Automatisation of FISH signals analysis in cells with preserved 3D nuclei

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Fluorescence *In Situ* Hybridisation (FISH) has been widely used to localize genes and DNA sequences in cellular preparations by hybridising complementary probe sequences. A method based on para-formaldehyde cell fixation has been developed to perform FISH in cells with preserved three-dimensional structure. This technique, named 3D-FISH, has opened the way to study organization of chromatins under context of chromosomes in interphase.

Individual chromosome has been shown to occupy discrete regions, called chromosome territories (Zorn *et al*., 1976). Relative position of a given gene to another in interphase nuclei, is important because it could have a major impact in chromosomal rearrangments such as inversion and reciprocal translocation. Indeed, some studies indicated that the spatial proximity of gene loci could be at the origin of chromosomal translocation. (Nikiforova *et al*., 2000, Science; Lukasova, Kozubek *et al*. 1997, Hum Genet).

To further test this hypothesis, a faithful measurement of the distance between gene loci which are potentially involved as partners of translocation in cells with preserved 3D structure is a prerequisite. Hundreds images need to be analysed in order to perform statistical analysis. A tailor-made software package is developed. It is available as a set of plugins for ImageJ software to automatically measure distances between two or more gene loci in 3D-FISH experiments. The software fully automates the process of segmentation and analysis of distances. It can handle any number of genes and incorporate the image of DAPI counterstained nucleus in order to assess the relative distance of these gene loci to the centre of the nucleus. It greatly facilitates the image processing and analysis by providing an useful tools to overcome laborious task of image segmentations based on user defined parameters (applicable up to more than 500 stacks) and to reduce subjectivity in data analysis. Up to 98% sensibility has been achieved.

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