Epigenetics & Chromatin

Imaging heterochromatin in human embryonic stem cells with light-sheet Bayesian microscopy

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We present a novel super-resolution microscope for the study of chromatin architecture. In eukaryotic cells, chromatin is organized into two distinct domains: lightly packed, and actively transcribed euchromatin, and highly condensed, and transcriptionally silent heterochromatin. While being transcriptionally repressive, heterochromatin is known to serve as binding sites for regulatory proteins, and therefore, plays an important role in the epigenetic regulation of gene expression [1]. However, despite its importance, the architecture of heterochromatin remains elusive, due to the lacking of powerful tools. Single-molecule super-resolution microscopy [2,3], invented by Betzig [4], Hess [5], and Zhuang [6], improves the spatial resolution of light microscopy by over an order of magnitude to ~20-30 nm, and therefore offers a new approach to examine the 3D architecture of chromatin. However, its fundamental requirement of high signal-to-noise ratio (SNR) of fluorescence signal to detect single fluorophore has limited its applications mainly to the study of the cytoskeleton and membrane proteins that reside near the surface of a cell [7-9]. To image beyond the surface of a cell where chromatin resides, we develop a Light-Sheet Bayesian Super-resolution Microscope (LSB-SRM). This microscope is a marriage between two recently developed techniques: light-sheet single molecule super-resolution microscopy and Bayesian super-resolution image reconstruction algorithm. The light-sheet condenser selective illuminates the nucleus of a cell, suppresses the fluorescence background from fluorophore in unwanted layer of the cell, and therefore increases the signal-to-noise ratio of the single-molecule detection. The Bayesian algorithm can efficiently resolve single molecules in images with noisy background. A combination of the two allows us to directly visualize heterochromatic structures in the nucleus of human

embryonic stem cells at super-resolution. Our study shows that the heterochromatin organize into nanometer scale domain structures in H1, human embryonic stem cells, which is consistent with the topological domain theory derived from recent sequencing experiments [10,11].

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