STED AND EXPANSION MICROSCOPY FOR THE STUDY OF NUCLEAR ORGANIZATION

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ABSTRACT

Expansion microscopy (ExM) is a super-resolution imaging method that does not require any exceptional optical microscope (1). The approach is based on uniformly chemically expanding a sample, thus increasing the relative distances among objects of interest as fluorescent molecules labeling specific components. Nevertheless, ExM is highly invasive; it involves fixation gelation and digestion steps that could introduce artefacts and heterogeneities in the relative spatial distribution of complex proteins in the cells. The combination of STED (2) and ExM (ExSTED) (3) allows an unprecedented resolution, providing high sensitivity to discover possible pitfalls and to quantify the expansion parameters, i.e., scale factor, isotropy, and uniformity (4). Our focus is on the cell nucleus, in particular on nuclear membrane and chromatin organization. We show that Nup153 (5), a filamentous subunit localized in the nuclear pore basket, is an excellent reporter to address the isotropy of the expansion process quantitatively. Since the quantitative analysis carried out on NPCs at different spatial scales validates its feasibility at the nanoscale (4), we investigated more the three-dimensional nuclear structure, from lamin to chromatin organization.

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