photosensitive cells. This concept has been copied in order to construct one of the most popular color system to represent colors. I'm sure you have already heard of RGB images. The exact format of this image format will be discussed in a following lecture. However we'll have closer look at the underlying principle to describe colors. In general, color schemes can be divided into two categories. The additive ones and the subtractive ones. The first one is, to my understanding, the more intuitive one. Here are colors formed by the mixing light of different wavelengths. This is a model which is based on a direct emission.

Notes

Summary

1m 48s

Seeing Colors



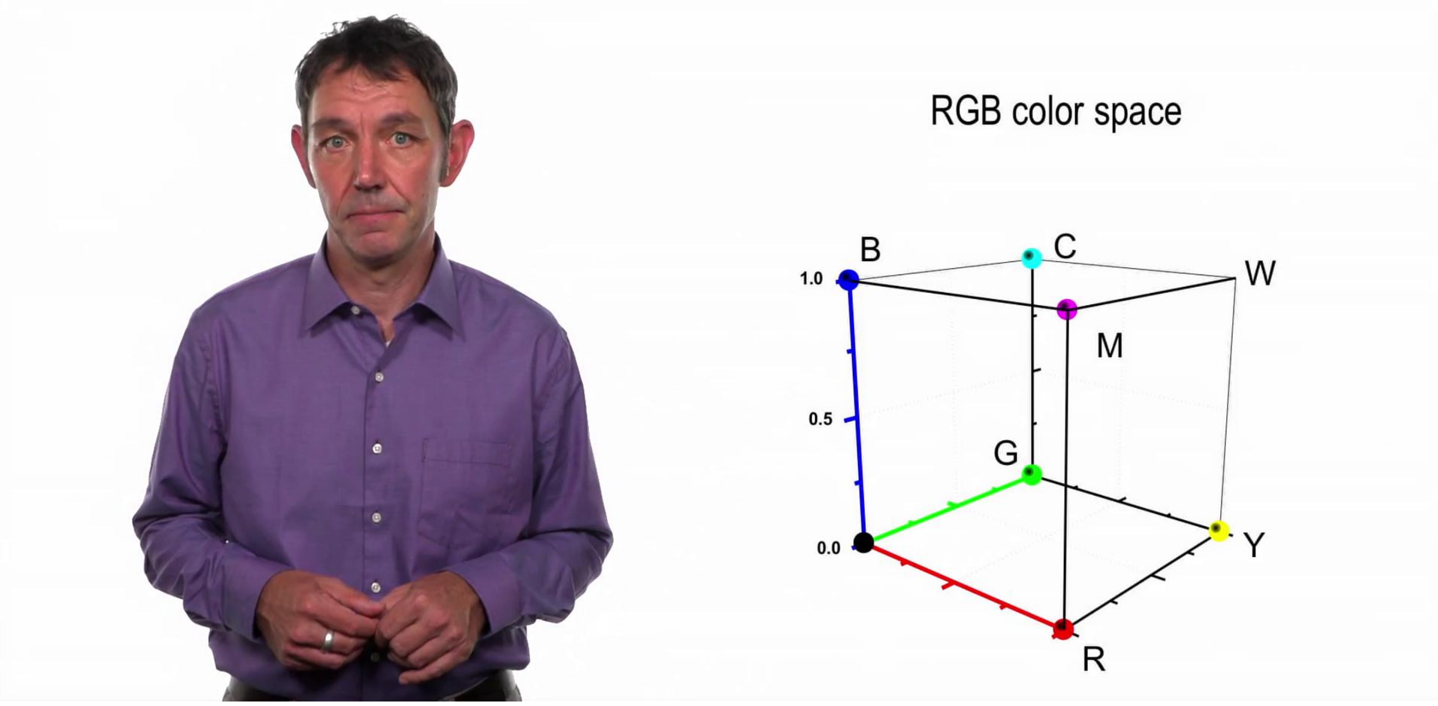
So we do not need an additional light source. In the subtractive color model, a light source is essential and needs to be taken into account. We might already have encountered this effect. The appearance of your most colorful T-shirt might be completely different at sunlight or in equally bright room light, where the light is coming from a neon bulb. Here the color is formed by the light reflected from the object. There the light of the light source and the absorption of the object need to be taken into account. Thus, it is more complicated than the additive color model. Let's have a closer look at the RGB color model. We do have 3 color channels. And, not too surprisingly, they are called: red, green and blue. To each of the color channels, we assign an intensity value. This concept is the same one which is used for grey scale images. But in the RGB model, we need 3 distinct intensities for the different colors. One representation of the independent parameters is a cube. We have 3 axes perpendicular to each other. The colors of the cubes are given by the pure individual colors red, green and blue. Now if you mix 2 of these colors, we come to 3 more colors named yellow, cyan and magenta.

Notes

Summary

3m 27s

Seeing Colors



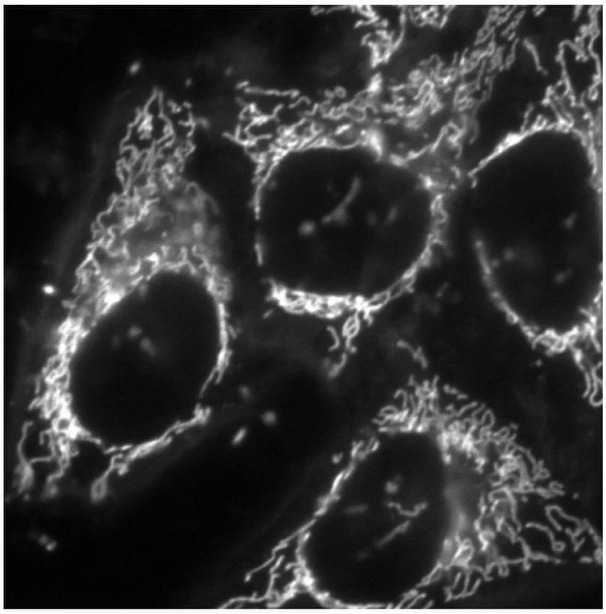
And you can put them in the corners between the individual colors. The 2 remaining corners are black, with no light to see, and white, with the maximum intensity in all 3 individual colors. The numbers of colors we can display depends on the numbers of intensity steps. If we allow over 256 steps for each color. We end up roughly with 16 millions individual colors.

Notes

Summary

4m 55s

Data and its Display



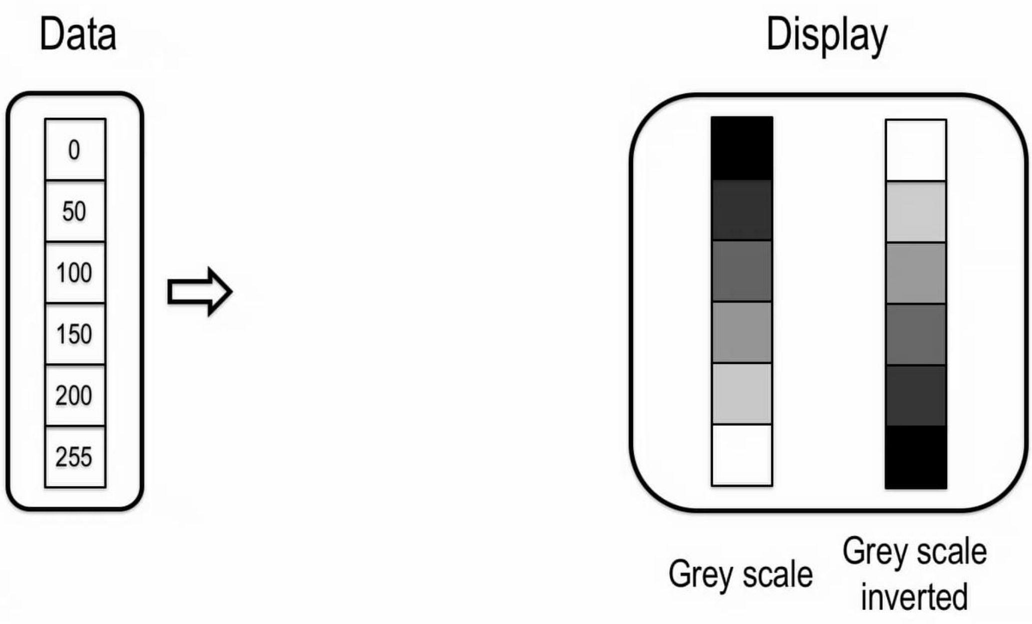
The exact format to store RGB images, will be discussed later. Now I want to introduce the concept of 'look up tables'. This is an extremely powerful concept, as it allows to color images without the need of changing underlying data. Please keep in mind that most of the detection devices contain no or little spectral information. Therefore adding color to an image can always be seen as a bias; or, as least, as an interpretation. In particular if it comes to image analysis, the preferred strategy is to preserve the original data, and have a colored display at the same time. This is realized in the concept of look up table. And I will show you how it works, and how you can color the images shown on the left.

Notes

Summary

5m 26s

Mapping Functions

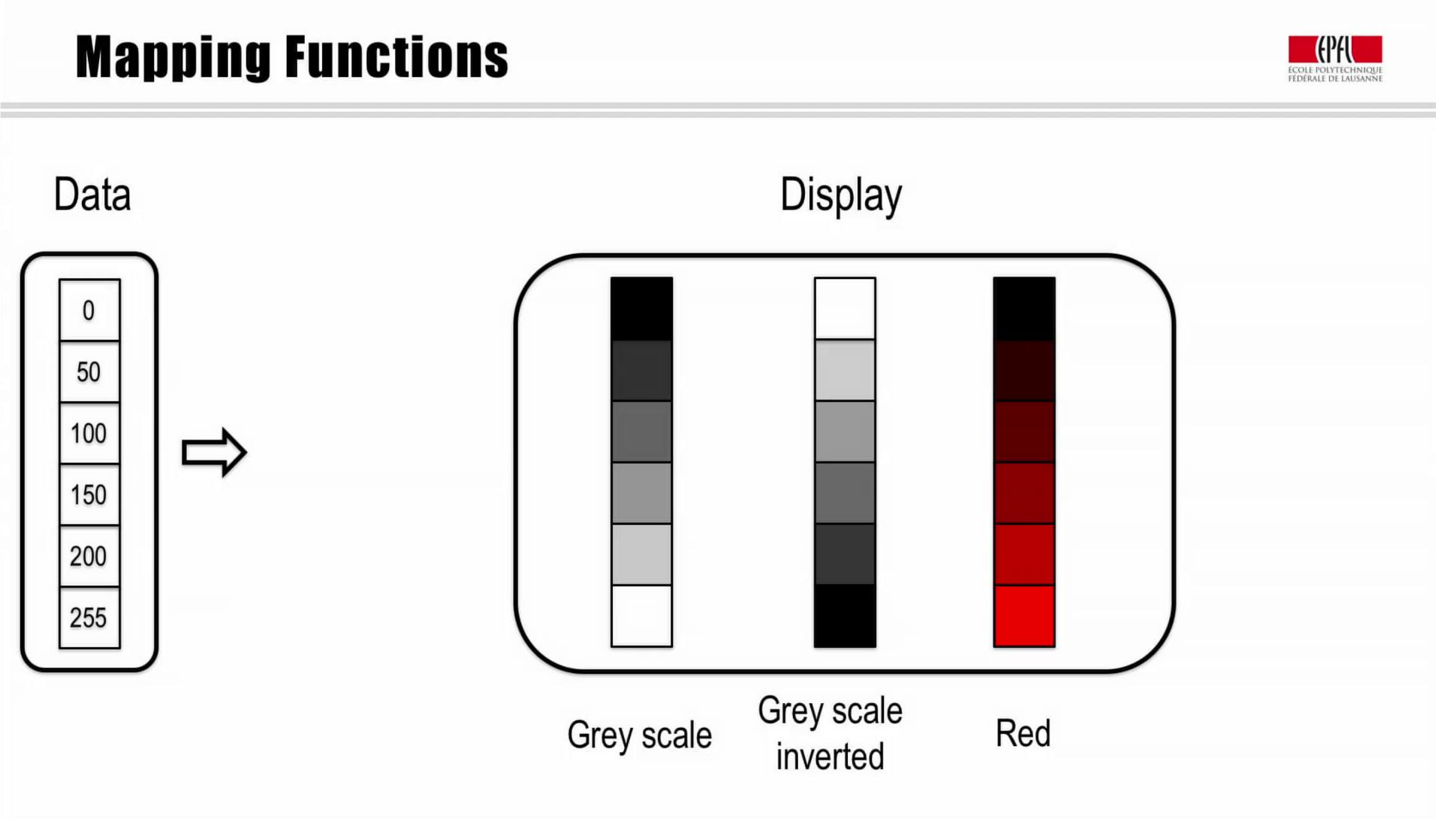


The concept of look up tables is comparable to the concept of adjusting the brightness and contrast of an image. You might remember that it was possible to change the display of an image on the screen without changing the underlying data. And we can extend this for the display of colors. For the so far used grey scale images, we defined that the pixel intensity value 0 is displayed as black. And the maximum pixel intensity value is displayed as white. What do we need to do if we want to display an image the other way around? We have 2 possibilities to obtain such an inverted image. The first one involves some math. We don't wan't to follow this approach any further here. But the ones interested can try to find the mathematical operation which is needed to come to such an image. The 2nd approach is not altering the data. Here we just change the mapping function. We define that the intensity value 0 is displayed white, and the maximum intensity value as black. And obviously, we obtain an inverted image with such a definition. This is what is called a look up table. It is just a convention on how we display data on a screen. The objectivity of the concept is that it can be easily extended and also be used in order to obtain colored images.

Notes

Summary

6m 19s



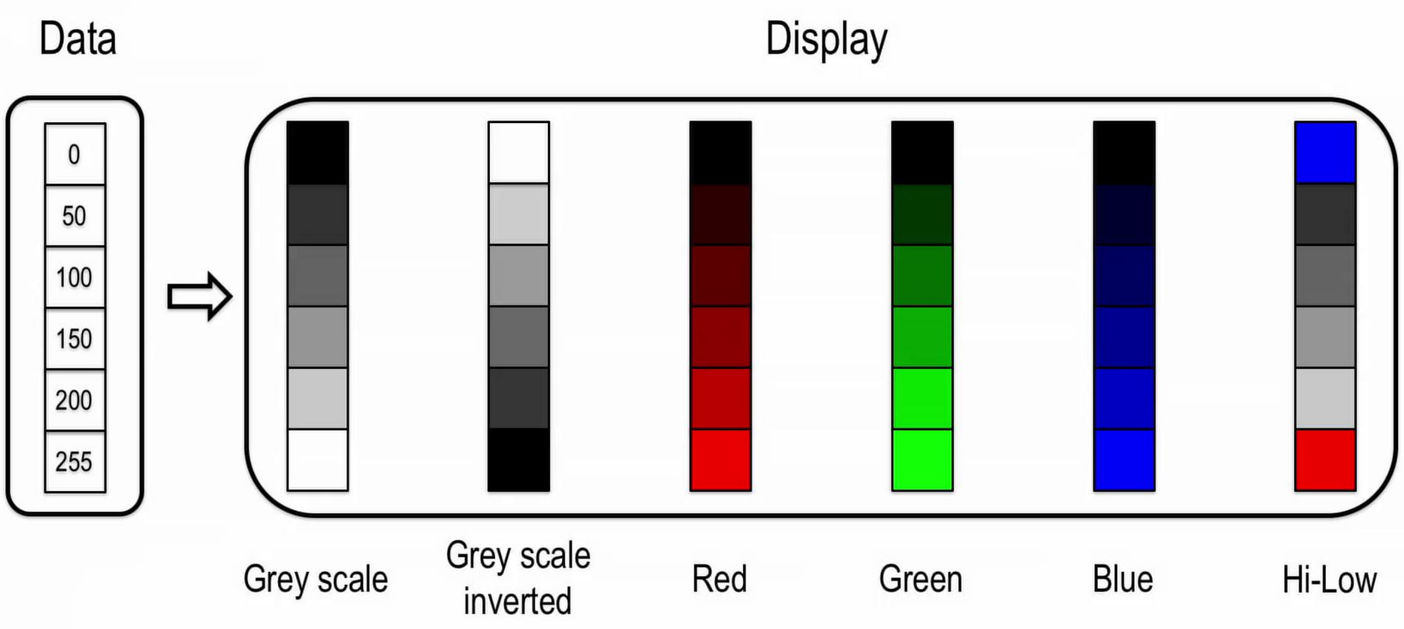
We just need another definition of our mapping function. In order to obtain red image we display the pixel intensity value of 0 as black; and the value of 255 as bright red. If you think about the cube of the RGB model, it means that we're moving along the axe from the origin to the red corner in order to diplay the values between 0 and 255.

Notes

Summary

7m 50s

Mapping Functions



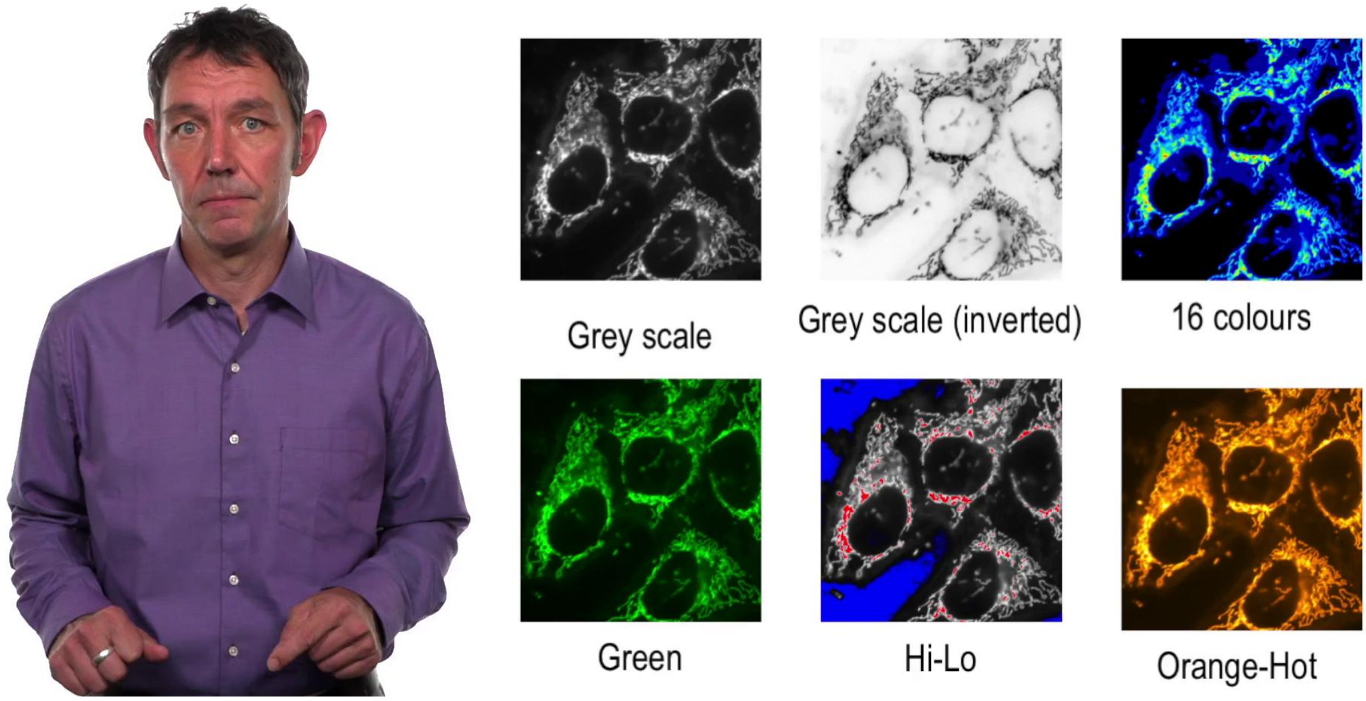
So if you repeat the procedure described before for the other 2 colors, we can easily produce images which are displayed red, green or blue. And please keep in mind that the data of the image remains unchanged. So far we have used a continuous mapping function. But Nothing hinders us to use a non continuous definition as well. Have a look at the "high/low" look up table. It is extremely similar to the grey scale one with which we started. Only for the values of 0 and 255 there is a difference. We just convene to display these intensity values either blue or red.

Notes

Summary

8m 14s

Look up Table (LUT)



By using such a look up table we can easily identify regions with the maximum intensity value, and if we apply it during image acquisition, we can avoid saturation over image easily. That was rather dry theory. Now let's have a look what kind of images can be obtained by just changing the look up table. On the left side, you see the image of fluorescently labelled mitochondria displayed with 6 different look up tables. Take your time, have a look at them and find out which one is your favorite.

Notes

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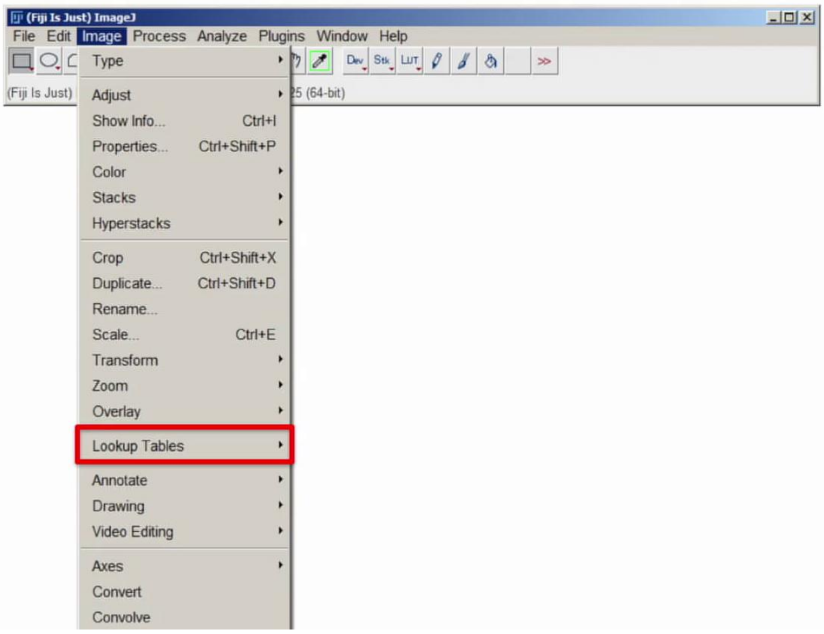
8m 56s

LUT in Fiji/ImageJ



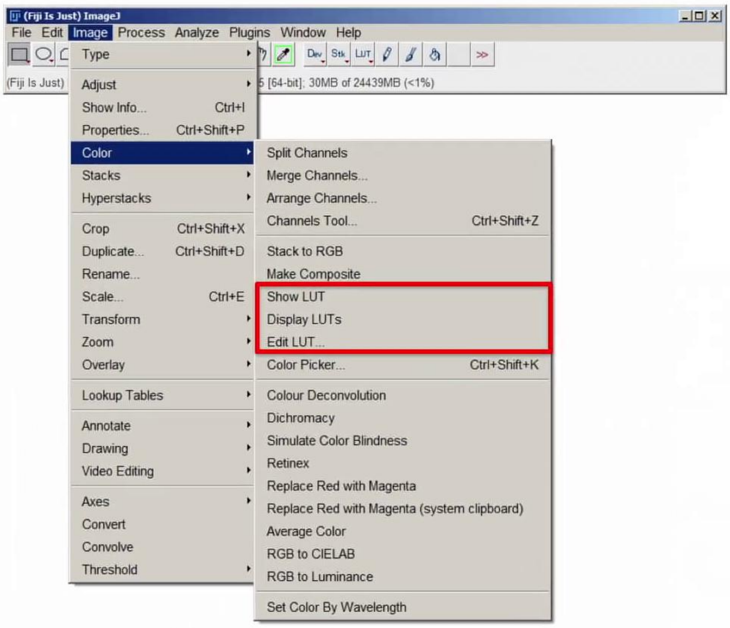
The concept of look up table is also available in Fiji. By clicking the little arrow in the red square (Image>Lookup Tables) all the available look up tables will be shown. And you can apply them to an image just by clicking.

Notes

Summary

9m 29s

LUT in Fiji/ImageJ



More information about the look up tables is available in the submenu shown on the left. You can show the look up table you are using. Display all available ones. Add an existing one. Or create your own one.

Notes

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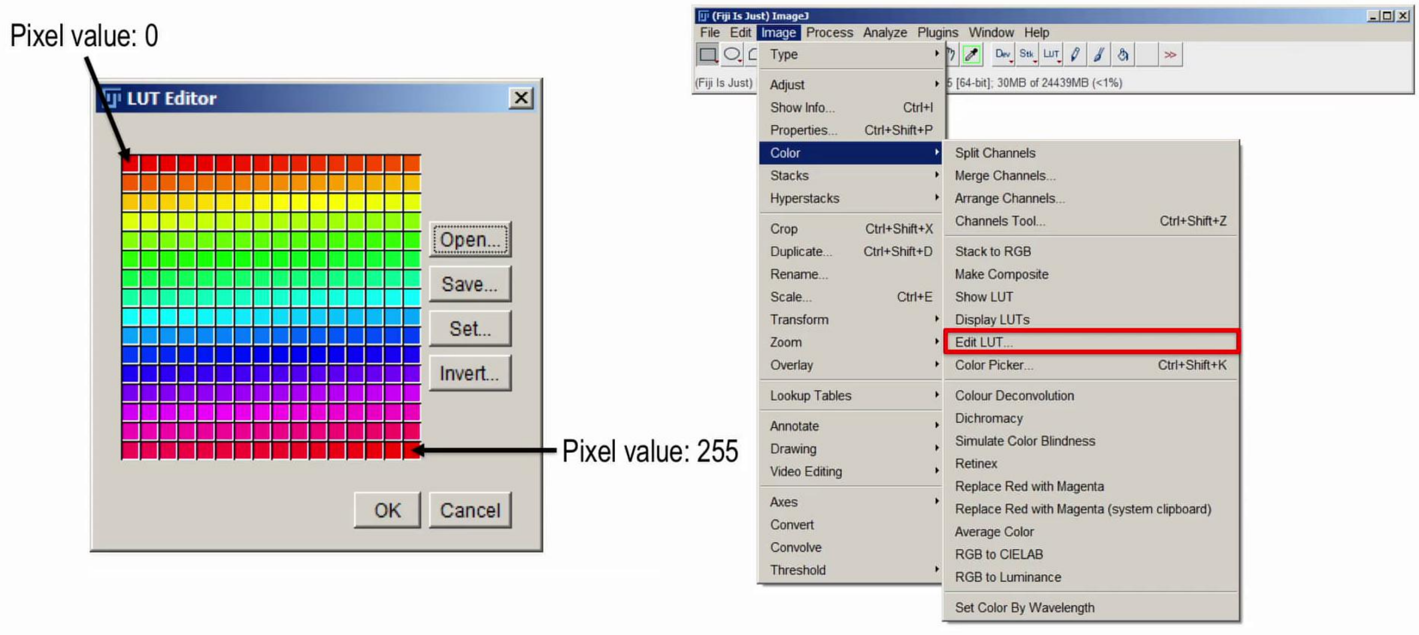
9m 44s

LUT in Fiji/ImageJ



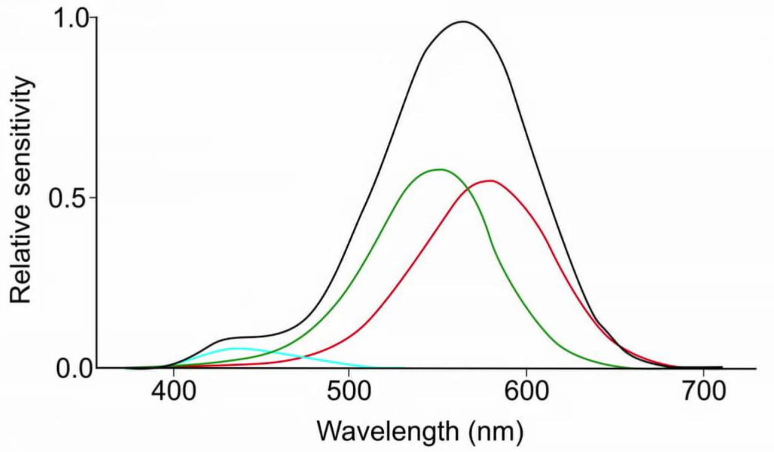
On the right hand side you see the LUT editor for the look up table called 'Spectrum'. The square in the upper right corner is corresponding to the pixel value of 0. The one in the left corner, to the pixel value of 255. Each pixel value is associated to an individual color. By clicking on an individual square, you can change the color and decide how this value is displayed. Thereby you can create your favorite look up table.

Notes

Summary

10m 00s

Pitfalls



Modified from:
Wald, G. The Receptors of Human Color Vision. *Science* **145**, 1007-1016 (1964).

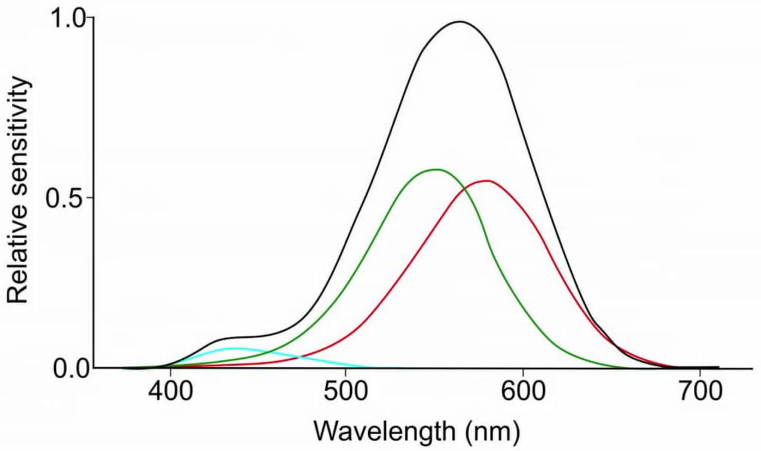
There are more LUTs than the 6 ones used to color up the images of mitochondria. Just have a look at the image on the left. There, all the LUT that can be currently found Fiji/ImageJ are displayed. Some of them are generally suited. Some of them are tailored for one particular need. But they all have in common that they are just mapping functions only affecting the display of the data on the screen. We'll later on see that the size of a colored image is a factor of 3 larger than the one of black and white images. One can also consider a LUT as a concept to reduce the data size of scientific data. Towards the end of this lecture I want to discuss pitfalls when using colored images. The most obvious drawback is that a part of the human population have difficulties to distinguish red and green colors. This is called dichromacy. Especially in images 2 different colors, this will become an issue for humans having this deficit. In order to avoid misinterpretations, using cyan, magenta and yellow instead of green, red and blue, can be a good strategy. And based on what we have heard about the human eye and RGB color model you shall be able to explain the reason of this approach.

Notes

Summary

10m 29s

Pitfalls



Modified from:
Wald, G. The Receptors of Human Color Vision. *Science* **145**, 1007-1016 (1964).

A little less known is the fact that the cone cells responsible for the detection of the blue color are much less abundant in the retina than the two other ones. Therefore the sensitivity to detect blue light is by one order of magnitude lower, compared to the detection efficiency of green or red light. When the same structure is displayed with a blue or with a green LUT, we have the impression that the green one is brighter. As brightness is intuitively often correlated with concentration, this can lead to wrong conclusions.

Notes

Summary

11m 52s

Summary

- Look up tables (LUT)
  - Mapping function
  - Only affects the display of the imag
  - Preserves the original data.
  - Portable

This again demonstrates that an analysis taking the real value into account is always the preferred solution, as human visual perception can add unwanted bias. However, in order to illustrate things, colors and especially, the concept of LUT is extremely powerful. So, it's time to summarize! We have discussed that color vision in humans is based on cones cells which occur in 3 spectrally distinct variants. The cell types are stimulated to a different extent by a given wavelength. The combination of the stimulation is perceived as an individual color. This concept of 3 independent color channels has been transfered into the most commonly used RGB color model. In order to avoid modifying data, the concept of look up table has been introduced. A LUT is a mapping function which allows you to display a colored image without the need to modify the underlying original data of the image. Have fun with the exercises! Color up your images! And I hope to see you next time. And take care!

Notes

Summary

12m 28s