Assessing co-localisation of nuclear proteins with super resolution microscopy: a comparison between Expansion Microscopy and dSTORM

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Abstract

In biology, co-localisation of fluorescently labelled proteins is an informative approach to study the spatial proximity of proteins and, in turn, to give insights about the biological mechanisms they are involved in [1]. However, co-localisation precision relies on the resolution of the microscopy technique. Tying to define molecular proximity in case of densely packed systems, such as most of nuclear proteins, often requires moving from the classical diffraction-limited microscopy to newly developed super-resolution protocols [2]. In particular, these might involve either a structural modification of the sample or more sophisticated microscopy techniques, and the choice of the suitable protocol is crucial for obtaining the correct results. In this work, we have explored co-localisation of nuclear proteins by using a) Expansion Microscopy, a novel approach that involves confocal acquisitions of expanded samples embedded within an acrylamide gel [3] and b) dSTORM, an advanced microscopy technique that allows the localisation of single fluorescent molecules [4]. In both cases, the resolution limit can be overcome by at least a 4X factor. From a biological point of view, we explore the co-localisation of ZC3H4, a nuclear protein involved in transcription termination [5], with other components of the transcription machinery and we compare the results obtained with the two different imaging protocols. The co-localisation analysis we perform involves both the use of existing software for co-occurrence, correlation and object-based co-localisation as well as ad-hoc tools developed for this purpose.

References

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