SLOW SCAN FCS AS A TOOL TO INVESTIGATE CHROMATIN COMPACTION IN THE EUKARYOTIC NUCLEUS.

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DNA is one of the most important biological macromolecules, since it harbors the genetic information of the cell. In order to fit in eukaryotic cell nucleus, DNA is highly compacted and packed by histones in chromatin. Chromatin compaction has recently been shown to be related to cell epigenetic states [1] and levels of commitment [2]. It seems also to be involved in important processes like senescence and cancer [3], [4]: it could be interesting to study chromatin degree of compaction in relation to cancer insurgence and progression. Fluorescence Correlation Spectroscopy (FCS) is a technique able to probe chromatin accessibility in living cells by measuring fast diffusion of molecules like GFP in the range between microseconds to milliseconds [5]. Here, in order to quantify local chromatin accessibility, we sample several positions within the nucleus by performing a slow orbital scan. The slow scan allows us to ignore the motion of the scanner and analyze the data as in single point FCS without losing temporal resolution. To extract the diffusion properties in distinct sub-nuclear compartments, we sort the sampled points based on the intensity value measured in the same or in another channel (e.g. Hoechst 33342) prior to averaging In this way we can rapidly compare, for instance, the diffusion properties inside heterochromatin and euchromatin. We discuss the advantages and limitations of the method, and its application for comparing chromatin accessibility in cellular models of different cancer stages.

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