

## The paradox of color space display

[https://en.wikipedia.org/wiki/HSL\\_and\\_HSV](https://en.wikipedia.org/wiki/HSL_and_HSV)

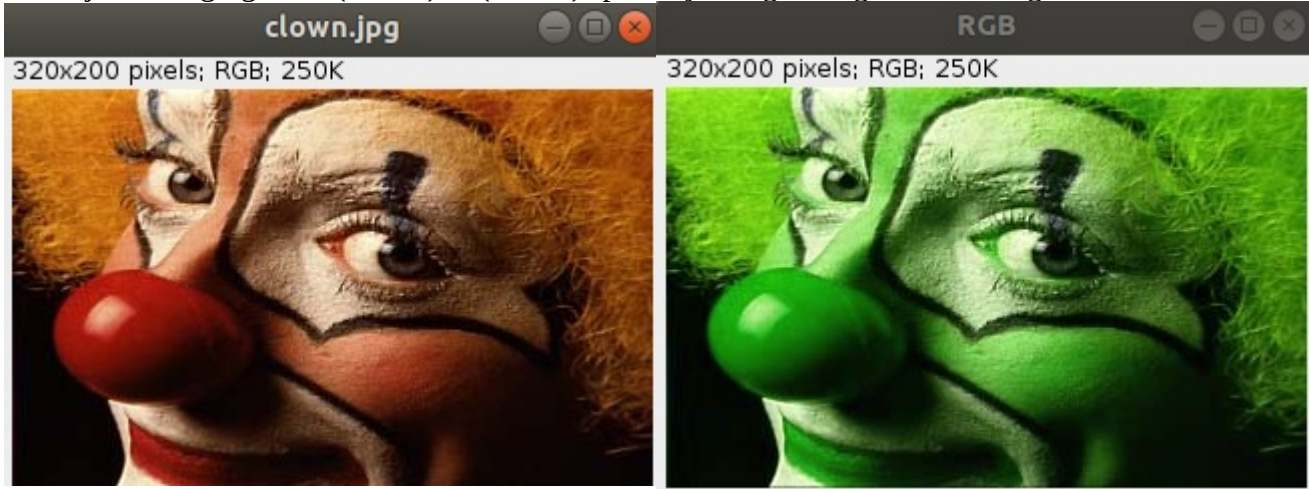
On your default display, the RGB additive way is used. So if you want to display another color space (or deconvoluted space or composite image) you have no other choice than putting channel 1 in the R channel, channel2 in the G channel and component 3 in the B channel. No choice but is it clearly that the Red channel displayed is not the real Red channel ? (i.e. in the case of Fluorescent image [https://en.wikipedia.org/wiki/Fluorescence\\_imaging](https://en.wikipedia.org/wiki/Fluorescence_imaging) or hyperspectral image for instance but just a trick that corresponds to our human visual system (<https://www.kaytee.com/learn-care/wild-bird/can-birds-see-color> birds have 4 channels).

Normandy image:



Split into the three channels : Image/Color/Split channels. Are the green and the blue channels really related and only related to the grass and the see respectively?

Then just changing from (R,G,B) to (G,R,B) space by using : Image/Color/Merge channels.



See the paradox or the ambiguity ? Play as well with arranging various channels.

So when you are dealing with many other space color representation the same issue arises.

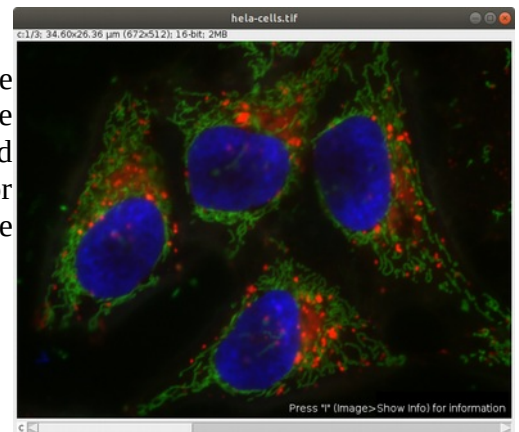
$$\begin{bmatrix} R_{\text{linear}} \\ G_{\text{linear}} \\ B_{\text{linear}} \end{bmatrix} = \begin{bmatrix} +3.24096994 & -1.53738318 & -0.49861076 \\ -0.96924364 & +1.8759675 & +0.04155506 \\ +0.05563008 & -0.20397696 & +1.05697151 \end{bmatrix} \begin{bmatrix} X_{D65} \\ Y_{D65} \\ Z_{D65} \end{bmatrix}$$

From <https://en.wikipedia.org/wiki/SRGB>

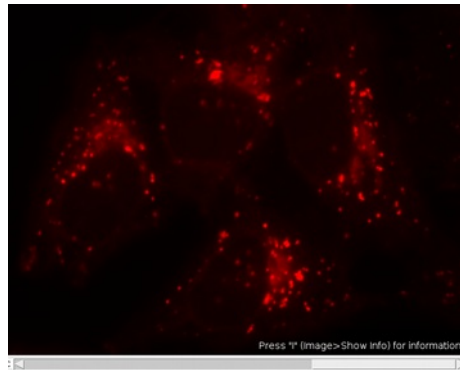
$$\begin{bmatrix} X_{D65} \\ Y_{D65} \\ Z_{D65} \end{bmatrix} = \begin{bmatrix} 0.41239080 & 0.35758434 & 0.18048079 \\ 0.21263901 & 0.71516868 & 0.07219232 \\ 0.01933082 & 0.11919478 & 0.95053215 \end{bmatrix} \begin{bmatrix} R_{\text{linear}} \\ G_{\text{linear}} \\ B_{\text{linear}} \end{bmatrix}$$

You will be able to play with these linear transformation with **color\_decon.py** code if you like in the Color short tutorial.

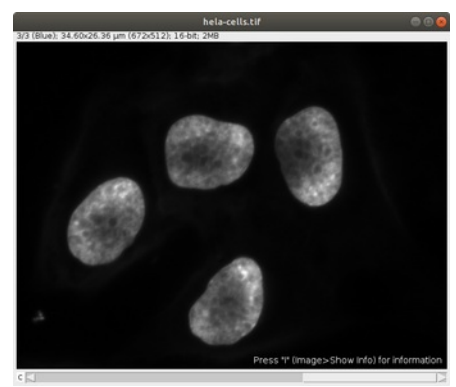
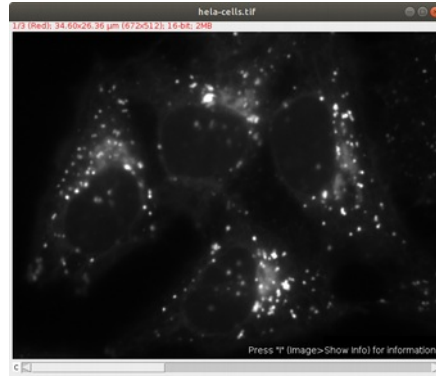
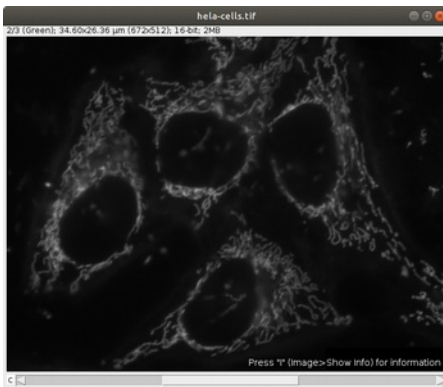
The same arises for multiplex fluorescent images. The composite image HelaCell versus the RGB channels corresponding to the various fluorescent markers. Play with arranging channels and color/composite display. If you use Shift+I you get yellow color corresponding to colocalisation of red and green. In composite images you can have more than 3 channels.







The color is related to the wavelength of excitation but the real value is the intensity for each channel possibly in grey level.



See <https://petebankhead.gitbooks.io/imagej-intro/content/chapters/colors/colors.html> for composite images explanation.

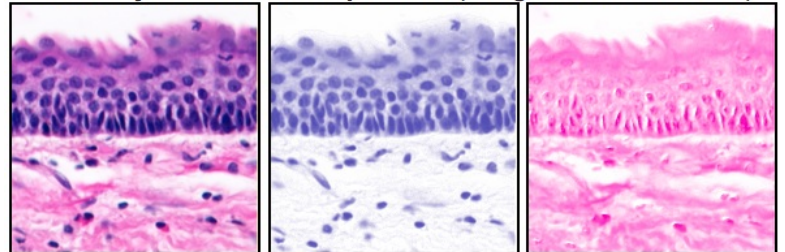
Once again the same arises but with different staining issues in digital histopathology (bright field microscopy). See the deconvolution part of the Color tutorial (Ruifrok et al. For unstaining images of cells stained with H&E for instance).

H : bluish– nuclei

E : pinky – cytoplasm

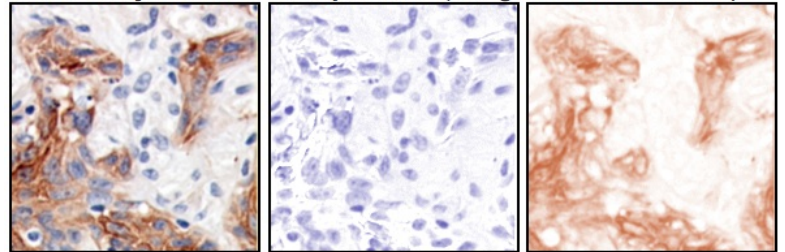
DAB : brownish : proteins like ki67 (3,3'-diaminobenzidine) is oxidized in the presence of peroxidase and hydrogen peroxide resulting in the deposition of a brown, alcohol-insoluble precipitate at the site of enzymatic activity. DAB (3, 3'-diaminobenzidine) produces a dark brown reaction product and can be used for both immunohistochemical and blotting applications. See

#### Haematoxylin and Eosin separation (using the built-in vectors).



From left to right: original, Haematoxylin, Eosin, virtually empty 3rd

#### Haematoxylin and DAB separation (using the built-in vectors).



<https://www.sinobiological.com/category/dab-ihc>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6779686/>

or