

QUANTITATIVE MEASUREMENT OF COLOCALIZATION IN CONFOCAL MICROSCOPY

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Since the early nineties, confocal microscopes are equipped with more than one detector in order to detect several fluorescence signals at the same time. This technique allowed to study in detail colocalization of three-dimensional objects. Colocalization of objects in dual color images was quantitatively analyzed for the first time by calculation of the Pearson's correlation coefficient (Manders et al 1992). Later numerous new methods were developed by several groups and some colocalization coefficients became accessible in commercial software packages. At this moment, colocalization measurement is a standard method in image analysis. With the commercial software it is very easy to produce numbers (colocalization coefficients) that should quantitatively describe the degree of colocalization. However, there are numerous effects that seriously bias the result of a colocalization measurement. Because measurement of colocalization is not as easy as it seems, we have organized this tutorial.

In this tutorial on colocalization measurement we will describe several methods, compare them, show the pitfalls and show how specific methods can be used in specific biological applications.