Appendix F

Hue-Saturation-Value Representation and Correlation of Multispectral/Multi-Modal Datasets

Approach

Combining multiple images is a powerful tool for analyzing data provided that it can be done accurately, without distortion to the original image. Combining multiple images corresponding to multispectral or multimodal data allows one to easily and quickly interpret all the data at once. It also allows one to identify correspondences and differences across modes. For example, the method presented in this paper was originally developed for combining images taken by an atomic force microscope and a fluorescence microscope simultaneously. Combining the images gives a detailed topographical image of the sample overlaid with the fluorescence image to show points of interest.

Traditional methods for combining multiple images use red-green-blue (RGB) color space[1], but this often distorts the contrast of the original image. Specifically, RGB images encode grayscale as well as color in each channel. When RGB images are co-added the result inherently becomes darker and darker as the grayscales sum. The author then processes this summed image, using a program such as Photoshop, at a minimum by adjusting brightness and contrast to achieve a visually pleasing image. This undocumented distortion is unrepeatable. At best the result is an image that is visually pleasing, qualitatively correct but quantitatively distorted.

Color can be represented by bases other than RGB and images can be converted between these different color spaces[2,3]. This means that image processing can and should be done in the most convenient format, regardless of the format required for publication or other use. While methods for image fusion have used intensity-hue-saturation (IHS)[4,5] and hue-saturation-value (HSV)[6] color spaces the use of these non-RGB color spaces has been restricted to combining existing multispectral color images. The method we develop here uses HSV to combine multiple monochromatic images that belong to a multispectral into one color image. It can also be used to combine each imaging modality of a multimodal data set to represent multimodal data sets in a single color image without data loss. Our method is based on performing the combination in the HSV color space as opposed to the RGB color space. In the HSV color space, grayscale and color information are in separate channels, so the combined images do not suffer from the same darkening contrast as images combined in RGB color space. Also, our method provides a visually acceptable image without manipulation through programs such as Photoshop. The result of our HSV-based method is a quantitatively accurate image. The method is repeatable, reversible, and does not suffer from the contrast distortion issues seen in RGB-based combination.

Methods: RGB- HSV Conversions

The conversion between RGB and HSV color spaces is a well-defined process; images can be converted between the two formats without loss of information.

We can convert from RGB to HSV color space using the following set of equations[6]:

$$V = Max(R,G,B)$$

$$S = \begin{cases} \dfrac{V - Min(R,G,B)}{V}, V \neq 0 \\ 0, V = 0 \end{cases}$$

If S = 0, then H = 0.
If R = V, then,

$$H = \begin{cases} \dfrac{60(G-B)}{V - Min(R,G,B)}, G \geq B \\ 360 + \dfrac{60(G-B)}{V - Min(R,G,B)}, G < B \end{cases}$$

If G = V, then,

$$H = 120 + \frac{60(B-R)}{V - Min(R,G,B)}$$

If B = V, then,

$$H = 240 + \frac{60 * (R - B)}{V - Min(R, G, B)}$$

Similarly, we can convert from HSV color space back to RGB color space using the following set of equations.

First, let

$$i = \left\lfloor \frac{H}{60} \right\rfloor$$

$$f = \frac{H}{60} - i$$

$$p = V(1 - S)$$

$$q = V(1 - S * f)$$

$$t = V\big(1 - S(1 - f)\big)$$

Then,

$R = V, G = t, B = p$	$i = 0$
$R = q, G = V, B = p$	$i = 1$
$R = p, G = V, B = t$	$i = 2$
$R = p, G = q, B = V$	$i = 3$
$R = t, G = p, B = V$	$i = 4$
$R = V, G = p, B = q$	$i = 5$

Methods: Combining two data sets using value and saturation

The following methods for combining images require that the images to be combined are of the same resolution. If they are not, then the individual images must be upscaled or downscaled to a consistent resolution.

To combine two data sets using the value and saturation channels of the HSV color space, one data set is assigned to the value and the other to the saturation. The value channel corresponds to a pixel's grayscale component and the saturation channel corresponds to a pixel's color intensity. For example, whether a pixel is bright red or faintly red is controlled by the saturation. In effect, this method overlays one data set in one color over a grayscale image. This is most useful when the value data set shows more information than the saturation data. The saturation data then points out areas of interest. The hue of the HSV color space image is set to a constant value corresponding to the color desired. The resulting image can be converted into RGB color space for compatibility with computer formats. This process is shown pictorially in Figure F.1.

Methods: Combining two data sets using saturation and hue

The method can be adjusted to combine two data sets using the saturation and hue layers. In this approach, the saturation layer is the average of the two data sets and the hue is set according to the relative values of the color images. The value is set to a constant value, but should not be set to 0 or 1 or the resulting image will be entirely white or black. The resulting image shows each data set as its selected hue with a relative mixture of hues where the data sets overlap. This method is useful for comparing data sets relative to one another. In areas where one data set has large values compared to the other, the hue will correspond to that data set. In areas where the two data sets have comparable intensity, the hue will be between the two individual hues.

More precisely, if at pixel(i,j),

$$data1(i,j) = d1$$

$$data2(i,j) = d2$$

And the hues to represent data1 and data2 are h1 and h2 respectively, then,

$$hue(i,j) = \frac{d1 * h1 + d2 * h2}{d1 + d2}$$

$$saturation(i,j) = \frac{d1 + d2}{2}$$

Methods: Combining three or more data sets

Three or more data sets can be combined using a combination of these methods. One data set can be represented by the value layer and two data sets can be represented using the saturation and hue layers. More data sets can be represented in the saturation and hue values by generalizing the method for two data sets in the saturation and hue layers. For n data sets,

$$hue(i,j) = \frac{\sum_n d_n * h_n}{\sum_n d_n}$$

$$saturation(i,j) = \frac{\sum_n d_n}{n}$$

Results

We compared a combination of two data sets using HSV and RGB-based methods (see Figure F.2). The HSV method uses the saturation and value layers to perform the combination. In the RGB method, one of the original images (Figure F.2a) is converted to grayscale and and the other (Figure F.2b) is added to the green channel. The resulting RGB image (Figure F.2c) has a green tinge to its background and the maxima of the original data are obscured. Furthermore, the horizontal lines from Figure F.2a are also blurred in Figure

F.2c. By contrast, the horizontal lines and the maxima can be clearly seen in the HSV images shown in Figure F.2d.

We also compared a combination of 3 data sets using our HSV-based method (Figure F.3a) and an RGB-based method (Figure F.3b). We start with a grayscale AFM image (Figure F.3c) which is overlayed with two circles, one in blue and one in green. The intensity of color of each of the circles decays with a Gaussian distribution from the center of the circle. In the RGB-based method (Figure F.3b), the contrast of the AFM image becomes distorted while in the HSV-based method (Figure F.3a) there is no contrast distortion.

Significance of results

Using HSV color space to combine multispectral data is an effective and accurate way to combine multiple images. Multispectral data is typically displayed by using the red, green, and blue channels to each display one layer of data. Another method is to color each layer of data differently and then combine the images using an image processing program such as Photoshop to adjust each layer's opacities so that all sets of data are visible. Both methods combine in the RGB color space because computers describe color in an RGB color space. In the first method, only 3 data sets can be effectively combined. In the second method, the manipulation of images in Photoshop could compromise the accuracy of the final image. By definition, adjustments made in Photoshop are done to please the eye. However those adjustments are not recorded or presented with the data. Therefore, the data is intrinsically altered, albeit unintentionally.

The HSV-based method can combine any number up to seven data sets for visual interpretation. There is no mathematical limit to the number of data sets which can be combined since each new data set is simply assigned a new hue. However, the practical number of data sets that can be combined is limited by the number of hues that the human eye can discern. In practice, this number of easily identifiable hues is six: red, yellow, green, cyan, blue, violet. A seventh data set can be displayed in the grayscale of the image. Although multiple colors can be used in RGB color space, multiple layers of data are not independent in the combination: each color is a linear combination of the red, green, and blue channels. For example, if one layer of data is represented using cyan (a combination of blue and green) and another layer of data is represented using green, when these two layers are added, both data sets are represented in the green of the resulting image. In contrast, the HSV-based method can filter out a certain hue to find the data that corresponds to it. In this way, the original data is preserved. Protecting the quantitative content of the data sets is important for lossless communication between team members. It is also important for subsequent researchers who want to replicate results, analytic conclusions, or apply new analytic tools to these data sets.

Such accuracy to the original data is the main advantage of the HSV-based method. For example, images created in HSV can be converted into an RGB representation for compatibility with digital systems, the new image can be converted back to HSV form since the conversions between RGB and HSV space are well defined. The ability to extract the original data from the HSV-based image is lacking from RGB methods. Combining images in RGB color space, especially in programs like Photoshop, can easily distort individual data sets. The original quantitative data is lost in this process. The HSV-based method for image combination presented in this paper preserves the original data both qualitatively and quantitatively in the final image.

References

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Figures

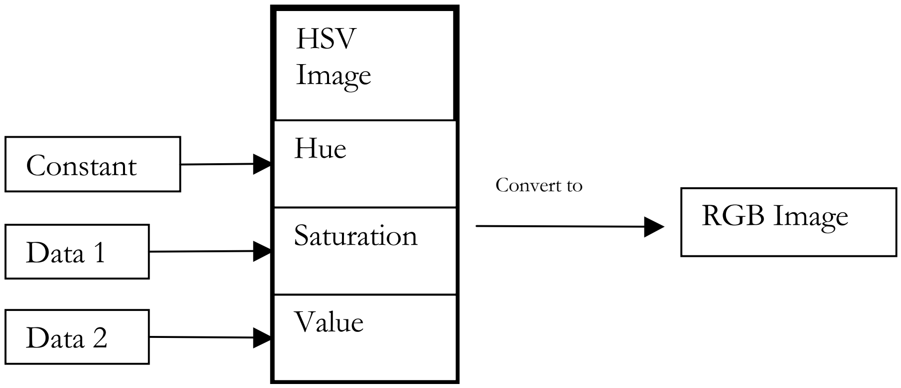

Figure F.1: Block diagram for combining two data sets using saturation and value layers. One data set is used for the saturation layer. The other data set is used for the value layer. The hue is set to a constant value. The HSV image can then be converted to RGB for general compatibility.

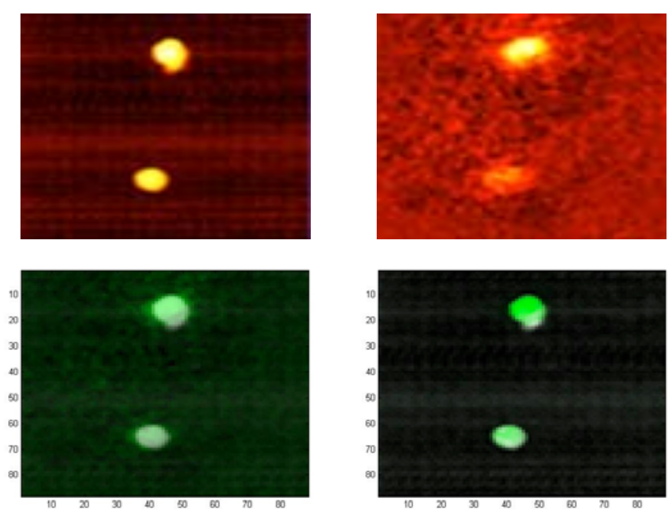

Figure F.2: Comparison of RGB-based and HSV-based image combinations. Images are 90 pixels x 90 pixels. a) First original data image. Yellow corresponds to higher intensity while dark red corresponds to lower intensity. b) Second original data image using similar coloring as (a). c) Combination of images using RGB-based method with (b) overlaid in green on (a). Note the washed out green across the image. d) Combination of images using HSV-based method with (b) overlaid in green on (a). In this image, the relative intensities of the original image (b) can be seen.

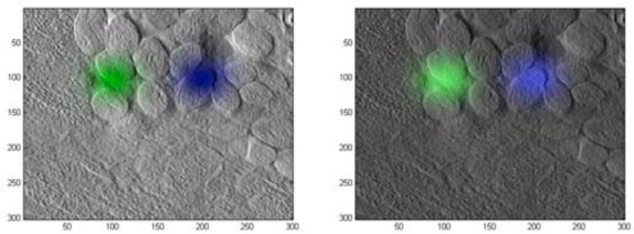

Figure F.3: The original AFM image (a) becomes distorted in the RGB-based image combination (b) as opposed to the HSV-based image combination (c). a) 300x300 HSV-based method for 3 data sets. Two Gaussian circles are overlaid, one in green and one in blue, on an AFM image. b) RGB-based method for the same 3 data sets in (a). c) Original background data set.