CORRESPONDENCE

Quantifying colocalization: the MOC is a hybrid coefficient – an uninformative mix of co-occurrence and correlation

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Herein, we challenge one of the main conclusions in the Review on colocalization recently published in Journal of Cell Science (Aaron et al., 2018), that the Manders' overlap coefficient (MOC) is a valuable coefficient for assessing colocalization by co-occurrence.

The underlying theme of the Review is that colocalization comprises two distinct phenomena, co-occurrence and correlation. We are pleased that our proposal that colocalization should be treated as two distinct but complimentary measures is gaining acceptance (Adler et al., 2008; Adler and Parmryd, 2007, 2013). The division is powerful, in that it allows the growing number of colocalization coefficients to be characterized and compared. Accordingly coefficients can be categorized as measuring either co-occurrence, the extent of a common distribution, or correlation, the strength of the relationship between intensities. This scheme also exposes a third group of coefficients that report a mix of co-occurrence and correlation, which we termed 'hybrids'. Our detailed studies conclude that both the MOC (Manders et al., 1993) and the more recently introduced H_{coeff} (Herce et al., 2013) are hybrid coefficients (Adler and Parmryd, 2010; Adler and Parmryd, 2014). The problem with hybrid coefficients is interpretation, since they fail to differentiate between widely differing combinations of co-occurrence and correlation (Fig. 1).

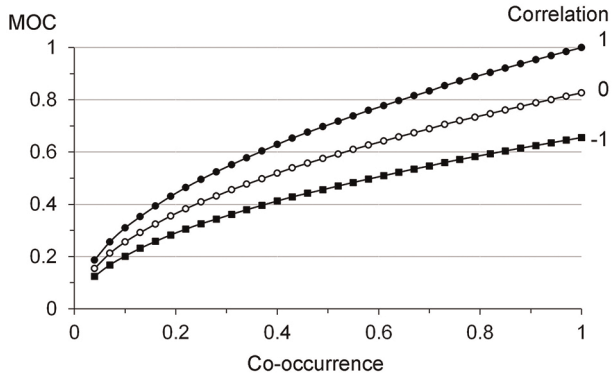

Fig. 1. The MOC reports co-occurrence poorly since it is affected by the degree of correlation. Three paired datasets (200×200 pixels) whose correlations extend across the full range (1, 0 and −1) had their co-occurrence (M1 or fraction of area common to both – in this simulation they are the same) progressively altered while their co-occurrence M2 remained maximal. The initial distributions were Gaussian, clipped to a range of mean±twice the s.d., with the lowest value above zero. The co-occurrence was progressively reduced by setting an increasing number of pixels in one dataset to zero.

In the datasets shown in Fig. 1, a MOC value of 0.6 covers a co-occurrence of between 0.37 and 0.82 (a scale of 0–1), depending on the correlation.

Aaron et al. discuss the MOC at some length and advocate its use as a measure of co-occurrence. We find this surprising since our earlier investigation concluded that the MOC had little value (Adler and Parmryd, 2010) and Aaron et al. present no observations to challenge or alter our conclusion. In fact, their data supports our view; in their Fig. 6, two cells with differing correlations (Pearson: 0.76 and 0.36) and co-occurrences (M1 and M2: 0.99 and 0.44, and 0.68 and 0.71) are nonetheless reported by the MOC to be almost identical (0.68 and 0.72, on a scale of 0–1). Hybrid coefficients confuse rather than inform, since different combinations of correlation and co-occurrence can produce the same numerical values.

We have for more than a decade advocated that the most efficient way of describing patterns of colocalization is to report both correlation and co-occurrence. Acceptance of this scheme leaves no role for hybrids like the MOC, and the additional evidence we have now provided strengthens our earlier conclusion that "The MOC is not suitable for making measurements of colocalization either by correlation or co-occurrence" (Adler and Parmryd, 2010).

Competing interests
The authors declare no competing or financial interests.

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The Pearson's correlation coefficient is not a universally superior colocalization metric. Response to 'Quantifying colocalization: the MOC is a hybrid coefficient – an uninformative mix of co-occurrence and correlation'

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In their correspondence, Adler and Parmryd reiterated their conclusion that "the Manders' overlap coefficient (MOC) is not suitable for making measurements of colocalization by correlation or co-occurrence" (Adler and Parmryd, 2010). As a result, they also challenge one of the main points of our Review (Aaron et al., 2018) in which we advocate that there is no one superior colocalization coefficient, and that complex biological situations would require the proper implementation of the optimal coefficient, either to measure signal overlap or to measure signal correlation. They claim that we have not provided any observation to alter their conclusion that the Pearson correlation coefficient (PCC) is superior.

Unfortunately, to make such an assertion is to completely miss the illustration presented in Fig. 4 of our Review (Aaron et al., 2018). In this figure, we present two situations wherein MOC and PCC offer two contrasting results, which are not merely interpretative, they are numerical and substantive. This observation shows that changes in co-occurrence can take place independently of any alterations in correlation, and vice versa. This is a main takeaway message of the article.

To further illustrate this point, we have expanded on Fig. 4A from our manuscript here. In Fig. 1 below, simulated data are presented in image 1–4, whereby an increasing amount of non-overlapping green signal is overlaid onto a static red signal. This represents a common type of experimental question wherein the primary parameter to be addressed is the extent to which two biological signals overlap. The correlation between the intensity signals would be of secondary importance. Calculation of the MOC and PCC values for each image indicates that while the overall co-occurrence decreases as expected, the change in correlation (as measured by the PCC) is negligible. This indicates that under some circumstances, the MOC will be sensitive to changes in image characteristics, while the PCC will not.

The figure above also highlights an important factor that Adler and Parmryd unfortunately seemed to have ignored in their steadfast claim that one coefficient is superior to another: given a multicolor image, the MOC and PCC are each calculated using different sets of pixel pairs. As described in Fig. 1 of our Review (Aaron et al., 2018), the MOC is applicable over the 'union' of the above-threshold regions of the two channels, while PCC should be calculated across the 'intersection' of the above-threshold regions of the two channels. This fact was, ironically, described by Adler and Parmryd (2010), and highlighted by others (Dunn et al., 2011). This distinction by itself should be more than sufficient to completely negate the assertion that one coefficient is inherently superior, since the two coefficients do not even consider the same set of pixel pairs. However, in keeping with the intended didactic spirit of our Review, we will explore this problem further using biological examples.

Based on the observation presented in Fig. 6 of our Review, Adler and Parmryd also argue that since PCC reported more dramatic difference between two image pairs than MOC, it cements their claim that the MOC is inferior to correlation-based methods. However, this claim has been challenged with actual biological examples by Dunn et al. (Fig. 4E in Dunn et al., 2011). In fact, Dunn et al. made the same conclusion as ours by stating that "even if two probes co-occur on the same cellular structures, there may be no reason that they should co-occur in fixed proportion to one another. … for studies in which proportional codistribution is not necessarily expected, PCC can provide a poor measure of colocalization."

To further the point, we considered a pair of two-color images, showing mouse embryonic fibroblasts (MEFs) stably expressing TOMM20–Halo, which is labeled with Janelia Fluor 646 dye, to mark mitochondria, and TFAM–mNeonGreen, to label the location of mitochondrial DNA, shown in Fig. 2. The experiments were designed to measure mtDNA release over time from macropores in mitochondria during the apoptotic cascade. The data shown here are two time points from one of many similar experiments, which has been previously published (McArthur et al., 2018), and are adapted with permission.

Similar to what is seen in Fig. 1, this example highlights a situation where an MOC measurement can detect changes between two multichannel images, while the PCC does not. In this case, the MOC allows us to better infer a relative change in association between mitochondria and mtDNA during apoptosis, leveraging signal overlap as a parameter and not signal correlation.

In addition, contrary to the assertion of Adler and Parmryd, there is nothing wrong with reporting 'hybrid' coefficients. As an analogy, while the information of height and weight of a patient are important to a physician, the hybrid value of body mass index or BMI would be a better predictor of cardiovascular health than the pure metrics of 'height' or 'weight'. It all depends on what information is important to the question being asked.

From these examples, we cannot accept the claim of Adler and Parmryd that PCC is simply superior under all conditions, as there is evidence presented here, in our original Review (Aaron et al., 2018) and elsewhere (Dunn, et al., 2011) to reject such an acceptance. Taken together, these observations leave no room for such a narrow interpretation of how these statistical models should be applied to a myriad of highly complex biological situations, wherein the signals, structures and hypothesis-driven questions vary widely. This is the essence of the scientific discourse presented here. The mathematics of any colocalization metric are readily available for all to implement accordingly. Subsequent wholesale advocacy for or opposition to a particular metric is erroneous, biased and misleading. Likewise, we

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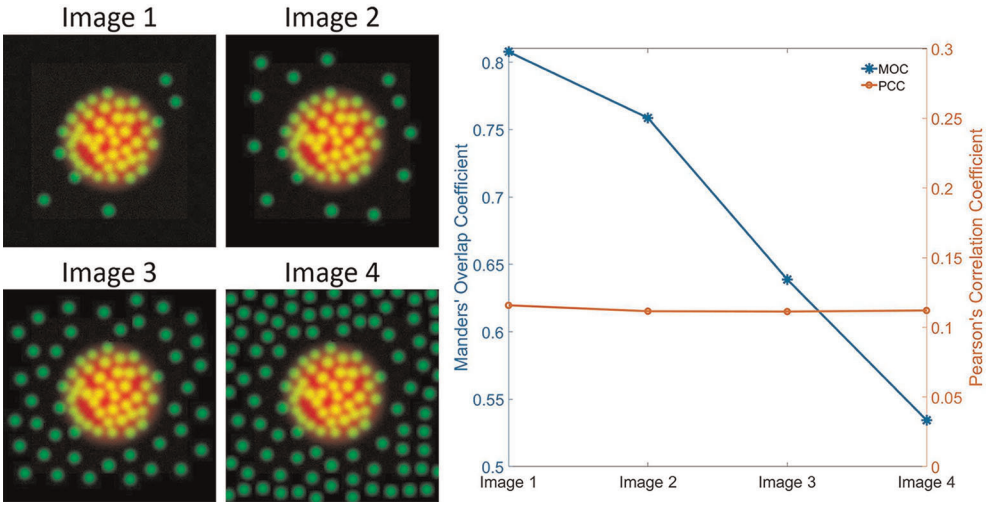

Fig. 1. Simulated data show that the MOC can detect changes in two-color images when the PCC does not. On the left, simulated data in Images 1–4 illustrate increasing amounts of non-overlapping green signal overlaid on a static red signal. Calculating the MOC and PCC values for these four images indicates a decreasing MOC value, while the PCC value remains essentially unchanged. This indicates that under some circumstances, the MOC can measure changes in image characteristics, while the PCC cannot.

are also fully aware that the group has published numerous papers advocating a certain approach to reporting colocalization. However, the fact that a quantitative approach has been advocated for more than a decade lends it no immunity from being reexamined and, more importantly, from being refined and improved when evidence warrants such an effort.

Ultimately, the only important metric that truly matters in practice is whether these coefficients constitute a sufficient toolbox for biologists to utilize when tackling a wide range of possible biological questions – from how strongly the signals of two channels correlate with one another, to the degree of overlap two biological structures exhibit. Evidence presented here proves that to advocate only a single coefficient is to deny biologists the full set of current tools. And with that, we return to the take-home message of our original Review. We firmly stand by our cautionary note that there is indeed no one 'superior' coefficient that is applicable to all biological scenarios, and that specific biological questions of researchers should guide the selection of MOC, PCC, or SRCC (or the right combination) as relevant measures of colocalization.

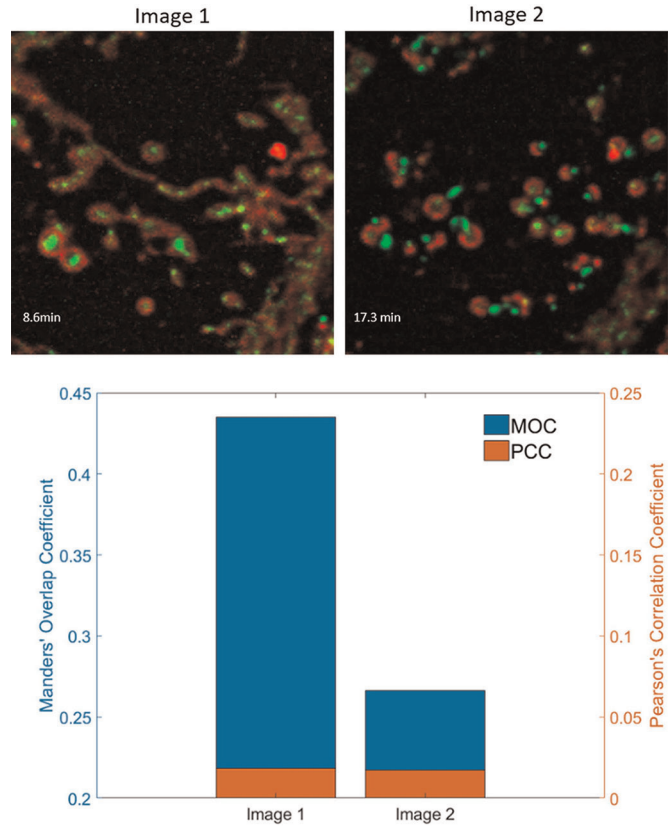

Fig. 2. The MOC can indicate mtDNA release over time, while the PCC remains unaffected. At the top, two time points from a live-cell imaging experiment, showing MEF cells stably expressing TOMM20–Halo, which is labeled with Janelia Fluor 646 dye (red), to mark mitochondria and TFAM–mNeonGreen, to label the location of mitochondrial DNA (green). The experiments were designed to measure mtDNA release over time from macropores in mitochondria during the apoptotic cascade. Quantification of the MOC and PCC values for these two images indicates a drop in overall signal overlap, while their correlation remains unchanged. Data adapted from McArthur et al. (2018) with permission from the AAAS.

Competing interests
The authors declare no competing or financial interests.

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