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Higher order chromatin structure: new insight with novel correlative approach

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The principles underlying spatial organization of chromatin mostly remain poorly understood. Among the methods that are frequently used in those studies microscopy occupies a prominent place. Thus, great amount of data was obtained with transmission electron microscopy. However, its main advantage – high resolution – comes at a cost: long sophisticated sample preparation requires a lot of time and inevitably casts doubts on structure nativity. The latter particularly raises concerns as chromatin is a very heterogeneous structure sensitive to the experimental conditions. Recent advances in super-resolution light microscopy allow to avoid those issues while keeping resolution relatively high. One of the most promising subdiffractional method is STORM, that can achieve resolution up to 20 nm and have both 2D and 3D modes. However, it is yet unclear to what extent STORM can be applied to studies of chromatin structure. Therefore, STORM results should be verified by method with higher resolution, such as electron microscopy.

Here we present CLEM procedure for combined STORM-TEM imaging of replicative labeled DNA which allows to obtain images of the very same structures using both fluorescent and electron microscopy. This protocol allows for high-efficiency replication labeling compatible with various 2D and 3D-electron microscopy (such as electron tomography) and fluorescent microscopy techniques. We also show that 2D-STORM can be effectively used in studies of replication domains spatial organization, whereas usage of 3D-mode seems to be problematic.

Materials and Methods: We adapted method of replicative labeling [1] for strong aldehyde fixation that ensures optimal preservation of chromatin near-native structure during sample preparation for electron microscopy. Our protocol includes Edu-labeling of newly synthesized DNA, label detection with Click-chemistry and biotin-streptavidin and subsequent Nanogold-Ag amplification for TEM. Click-chemistry provides simple and extremely selective labeling of replicated DNA and the use of streptavidin-Nanogold conjugates provides better penetration efficiency even into glutaraldehyde-fixed samples due to the relatively small size of the probe. This approach was applied for correlative superresolution and TEM microscopy by using a mixture of biotinylated and fluorescent (AlexaFluor-647) azides and sequentially imaging the samples by STORM microscopy and labeling the same replicative domains with streptavidin-Nanogold.

STORM images were processed with Thunder-Storm ImageJ plugin and then analyzed with SR-Tesseler (for 2D images) or Python3.7 libraries sklearn and tensorflow.

Results: Our modified protocol allowed us to perform electron tomography of labeled DNA with near-native structure. It enabled visualization of chromatin fibers that are

mostly 140–200 nm wide. We were also able to compare images of labeled newly synthesized DNA obtained with STORM and TEM. Those images showed striking similarity, indicating that STORM resolution is more than enough to investigate structure of replicative domains study. Apart from that STORM images can be easily analyzed with multiple tools since STORM presents tables of dots coordinates. Those dots can be clusterized (with DBSCAN for instance) and then a large set of geometrical parameters can be measured. Together with relatively short sample preparation it makes 2D-STORM a promising method in such studies.

STORM-3D turned out to be much more difficult to apply to that issue. This method is based on usage of cylindrical lense and hence astigmatism, which together with calibration data can be used to calculate the Z coordinate. However, dot distribution upon the Z axis seems to be extremely sensitive on certain calibration, which indicates low reliability of such data and makes it almost impossible to compare images obtained using different calibrations.

Conclusions: Our protocol allows for high contrast high-efficiency pre-embedding DNA labeling compatible with various light and electron microscopy techniques, for instance TEM, electron tomography, STORM.

Electron tomography reveals the existence of 140–200 nm fibers of 2 hours replication labeled DNA.

2D-STORM is a fast, effective and relatively simple method to examine spatial organization of chromatin.

3D-STORM requires further adjusting to perform properly and produce trustworthy results.

References

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