# COLOCALISATION OF DYNAMIC OBJECTS IN DIGITAL FLUORESCENCE MICROSCOPY MOVIES BASED ON CORRELATED MOVEMENT

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The interaction of two different species of objects is frequently investigated by labeling them with spectrally different fluorophores and examining the colocalisation of both colors in fluorescence microscopy images. If the two species are resolvable as distinct fluorescent objects, object-based colocalisation algorithms can be used to quantify the degree of colocalisation. Existing object-based colocalisation algorithms look for a spatial overlap in single images. However, in still images it is often difficult to determine if the colocalisation is significant, for example when the objects of one or both species are densely packed. This is especially the case for widefield fluorescence images where the out of focus fluorescence signal can add to the confusion.

Here we report a new object-based colocalisation approach making use not only of the spatial information, but also of the temporal information in dual color fluorescence time-lapse movies. In all frames of the movie and for both colors, the objects are identified and their centre location is calculated. Similar to what is done in Single Particle Tracking, a nearest neighbor algorithm is used to calculate the trajectories of the objects in the movie. If two objects are interacting with each other, their movement will be correlated. Therefore, we have developed a statistical method that defines colocalisation of two objects based on the correlation of their trajectories. The method takes into account the limited localisation accuracy of the objects and is capable of detecting transient interactions. One particular property of the method is that it does not depend on the distance between both objects.

The method is first validated using simulated diffusion trajectories, as well as experimental trajectories of double labeled nanospheres diffusing in water. As a first application, the method is used to study the stability of double labeled liposome-pDNA complexes which are being developed for use in gene therapy.