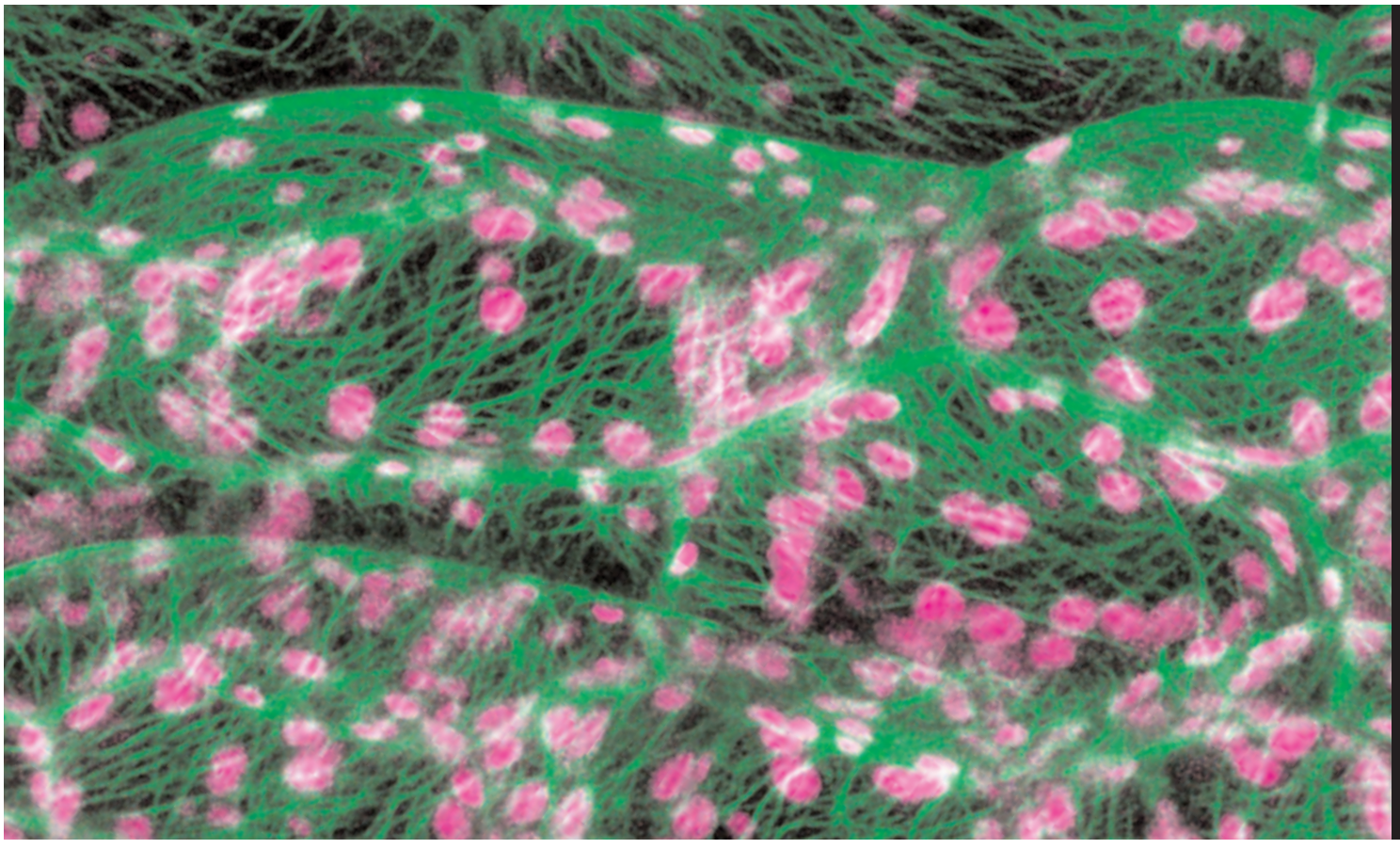

Using colour in figures: some colours are more equal than others

A picture can paint a thousand words, and cell biologists are among those who tend to put a high value on pictorial representations. With the advent of modern technology, it has become standard practice to use colour in a wide range of pictures, from graphs to micrographs. However, colour images produce problems of accessibility for a minority of people who do not have the full range of colour vision. This article suggests ways to maximise sharing of information with this minority.

The most common differences in colour vision are caused by the visual pigment protein (opsin) in either the red or green cones being missing or anomalously similar to the opposite channel (green or red respectively). Opsin genes are on the X chromosome, so the minority affected by such allelic polymorphisms are mostly male. The size of this minority varies between different ethnic groups, for example: in Caucasians: 8% of males and 0.5% females. This means that a paper using colour sent to three reviewers two of whom are male has a 16% chance of being seen by someone whose colour vision is of the minority type. Such people are commonly referred to as colour-blind, but this term is not accurate. Also, it is not always desirable or true to consider the genetic majority of people (so-called "normal") as better, particularly relating to colour vision [1], which is the source of social discrimination in some countries. To avoid this, colour vision is here described as being either the majority or the minority type.

For all computer generated images, the data is digital, and so colour can be applied or varied according to one's choice using software such as Microsoft Excel™ or Adobe Photoshop™. Producing an image accessible to all depends on the type of information it contains. I have identified three categories: diagrams, simple two colour pictures, and complex two-colour or three-colour images.

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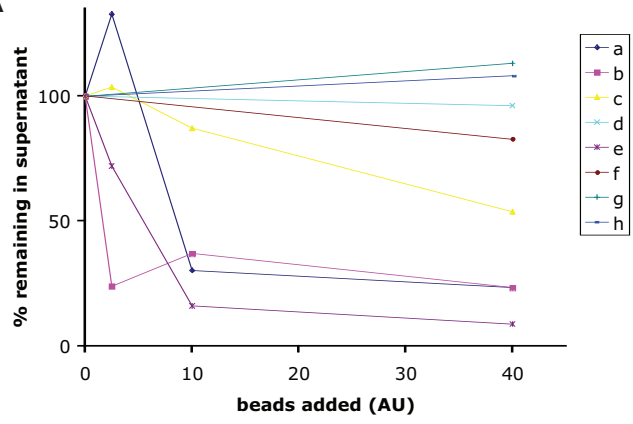
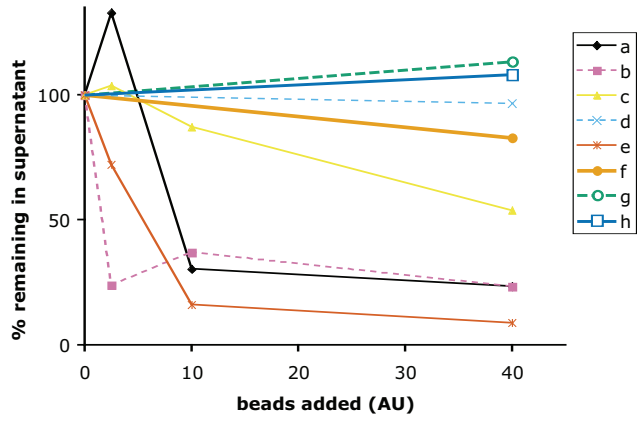
Figure 1. Some colours are more equal than others

A





The two graphs both show a single experiment relating to a pull-down of an activity on beads.

(A) uses the default settings provided by Microsoft Excel™.

(B) has been adjusted to make the data sets clearly identifiable. In general, diagrams should be designed in black and white, so that they work as far as possible without colour, which is added as ornamentation only. Thus, the most important change is enlarging symbols and lines. When choosing colour, avoid pure red, green or blue, and vary brightness as well as hue (see Fig. 3). Also, avoid using colour names to identify objects, as this will confuse the minority.

A palette of colours suggested by Kei Ito (Tokyo) is:

	CMYK (%)	RGB (0-255 scale)	RGB (% approx)
Black	0,0,0,100	0,0,0	0,0,0
Reddish purple	10,70,0,0	204,121,167	80,50,70
Yellow	10,5,90,0	240,228,66	95,90,25
Sky blue	80,0,0,0	86,180,233	35,70,90
Vermillion	0,80,100,0	213,94,0	80,40,0
Orange	0,50,100,0	230,159,0	90,60,0
Bluish green	97,0,75,0	0,158,115	0,60,45
Blue	100,50,0,0	0,114,178	0,45,70

Category 1

Category 1 applies to all diagrams, including graphs (Fig. 1), where colour allows more information to be highlighted. Here, the minority types of colour vision still allow detection of many colours (in addition to black, white and greys in-between), but the choice of colours should be made carefully (Fig. 1B).

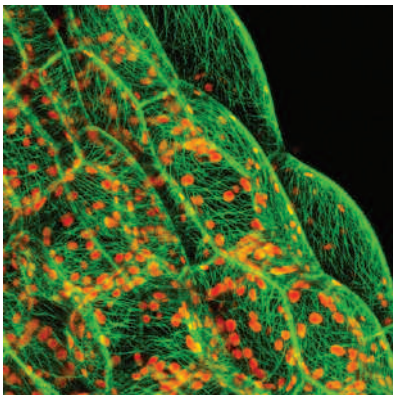
Category 2

In Category 2, two sets of information that are inherently quite different from each other are superimposed. In cell biology, this applies to images where two unrelated cellular structures are imaged together (Fig. 2, also see cover), and colour is used to demonstrate the two contrasting distributions. Although the colours may overlap, the overlap itself does not contain critical information.

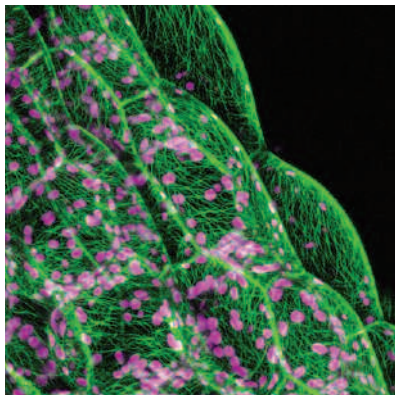
In the example shown, one channel sets landmarks for the other. In these instances, two colour images are typically shown as a single merged panel. But because loss of red/green discrimination is the most common phenotype of the minority with altered colour vision,

Figure 2. Recolouring simple two-colour micrographs where overlap is not crucial

A



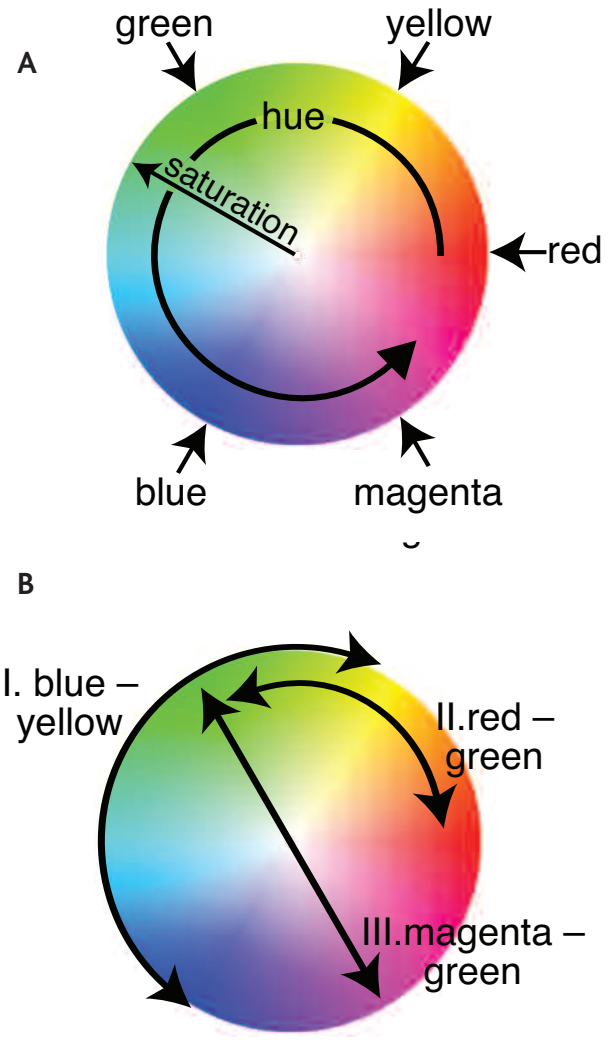
B



(A) A red/green image of *Arabidopsis* hypocotyl cells with the chloroplasts fluorescing red, and microtubules decorated with GFP.

(B) A magenta/green image of the same data. In Photoshop™ the image was converted to RGB mode, all the information in the red channel was copied into the clipboard, and pasted into the blue channel. The same result might be achieved during initial imaging, if software allows the Look Up Table (LUT) of the original red channel to be changed to magenta. This works well for the minority, and does not reduce information for the majority, because colour comparison on a pixel by pixel basis is not important. Image kindly provided by Juliet Coates.

Figure 3. The human visual system is trichromatic, but does not treat colours equally



(A) Hue and saturation represented in a single two dimensional colour wheel. Here all colours are at maximum brightness (i.e. with no added black).

(B) The same wheel used to represent three colour axes: I. blue/yellow (via green), II. red/green (via yellow), and III. magenta/green (via white).

Both the human visual system and digital cameras are trichromatic, but the visual system does not treat the three colours equally. Instead it concentrates on two axes: blue vs. yellow (which in trichromats is the sum of red and green), which evolved many millions of years ago; and red vs. green, which arose with the duplication of the red/green opsin recently in primate evolution. The colours at the poles of these axes are described as complimentary, meaning that mixtures between them are not perceived as such: we do not experience reddish-green or yellowy-blue, but we can locate colours along either axis. Thus, red/green images used in cell biology approximate the naturally occurring red-green axis, with overlap perceived in the spectrum of hues red<–>yellow<–>green.

By comparison, magenta/green images use a computer-created spectrum of magenta<–>white<–> green. Although guaranteed to be detectable by the minority, it only has two hues, and varies by degree of saturation (white is 0% saturated, green and magenta 100%). The key issue is that this type of spectrum does not maximally use the ability of the majority type of colour vision to discriminate hues, so these people find it less informative.

In conclusion, no single solution suits all, and a happy compromise might be to use two systems in parallel, one for the majority and one for the minority (see Figure 4).

almost any combination of colours is preferable to red and green. A simple way to generate an alternate colour pair is to convert the red channel into magenta (Fig 2B).

A similar approach can be extended to three colour images (say red/green/blue) only if the types of information being conveyed are radically different, but for three colours the manipulation of channels is more complicated, as there is no empty channel to paste data into, and so two signals have to be combined within a single channel, for example: a red signal has to be converted to magenta by adding it to the blue channel which already contains the nuclear stain. This can be done in Photoshop™ by pasting extra data into a new layer.

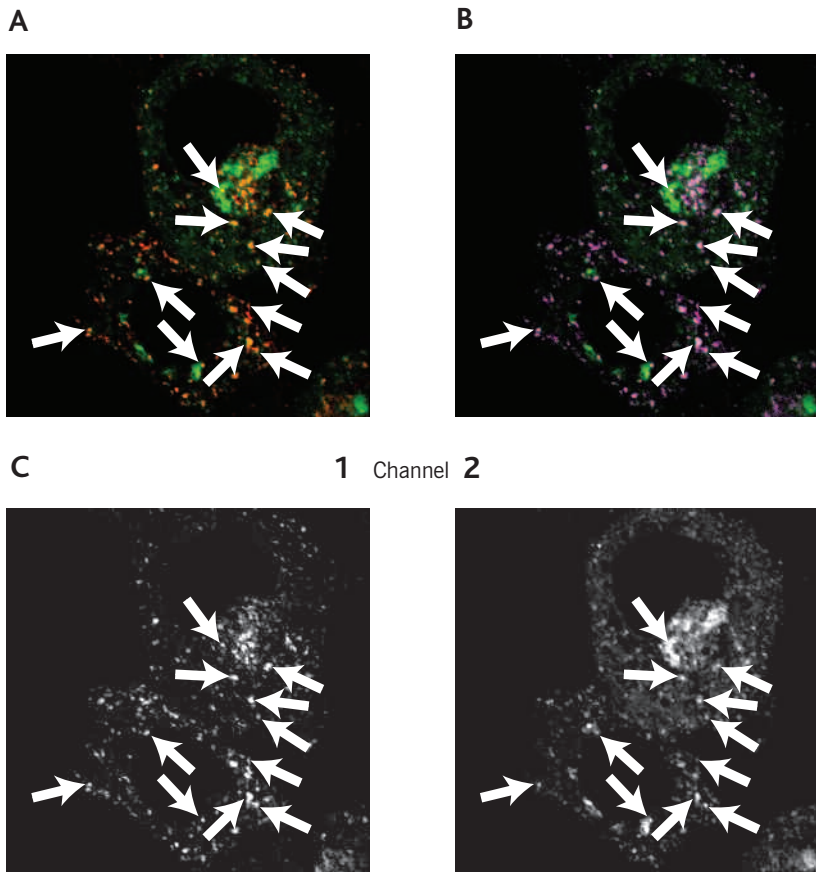
Alternatively, www.vischeck.com/daltonize, run by Bob Dougherty (Stanford) and Alex Wade (Smith-Kettlewell), performs an on-line separation of red and green on two and three colour images using a more complex algorithm that manipulates brightness as well as colour. However, three colour images might best be allocated to Category 3 (see below).

Within the community of cell biologists, it has been reported that the magenta/green approach does not work well [2], while others stress its importance [3, 4]. In my opinion the problems arise with a specific type of image that contains two sets of highly overlapping information, in particular two possibly colocalised intracellular markers with punctate distributions, a situation common in membrane cell biology, where overlap is demonstrated by the way the two colours merge to create an entire spectrum of colours, so that the precise extent of overlap is determined by the colour. As explained in Fig. 3, the combination of red and green for this type of image is particularly advantageous for the majority, because of the way information on colour is treated by the visual system. By contrast, magenta/green images do not use the natural system of colour mixing, and do not convey the full range of information to the majority of people. As the merging of colour is treated very differently by the visual system and by computer software, there appears to be no cure-all approach to colour manipulation in images that is guaranteed to satisfy 100%.

Category 3

Therefore, I suggest a third category for images where two markers overlap or are highly similar. To present these images, the separate channels are shown individually (i.e. not overlapped) (Fig. 4). Sometimes, two intracellular markers might be highly related in distribution, but in fact be adjacent with marginal overlap. Therefore, it is important that the typical relationship between the two markers be indicated with a set of arrows placed in precisely the same place on the two separate images. This can be achieved using the "Align" functions in programmes such as Adobe Illustrator™ or Microsoft Powerpoint™. As someone with minority colour vision, I can vouch for this approach personally.

Figure 4. Treating complex two-colour micrographs where overlap is crucial



(A) A red/green image of two markers with punctate distributions inside mammalian cells detected by immunofluorescence (kindly provided by Adam Grieve).

(B) The same data as a magenta/green image.

(C) The two separate images in black and white. Arrows mark the most prominent double-positive puncta. While an image such as (A) contain maximal information for the majority, it is largely useless for the minority. Images such as (B) and (C) should be made available to allow the minority to assess overlap. (C) provides extra light, but the appreciation of overlap must be indirect; (B) does not use the trichromatic colour system of the majority to maximum advantage.

Even though neither image is ideal, the combination offers the best chance for the minority to access this category of image.

A mistake that is often made is that false colour (e.g. red or green) is used for single channel images. While this may help identify the channel, a simple label suffices for that, while the false colour causes a considerable reduction in the information conveyed, no matter what colour vision capability. The biggest problem occurs when looking at printed images. CMYK inks do not reproduce RGB colours, and the inks saturate, failing to show the higher range of signal intensities, in particular for green (all pixels above 50% green appear the same). Therefore, greyscale (black & white) should be used for all single channel images. Even on computer screens using red or green is also not as good as greyscale, which produces more light, and so provides more visual information. In journals that are pressed for space, if it is not possible to show the extra images next to the colour merge, then it would be acceptable to make the extra black/white panels available as supplementary information on the web. The inclusion of a magenta/green merge might be helpful, although more experience of this is needed.

Here I have suggested a set of adaptations to colour images that increase access for people with minority types of colour vision. A much more important step will be the definition of standards for the use of colour in society at large. Progress in this area is being helped by the efforts of a few individuals (Kei Ito has successfully introduced changes to maps and all signs, i.e. Category 1, in the Tokyo underground system), and by public knowledge that some members of the minority are highly influential (Bill Clinton for one). To address the concerns of both majority and minority [2-4], it will help if a constructive debate is opened. Maybe our field can lead the way, and aim to reach a consensus view that can be adopted by national societies of cell biology and the relevant international journals. But, as hinted at by the majority/minority terminology used here, the question is political, where every colour image counts as a vote.

ACKNOWLEDGEMENTS:

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