

# QUANTITATIVE COLOCALISATION OF DYNAMIC OBJECTS IN FLUORESCENCE MICROSCOPY MOVIES

**Hendrik Deschout, Dries Vercauteren, Jo Demeester, Stefaan De Smedt, and Kevin Braeckmans**

**Lab. General Biochemistry & Physiscal Pharmacy**

**Ghent University**

**Harelbekestraat 72, 9000 Ghent, Belgium**

**E-mail : Hendrik.Deschout@UGent.be**

**KEY WORDS:** colocalisation, fluorescence microscopy, time-lapse imaging

The interaction or association of objects in fluorescence microscopy images is often investigated by colocalisation analysis. Different types of objects are first labeled with spectrally separated fluorophores and images are recorded in the corresponding colors. Most current colocalisation methods subsequently either look for correlation between the pixel values of the images or identify the objects in the images and compare their positions. Both approaches however have the same fundamental limitation that they only consider one time point, which makes it difficult or sometimes impossible to distinguish for example coincidental colocalisation from real interaction.

We have developed a new quantitative approach that minimizes such errors by taking both spatial and temporal information into account. This is done by acquiring multi-color fluorescence microscopy movies rather than single images and tracking the objects of interest. If there exists some form of interaction between two objects, their trajectories will be correlated. Therefore, colocalisation of two objects is defined in this approach as a statistically significant correlation between their corresponding trajectories. The reasoning is illustrated in Figure 1, showing two trajectories of objects derived from microscopy time-lapse movies in the green and red channel. Trajectories 1 and 1' are correlated, while trajectories 2 and 2' are not correlated. The proposed method will only identify 1 and 1' as colocalised, while current methods might also find that 2 and 2' are colocalised, based on nearly coinciding positions at some time points. The method has been validated with simulations as well as experimentally by using mixtures of single and double labeled fluorescent polystyrene microspheres diffusing in water.

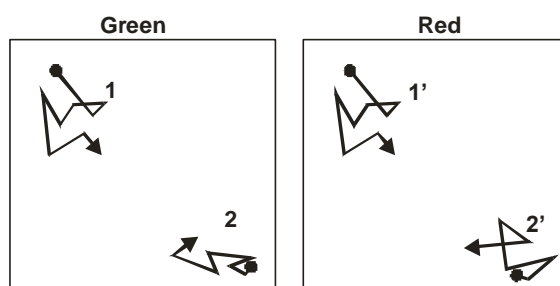


Figure 1 : A schematic representation of colocalisation based on correlated movement.

As a proof of principle study, the stability of liposome-pDNA complexes diffusing in water is monitored against the detergent Triton, which dissolves the lipids and thus releases the pDNA. In a second application we quantified the colocalisation of endocytosed polymer-pDNA complexes with different types of endosomes of living cells in confocal time-lapse movies.