Colocalisation – What is it and how do I Measure it?

Colocalisation, simply put, is the appearance of two molecules of interest at the same place in your sample at the same time. Notice that this doesn't say anything about whether these molecules are interacting or not, just that they are co-locating. There are several ways of showing whether two substances are colocalised. The simplest and most common way of showing colocalisation is generally to overlay red and green channels and then to assert that anything that shows up as yellow shows colocalisation.

There are some problems with this approach, not the least of which is that about ten percent of the male population (meaning between five and ten percent of reviewers) is red/green colour blind meaning that they *cannot see* the important bits of your data. The easy solution to this problem is to represent your data as complementary colours such as green and magenta or cyan and red.

Another problem is that yellow pixels only form where the intensities of the red channel and the green channel are approximately equal. If the intensity of the red channel is greater than that of the green channel – regardless of whether there is green signal at all – the pixel will appear to be red, and *vice versa*. In this case you will have missed that your molecules of interest are located in the same place. The solution to this would be to use a quantitative measure of colocalisation.

There are several ways of measuring the amount of colocalisation in an image. The most common measure of colocalisation is the Pearson's correlation coefficient, r_p. This measure describes the linear relationship between the grey levels (what people often refer to as the "intensity" in their images) in two images. If the image in one channel always gets brighter as the image in the other channel gets brighter and darker as the other gets darker (and by the same amounts), then the Pearson's coefficient is 1. If one channel gets dimmer where the other gets brighter and brighter where the other gets dimmer, then the Pearson's coefficient is -1. Real data will lie somewhere in between.

This method of using Pearson's correlation coefficient is not without its limitations. An important limitation to bear in mind is that image noise can greatly affect the measured coefficient, with greater noise pushing the coefficient towards zero. This means that sample preparation and sample quality can greatly affect the value obtained for r_p. Also, Pearson's correlation coefficient measures only *linear* relationships between variables – if the relationship between the brightnesses of your probes is not linear then this metric will not return an accurate result.

Alternately, one may use Manders' overlap coefficient. This value simply describes the amount of overlap between two channels, i.e. how much of the localisation in the red channel overlaps localisation in the green channel and vice versa, without respect to the linearity of the relationship. This coefficient can be split into two separate coefficients, k_1 and k_2, indicating the dependence of the red channel on the

green channel and the dependence of the green channel on the red channel. Finally, there are Manders' colocalisation coefficients, M_1 and M_2. These coefficients yield estimations of the fraction of one molecule that colocalises with the other and vice versa.

For more information on colocalisation and how it is measured, please see:

https://svi.nl/ColocalizationBasics
https://svi.nl/ColocalizationTheory
https://en.wikipedia.org/wiki/Colocalization
http://olympus.magnet.fsu.edu/primer/techniques/confocal/applications/colocalization.html

Dunn, K. W., Kamocka, M. M., and McDonald, J. H., **A Practical Guide To Evaluating Colocalization In Biological Microscopy.** Am J Physiol Cell Physiol. 2011 Apr; 300(4): C723–C742

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