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Super-Resolution as a Tool to Understand Nuclear Organization

Rohini R Nair*

Chromatin Dynamic Unit, University of San Raffaele, Italy

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***Corresponding author:** Rohini R Nair, Chromatin Dynamic Unit, University of San Raffaele, Milan, Italy, Email: rohini.nair07@gmail.com

Introduction

Light microscopy has allowed the visualization of cellular and subcellular structures in living and fixed samples. This non-invasive method allows the use fluorescent probes facilitating high contrast and high molecular specificity. However the diffraction limit of the light microscope prohibits in resolving the structure smaller than ~200-300nm.

Chromatin is made up of DNA wrapped around the nucleosomes which are formed by core histone protein H2A, H2B, H3 and H4. In vitro studies, have apparently established the hierarchical model of chromatin compaction, where 10-nm beads-on-a string chromatin fibres fold into 'higher-order' structures that are ~30nm in diameter which further compact to form 100-200nm chromonema structure [1-3]. Understanding the structure of chromatin is essential since it not only functions in assisting the six feet of DNA to condense and fit inside the nucleus but also assists in gene expression and prevents the DNA from being damaged [4]. However with the spatial resolution of ~200-300nm of a light microscope it is not feasible to study chromatin structure.

The introduction of super-resolution microscope has been able to overcome the diffraction limit extending the spatial resolution of light microscope to length scale as small as 10-20nm. Super-resolution microscopy methods can broadly be categorized in two types. Structured Illumination Microscopy (SIM) and Stimulated Emission Depletion (STED) takes advantage of patterned illumination light to improve spatial resolution of light microscope. Stochastic Optical Reconstruction Microscopy (STORM) and (Fluorescence) Photo activation Localization Microscopy- PALM and fPALM take advantage of single molecule detection and localization to break the diffraction limit hence both are also referred as Single Molecule Localization Microscopy (SMLM). There are relative benefits and limits of super-resolution microscopy mentioned in Table 1 adapted from Jennifer et al. [5].

Table 1: super-resolution microscopy.

Modality	Resolution	Acquisition Time	Data Size	Other Concerns
SIM	100nm	Short (seconds)	Small (9-15 frames)	Out of focus signal and sample thickness
STED	30-70nm	Short (seconds)	Small (1 image)	Photobleaching
STORM	10-55nm	Long (minutes)	Large (many frames)	Over/under labelling artefacts
PALM/fPALM	10-55nm	Long (minutes)	Large (many frames)	Over/under labelling artefacts

Super-resolution has been extremely useful technique to study chromatin structure. SIM was the first super-resolution method to visualize nuclear structures [6]. Using this approach visualization of chromatin of nuclear pore complex and of nuclear lamina was achieved. It made the precise measurement of chromatin components at higher resolution. SIM was used to further study nuclear compartmentalization and nuclear function [7]. However the SMLM enable for the first time to image histone at high resolution within the intact nuclei [8]. SMLM has also been used in visualizing chromatin plasticity by labelling histones at the nanoscale level as a function of cell state such as pluripotent or differentiated state [9]. Apart from histones SLMN was also used to image DNA [10].

Super-resolution imaging techniques have made it possible to evaluate the organization and dynamics of chromatin at single

cell level both in inter phase [8,11] and dividing cell nuclei. It also facilitates to understand the organization of single or groups of nucleosomes and the overall nucleosome occupancy level of DNA [12]. Using quantitative approach the authors identified a new model of chromatin assembly. The results stated that nucleosomes arranged in heterogeneous groups, which the authors named ''nucleosome clutches'' which were separated by nucleosomedepletedregions.

Hence, super-resolution imaging of histones, DNA RNA polymerase and other nuclear structures in living cells is certainly one of the most interesting fields in the next coming years. I will enable to understand the function of genes at single cell level. However more quantitative methods have to be developed to builds predictive model about gene function. Hence, using super-resolution microscopy it not far to view the activity of gene at single cell level.

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