

Optimisation of colour schemes to accurately display mass spectrometry imaging data based on human colour perception

Alan M. Race · Josephine Bunch

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Abstract The choice of colour scheme used to present data can have a dramatic effect on the perceived structure present within the data. This is of particular significance in mass spectrometry imaging (MSI), where ion images that provide 2D distributions of a wide range of analytes are used to draw conclusions about the observed system. Commonly employed colour schemes are generally suboptimal for providing an accurate representation of the maximum amount of data. Rainbow-based colour schemes are extremely popular within the community, but they introduce well-documented artefacts which can be actively misleading in the interpretation of the data. In this article, we consider the suitability of colour schemes and composite image formation found in MSI

literature in the context of human colour perception. We also discuss recommendations of rules for colour scheme selection for ion composites and multivariate analysis techniques such as principal component analysis (PCA).

Keywords Mass spectrometry imaging · Colour scheme · Data visualisation

Introduction

Mass spectrometry imaging (MSI) is a broad term encompassing many spatially resolved techniques capable of localising molecules such as drugs, lipids, metabolites and proteins as well as elemental composition in a wide variety of applications such as drug discovery, forensic science, biomarker discovery and surface analysis. These techniques include matrix-assisted laser desorption/ionisation (MALDI), secondary ion mass spectrometry (SIMS), inductively coupled plasma (ICP) and ambient techniques such as desorption electrospray ionisation (DESI), each of which utilise different physical phenomena to ionise a sample and therefore are better suited to different analytes.

Each recorded spatial location has a corresponding spectrum detailing the chemical composition in the form of (mass-to-charge ratio (m/z), intensity) pairs. The mass analyser and detector combination used dictates the mass resolution and therefore the number of pairs recorded in a single spectrum as well as the relative intensities of peaks. Depending on the mass spectrometer used, recorded intensities can span between one and eight orders of magnitude within a single spectrum.

Mass spectrometry imaging data are typically presented in the form of an ion image, which can be generated by integrating over a given peak (or m/z range) in every pixel within the

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A. M. Race
School of Chemistry, University of Birmingham,
Birmingham B15 2TT, UK
e-mail: alan.race@npl.co.uk

J. Bunch
Surface and Nanoanalysis, National Physical Laboratory,
Teddington, Middlesex TW11 0LW, UK
e-mail: josephine.bunch@npl.co.uk

J. Bunch (✉)
School of Pharmacy, University of Nottingham,
Nottingham, Nottinghamshire NG7 2RD, UK
e-mail: j.bunch@nottingham.ac.uk

Present Address:
A. M. Race
Surface and Nanoanalysis, National Physical Laboratory,
Teddington, Middlesex TW11 0LW, UK

image. This produces a 2D spatial distribution of the selected ion, assuming well-resolved peaks.

Data visualisation is defined as the process of generating and presenting a visual representation of the data [1]. This is vitally important in MSI, as conclusions are drawn based on the relative intensities of perceived structure(s) present within ion image(s). The intensity scale dictates the relative measured concentration of a given analyte which, as stated above, can span many orders of magnitude, presenting a difficult visualisation task.

Due to the large amount of information present in a single MSI data set, presenting multiple ion images at once is often desirable when attempting to determine the co-localisation of molecules. These are typically presented as RGB composite images, where each colour channel (red, green and blue) is assigned to a different ion image, with the resulting colours indicating the pixel-wise proportional contributions of each analyte.

The style and colour selection used for display has a dramatic effect on the perceived structure within the data [2]. Different aspects of the colour signal communicate different characteristics of the data, and to properly take this into account when defining a colour scheme requires knowledge of the physical and psychological properties of colour [2]. To reduce the impact of this, visualisation software typically include multiple colour schemes, with a preferred scheme employed as the default, and rely on the user to select the most appropriate. However, users tend to select visually appealing, vibrant colour schemes such as the rainbow colour scheme over more appropriate, “dull and ugly”, colour schemes [1]. The rainbow colour scheme, as its name suggests, is based on the visible wavelengths of the electromagnetic spectrum, which in theory seems like a sensible phenomenon to base a colour scheme on. However, multiple articles over the past few decades have illustrated reasons why it should not be used and the ways in which it can be actively misleading [2–5].

In order to evaluate colour schemes effectively, it is important to consider colour spaces, human vision, and how they relate to one another.

The two major photoreceptor cells found in the retina are rods and cones. Rods are extremely sensitive, capable of functioning at low light levels, and are responsible for scotopic vision, but do not provide any colour differentiation. Cones are responsible for colour vision, with three different types present in a normal eye (S, M and L, corresponding to short-, medium- and long-wavelength peak sensitivities, respectively). There are substantially fewer S cones than there are M or L cones in the eye.

Human perception is less sensitive to changes in hue than luminance [1], with high spatial frequencies being perceived through changes in luminance [3].

Various colour spaces have been defined for different purposes. For a more detailed review and description, see [6]. Here, we will simply focus on RGB and CIELAB.

RGB is a device-dependent colour space based in the human perception of colours, with each channel roughly corresponding to the region of the visible spectrum that the L, M and S cones, respectively, are sensitive to. Disadvantages of the RGB colour space include its psychological nonintuitiveness and the perceptual nonuniformity (low correlation between the Euclidean distance in the RGB space and the perceived difference in colour) [6]. Despite these issues, RGB is the most commonly used colour space for defining colours, as it maps directly to electronic displays.

The main goal of the CIELAB colour space was to provide a perceptually equal space, meaning that Euclidean distances between colours in this space correlate strongly with human visual perception [6]. In this space, the three axes correspond to lightness (L), the position between red and green (a, where positive values indicate red and negative values indicate green) and the position between yellow and blue (b, where positive values indicate yellow and negative values indicate blue).

In this article, we review colour schemes that are used in the MSI literature, relating them to human vision and perception while also highlighting any artefacts that can be introduced. We make recommendations of rules to follow in the selection of colour schemes in order to avoid introducing unnecessary artefacts and to produce perceptually accurate representations of data.

Methods

MALDI MSI data of a 12- μm sagittal section of mouse brain, which was coated with 15 mL of 10 mg mL⁻¹ CHCA via airspray, were acquired using a QSTAR XL (AB Sciex, Framingham, MA, USA) in positive mode over the range m/z 50–1000. The dataset used in this article is available from <http://www.imzMLConverter.co.uk>. Data were converted from mzML to imzML using imzMLConverter [7]. All data processing was performed using in-house software written in MATLAB, using MATLAB 2011b (MathWorks, Natick, MA, USA).

A common m/z axis was calculated for the dataset, as described in [8]. Each spectrum was smoothed with a dual-pass Savitzky–Golay (window size of nine bins) filter and summed to generate the total spectrum. Peak detection was performed on the total spectrum using a gradient method, as described in [8]. The intensity of each peak was extracted from each spectrum in turn to generate a datacube, which was outputted to imzML.

Memory-efficient principal component analysis was performed as described in [8], with the preprocessing altered as described above.

Dichromacy simulations were performed using the Vischeck ImageJ plugin [9].

Articles were selected by searching Google Scholar for three keywords (“mass”, “spectrometry” and “imaging”). Articles primarily consisting of reprinted figures, such as reviews, and articles containing no ion images were omitted. The first 100 articles meeting these criteria were selected. Articles from the current year were also selected, using the same criteria as above except with the limitation that the year in the search was set to 2014. The first 20 articles meeting these criteria were selected. A complete list of the 120 articles evaluated, and which colour scheme(s) were present in each, is included in Table S1 of the “Electronic supplementary material” (ESM).

Results and discussion

A colour scheme is a series of colours defined in a chosen colour space (typically RGB) that have a corresponding value such that each value within the data can be replaced with, or mapped to, its assigned colour for display. Accurate representation of the structure in the data largely depends on the type of

data being visualised, and no colour scheme is ideal in all situations. Types of data can be broadly classified as either nominal, ordinal, interval or ratio. Rough guidelines for each type of data are provided elsewhere [2]. Here we expand on these within the context of MSI data visualisation scenarios.

Ion images

Ion images are the most commonly displayed form of data within the MSI literature, mapping the intensity of a selected ion at every spatial location within the acquired image region. After evaluating 120 MSI articles, it was found that over 30 different colour schemes were used, with some articles containing up to 8 unique colour schemes [10]. A selection of the colour schemes present in the literature were applied to the same unnormalised ion image (mouse cerebellum, m/z 826 with values spanning 0–100 counts), as shown in Fig. 1. As stated in [2], how the data are represented visually can greatly influence the perception of the structure within the data. This is especially evident when comparing Fig. 1a with Figs. 1o, q

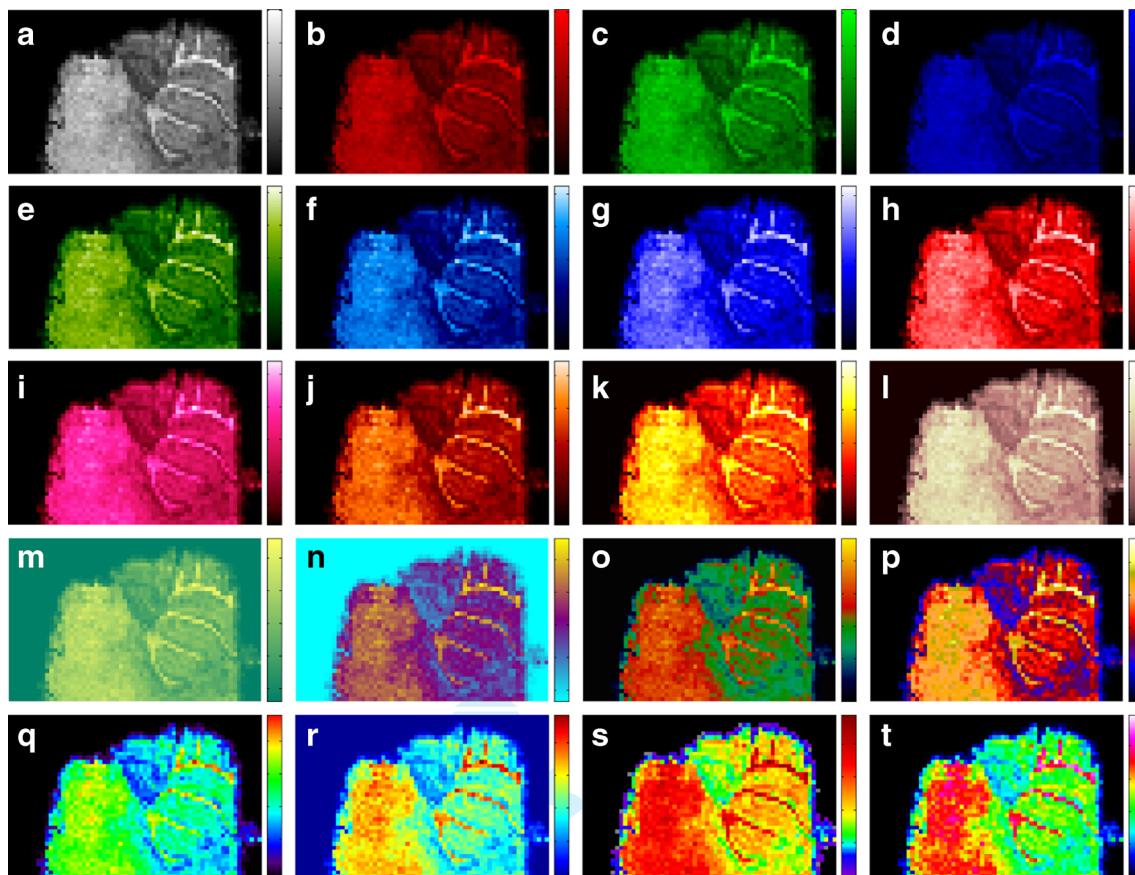


Fig. 1 a–t Visualisation of the same data (unnormalised m/z 826 from the cerebellum region of a mouse brain) using colour schemes found in the MSI literature. Intensity spans from 0 to 100 counts. a Grayscale, b red, c green, d blue, e green to white, f cyan to white, g blue to white, h red to white, i pink to white, j copper to white, k hot, l pink hot, m green to yellow, n cyan to magenta to yellow, o double scale (blue to green, red to yellow), p temperature-based, q–t rainbow-based

and r (at least one of which is present in 34 of the reviewed articles), where contrast is lost between the arbor vitae and the cerebellar cortex (see Fig. S8 of the ESM for labelling).

The simplest, most common (appearing in 40 of the surveyed articles) and previously cheapest to publish colour scheme is grayscale (see Figure 1a). The benefit of grayscale is that it is a simple task to perceptually order shades of gray based on their lightness, allowing intuitive interpretation of the data. However, this comes with a caveat: human perception of brightness is affected by the brightness of surrounding regions [1, 11]. More generally, the perceived appearance of a colour is affected by its surroundings, most notably when they are complementary colours. This effect is referred to as simultaneous contrast.

A grayscale colour scheme, or any colour scheme which only varies luminance, cannot accurately communicate gradual changes (low spatial frequency) in structures present within the data [2]. The number of shades of gray that can be reliably distinguished is dependent on the luminance of the display device used, essentially limiting the number of unique values that can be displayed in a given image [12]. The addition of colour is therefore beneficial, as it increases the dynamic range of a colour scheme [1].

The most frequently used colour scheme group is the group of rainbow, or rainbow-based, colour schemes (Figs. 1q–t), featuring in 48 articles. These colour schemes are so-called due to their basis in the colours of each wavelength in the visible portion of the electromagnetic spectrum. There are significant disadvantages with using rainbow colour schemes, which can result in an actively misleading representation of the data [3]. These are excellently demonstrated in [3], but will be summarised here. Firstly, there is no perceptual ordering, with experiments showing that people will order rainbow colours in numerous ways [1]. This results in confusion due to the fact that greater-than and less-than relationships are not immediately obvious and must be inferred through memory (error-prone) or by consulting a legend (needless distraction). Next, as large regions of the rainbow colour scheme are isoluminant, small details and sharp features that fall within these regions are obscured. This is evident in Fig. 1q, where it is difficult, if not impossible, to distinguish between the lower regions of the arbor vitae and the cerebellar cortex. Finally, artefacts are introduced in the form of apparent bands of data resulting from sharp transitions between hues, when in reality there are only small differences between the values. This is particularly evident on the tissue boundary in Fig. 1s. Although this is an artefact of all of the rainbow colour schemes, it is most notable in Fig. 1s due to the fact that over 50 % of the colour scheme is varying shades of red and the remainder includes six colour changes.

A further issue with rainbow colour schemes is that perceptual changes in the colours are not uniform, with changes appearing faster in cyan and yellow than in green (shown in

Figure 1q). This further complicates the interpretation of the values of specific colours and can cause false contrast. Attention is drawn to the yellow areas due to their brightness, not necessarily because they are the most important [2].

The most common oversight when considering colours to use when presenting scientific data is the resulting perception by individuals with colour-deficient vision [4]. Colour vision deficiency (CVD) refers to the decreased ability, or inability, to see colour, affecting 8 % of men and 0.5 % of women [13]. Anomalous trichromacy, the most common form of CVD, results in one type of cone having a sensitivity shift to one end or the other of the visible spectrum [14]. Dichromats lack either the L (protanopes), M (deutanopes) or S (tritanopes) cones, resulting in dichromatic vision. The most severe, and also extremely rare, form of CVD is monochromacy—complete colour blindness.

Simulating deutanopia, where the M (medium-wavelength, roughly centred around green light) cones are absent, for Fig. 1 further shows the unsuitability of rainbow or rainbow-based colour schemes, as multiple values are mapped to the same colour. This is especially evident in Fig. 2t, with the appearance of slight holes in the white matter region of the tissue (dark yellow in Fig. 2t), as the high-intensity pink pixels instead appear as a blue colour, indicating low intensity. This renders the data impossible to accurately interpret for any deutanope. Similar effects are observed with other forms of dichromacy (shown in Figs. S1 and S2 of the ESM).

The frequency of occurrence of the rainbow-based colour schemes, despite their well-documented flaws, is likely due to the fact that these colour schemes are often used as default in the instrument vendor's software (Fig. 1t: flexImaging (Bruker); another rainbow scheme that is similar to Fig. 1q but not shown here: ImageQuest (Thermo Scientific)), in vendor-neutral software (Fig. 1r: MATLAB (MathWorks); MSiReader [15]; omniSpect [16]) and in commercial software (Fig. 1r: SCiLS Lab (SCiLS GmbH)).

The next most frequently used set of colour schemes is the linear red, green and blue, as shown in Figs. 1b–d, respectively, at least one of which appears in 31 of the reviewed articles. These are often presented as a precursor to displaying an RGB composite image portraying the spatial distribution of three ions within a single image (discussed in “Overlapping data” below). Although the same data are shown, the apparent contrast and brightness is much lower in the blue colour scheme than it is in the green. The distance between colours in the CIELAB space should correspond to perceived changes in colour, but the Euclidean distance was found to have flaws, namely poor performance in the blue region [17]. The CIEDE2000 distance metric (ΔE_{00}) was developed to address these issues and provide distances that correlated more closely with human perception. Using CIEDE2000, as implemented in [18], it is possible to determine the distance between the

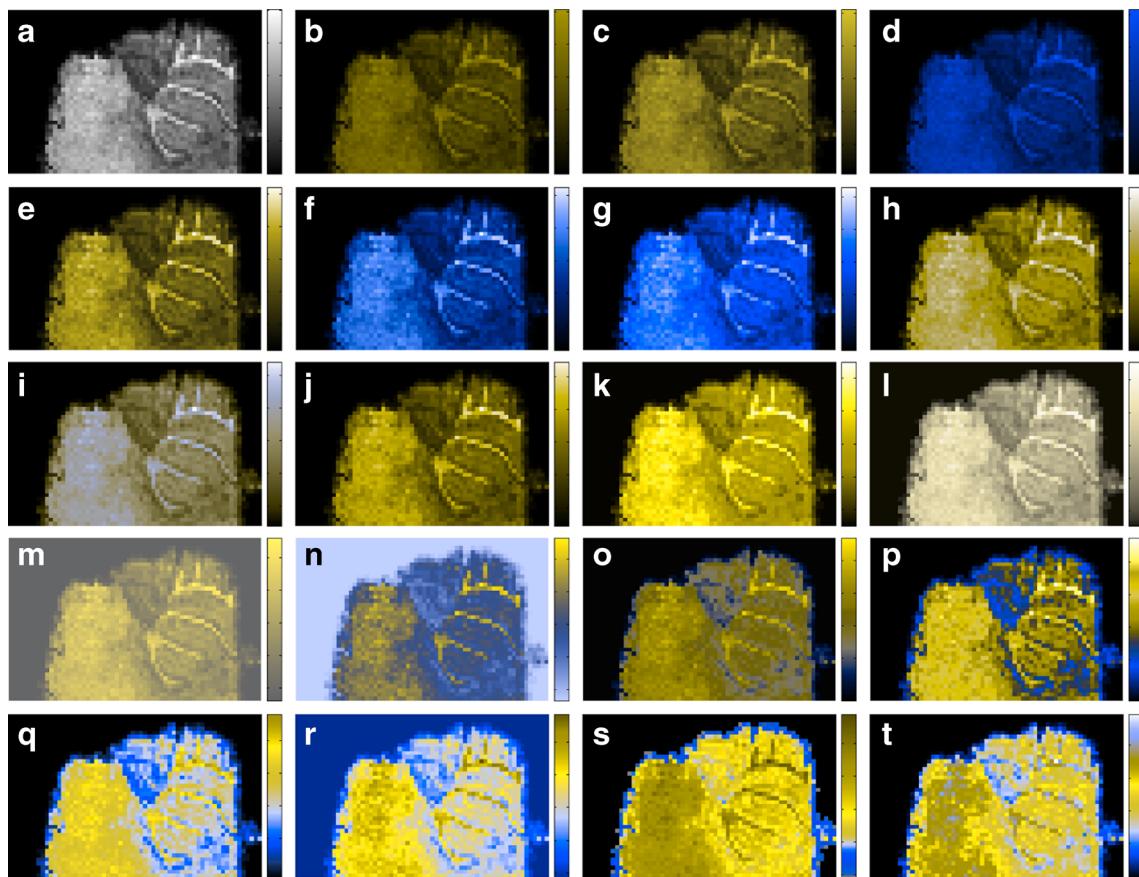


Fig. 2 **a–t** Deutanope simulation of colour schemes found in the MSI literature (corresponding to those shown in Fig. 1). The same colour appears at different values along the colour scale in panels **o–t**, rendering it impossible to accurately interpret the data from the visualisation alone

colours representing the minimum and maximum within a colour scheme, where the distance between black and white is 100 and the smallest perceivable difference is approximately 1 [19]. Assuming a colour scheme defined by a straight line in the CIE space, a smaller ΔE_{00} results in less perceivable colour changes throughout the colour scheme. Calculating this for the linear forms of Figs. 1b, c and d results in ΔE_{00} values of 51.34, 87.80 and 38.45, respectively. As the distance between the black and blue is significantly smaller than the distance between black and green, there are a reduced number of perceptually distinct colours within the blue colour scheme when compared with the green. So, although unintentional, more detail is apparent in whichever ion image is assigned to the green channel.

Calculating the distance between each subsequent colour in each colour scheme provides an easy way to evaluate its perceptual linearity. Plots for each of the colour schemes presented in Fig. 1 are provided in Fig. S7 of the ESM. Perceptually linear colour schemes have constant distances across the entire range, with the colour scheme in Fig. 1m being the closest to achieving this. The colour schemes shown in Figs. 1o and p (which is the default colour scheme in HDImaging (Waters))

and the rainbow colour schemes (Figs. 1qt) show the least linearity.

Application of the colour scheme presented in Fig. 1n gives the false illusion that there is a tear or part of the tissue missing because parts of the tissue have the same colour as the background.

The presentation of the data using the colour scheme in Figure 1o shows significant contrast between the grey and white matter regions of the tissue due to the change in colour from green to orange. However, this is an artificial sharp boundary introduced at the 50 % intensity mark, where values within the 50–55 intensity count range have 2–5× the contrast (according to ΔE_{00} , see the central peak in Fig. S7o of the ESM) when compared with other same-size ranges. In the CVD simulation (shown in Fig. 2o), the previously significant contrast between white and grey matter is lost.

As the magnitude is important in the display and interpretation of ion images, equal increases in ion intensity should appear as steps of equal perceived magnitude in the visualisation. Monotonic increases in luminance and saturation have both been individually shown to result in perceived monotonic increases in magnitude [2].

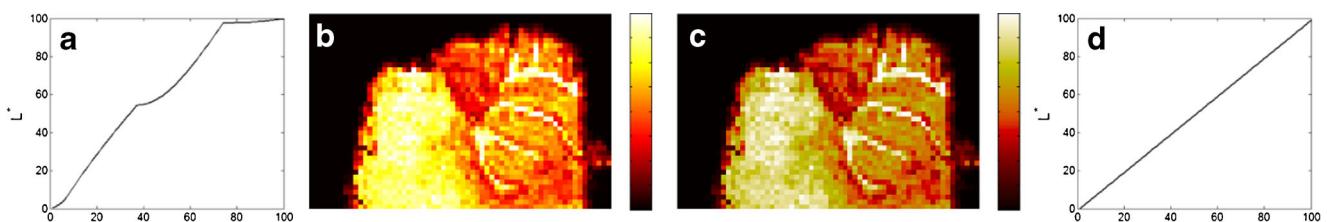


Fig. 3 **a–d** Same ion image displayed with a monotonically (but not linearly) increasing lightness colour scheme and the linearised lightness form. **a** Lightness plot for the nonlinear lightness colour scheme. **b**

Nonlinear lightness colour scheme. **c** Linear lightness colour scheme. **d** Lightness plot for the linear colour scheme

The use of both is necessary to provide a suitable colour scheme, as luminance is important for conveying high spatial frequency information, while hue and saturation convey low spatial frequency structures [2].

By defining colour schemes in the CIELAB space, it is much easier to ensure that changes in colour correspond to perceived changes in magnitude. A colour scheme which follows the rules above (monotonic luminance and changing hue) but is defined in the RGB colour space is shown in Fig. 3b. As shown in Fig. 3a, the lightness increases monotonically, but the final quarter of the colour scheme covers just 5 % of the lightness range, resulting in low contrast for values between 75 % and 100 %. It is difficult to differentiate regions when luminance is equal, even with large chromatic differences [20]. Linearising the lightness increases contrast in this range, as shown in Fig. 3. This improved contrast over small intensity changes is progressively important for distinguishing different intensities as the range of values displayed increase in orders of magnitude.

Overlapping data

It is occasionally beneficial in MSI to overlay data, such as an ion image and a photograph or a corresponding histological image, to determine the co-localisation of features between the two imaging modalities. This is done by presenting the ion image as a transparent layer over the optical image. However, there are perceptual pitfalls in this approach whereby the contents of the layers can interfere with one another, sometimes to the extent that it is impossible to determine which layer a given object belongs to [20]. For example, if a translucent green object is placed over a blue and a pink object (such as in the case of H&E stain images), the expectation is that the objects would maintain their colour but with a green tint. This is the case for the blue objects, but the pink objects now appear orange (see Fig. S6 of the ESM). In order to minimise interference, maximal separation in colour, texture, motion, and stereoscopic depth is required [20]. In the case of MSI, the simplest solution is to ensure that the colour scheme(s) selected do not contain any duplicate colours. For example, overlaying an ion image with the rainbow colour scheme in Fig. 1t onto an H&E stain image would result in duplicate colours

(blue, pink and white), making it difficult to determine which colour belongs to which layer.

Composite images are frequently employed in MSI publications (29 out of the 120 surveyed) to show distributions of multiple ions within the same image. A full list of all composite combinations and accompanying references is included in Table S2 of the ESM. Two-colour composites are primarily (in 14 articles) displayed using the red and green channels. This has severe implications for any reader who suffers from either protanopia or deutanopia, as the two ions will be difficult, if not impossible, to separate. Safer options for displaying two-colour composites are to use green–magenta or blue–yellow.

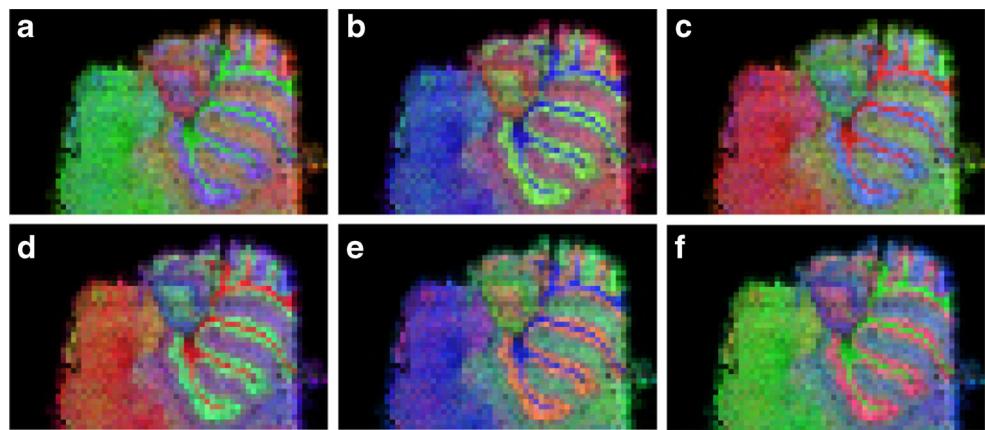
Display of three-colour composites using RGB was found in 12 articles. The order in which the ion images are selected for the red (R), green (G) and blue (B) channels can impact the perceived importance of certain regions due to the brightness of the green channel compared with the red and blue, as is evident in the bright green regions in Fig. 4. The chosen channel assignment also affects the perceived structure of the data for people with CVD because colours become indistinguishable, as shown in Figs. S3–S5 of the ESM. Although there is no simple solution to this visualisation requirement, care should be taken when drawing conclusions from three-colour composite images alone.

Multivariate analysis images

In some cases the data to be displayed has both a positive and a negative component. Such is the case in principal component analysis (PCA) score images. PCA is an unsupervised multivariate analysis technique which transforms a set of observations into a set of orthogonal variables, called principal components. The first principal component is the linear combination of variables (for MSI, this is generally peaks and occasionally m/z bins) that explains the largest proportion of the variance within the data. Each subsequent principal component is the linear combination of variables that explains the largest remaining proportion of the variance, with the requirement that it is orthogonal to all preceding principal components.

Once the principal components have been determined, the original data is projected into the new principal component space. These projected data are referred to as the scores. These

Fig. 4 a–f Composite RGB images of m/z 713, 826 and 844 where: **a** R is m/z 713, G is m/z 826 and B is m/z 844; **b** R is m/z 713, G is m/z 844 and B is m/z 826; **c** R is m/z 826, G is m/z 713 and B is m/z 844; **d** R is m/z 826, G is m/z 844 and B is m/z 713; **e** R is m/z 844, G is m/z 713 and B is m/z 826; **f** R is m/z 844, G is m/z 826 and B is m/z 713



are most commonly displayed by plotting one principal component against another to determine the separation (or lack thereof) of different data categories. By retaining the original spatial position of each data point, it is possible to generate score images for individual principal components by assigning each pixel the score of the corresponding data point.

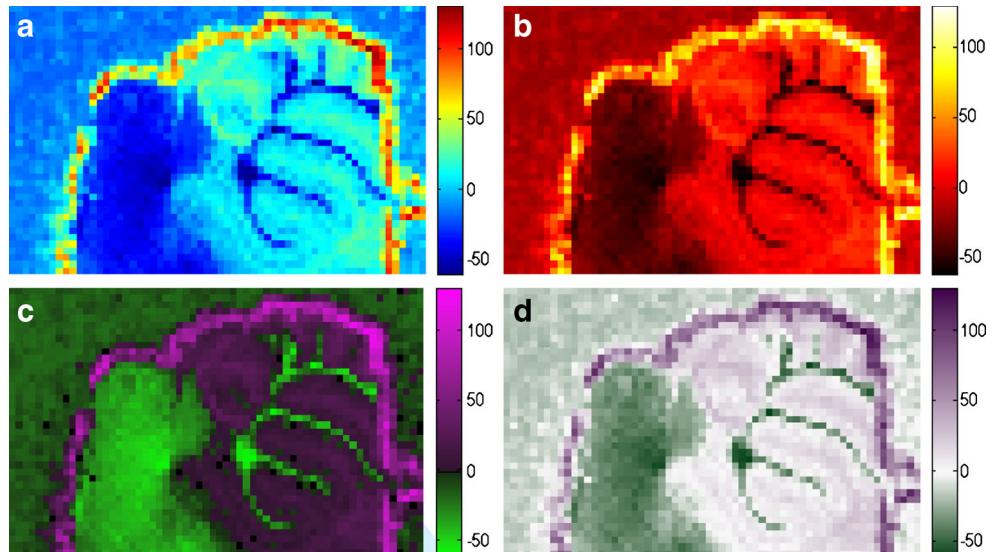
Only two of the reviewed articles contained PCA score images, one using the rainbow colour scheme from Fig. 1r [21] and the other using the hot colour scheme shown in Fig. 1k [22]. The drawback with using such schemes, apart from the reasons listed previously, is that it becomes a colour-matching exercise to determine which values are positive and which are negative. In such situations, the use of a diverging colour scheme centred around zero can provide a much more intuitive and informative representation of the data, as has been shown previously for SIMS data [23]. It is immediately evident which regions of the image have a positive value and which are negative in Figs. 5c–d. These can either be centred around black, as in Fig. 5c, or around white, as in

Fig. 5d (in which case it is useful to use the Msh colour space to avoid Mach band artefacts [1]).

Segmentation images

Segmentation images are often employed as either region labels (based on histology) or clustering results. These are generally a form of nominal data, as there is no order to the labels or clusters. Ideally the objects should be distinguishably different, but without perceptual ordering [2]. Care should be taken when selecting colours for this task, as certain colour combinations can result in over- or underestimating the comparative areas [24]. In general, more pastel colours result in more accurate perception of area coverage. A further consideration should be to ensure that the colours chosen are safe for people with CVD, otherwise multiple clusters or segments may be indistinguishable. This becomes increasingly difficult as the number of unique groups to display increases. A useful

Fig. 5 a–d Principal component 2 score image displayed using **a** the rainbow colour scheme shown in Fig. 1r, **b** the hot colour scheme shown in Fig. 1k, **c** a diverging colour scheme with black at 0, or **d** a diverging colour scheme with white at 0



resource for selecting colours that take into account these effects is ColorBrewer [25].

Summary

The use of CVD simulation tools, such as Vischeck (<http://www.vischeck.com/>), is a simple and easy way to ensure that figures are suitable for a wider audience. Abandoning rainbow or rainbow-based colour schemes and instead using perceptually linear colour schemes—which make use of as much of the lightness range as possible—to display ion images allows intuitive interpretation of the underlying data. The use of the CIEDE2000 distance metric provides a simple way to determine the perceptual linearity of any given colour scheme.

In MS imaging, the intensity indicates the relative measured concentration of a given analyte, and we have illustrated how the apparent structure within an image differs according to the colour scale selected. To unambiguously understand the analyte concentration, additional experiments to achieve absolute quantitation should be performed alongside imaging experiments.

We propose that, wherever possible, the community should strive to use perceptually linear colour schemes, which denote the minimum and maximum values that the colour scheme spans, when presenting MSI imaging data. In cases where a different colour scheme is used or where the colour scheme is adjusted for specific emphasis, the same data, but displayed with a perceptually linear scheme, should be included in the supplementary information to provide the reader with a better overview of the data as a whole, and to ensure that the article is accessible to a wider audience, including people with CVD.

We don't propose a single colour scheme that should be used ubiquitously, but rather a set of rules and metrics for designing and determining colour schemes that provide an accurate presentation of imaging data that takes into consideration human perception of colour. While the colour schemes that fit these rules may not be as visually appealing as some, they avoid a large number of artefacts that can result in misleading representations of data, which could have severe implications as MSI moves towards clinical applications.

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