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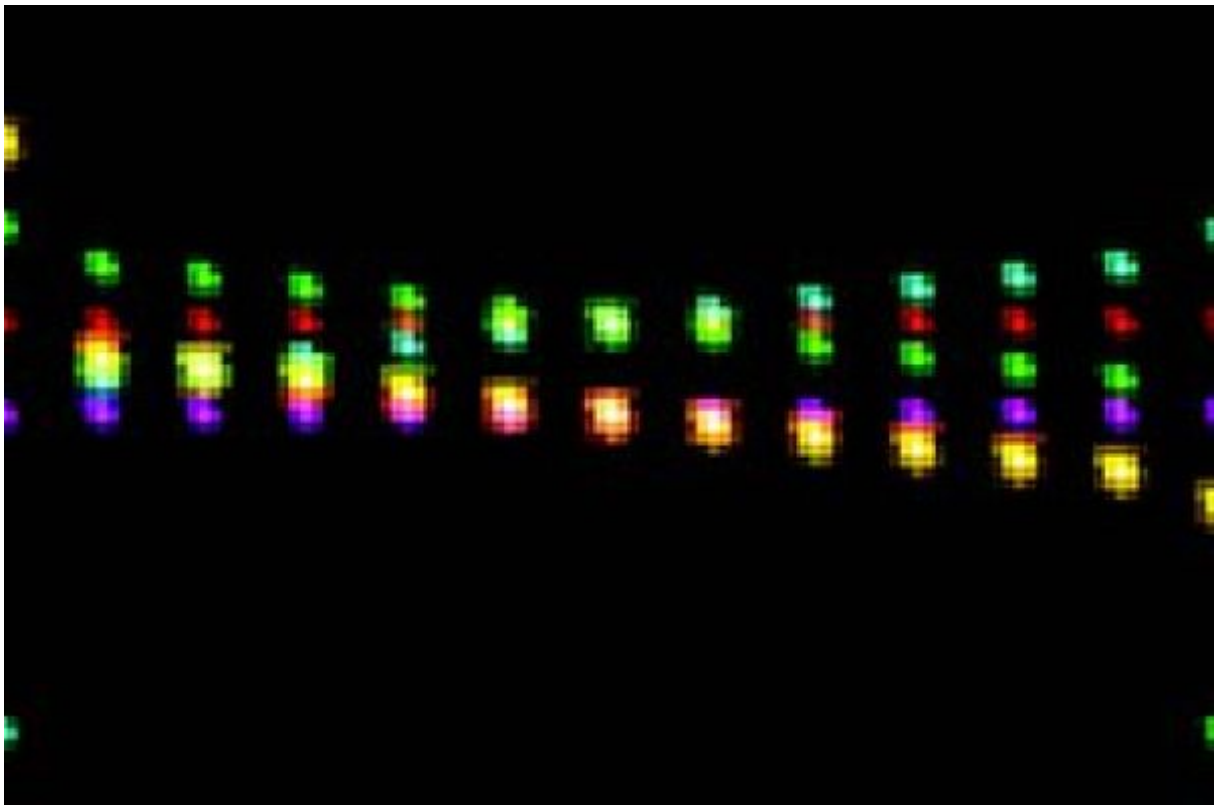

PhD Thesis Defense PABLO GOMEZ GARCIA 'Development and Application of Localization-Based Microscopy Methods to Study the Structure and Dynamics of Chromatin Through the Process of Cellular Differentiation'

PABLO GOMEZ GARCIA

April 24, 2020

15:00

Online (Teams)

Thesis Defense, April 24, 2020, 15:00. Online

PABLO GOMEZ GARCIA

Biophotonics (SLN Team Wieser - CRG)

ICFO-The Institute of Photonic Sciences

Recent advancements in single-molecule localization-based microscopy have made it possible to visualize biological structures and dynamic processes within the cell with unprecedented spatial resolution. Determining the spatial organization of these complex structures, like chromatin, under physiological and pathological conditions is an important biological goal. Currently, one of the main limitations of this family of techniques is the difficulty to extend them to multiple colors, so that multiple target molecules can be imaged simultaneously. We developed an approach for simultaneous multi-color super resolution imaging which relies solely on fluorophore excitation, rather than fluorescence emission properties. By modulating the intensity of the excitation lasers at different frequencies, we show that the color channel can be determined based on the fluorophore's response to the modulated excitation. We use this frequency multiplexing to reduce the image acquisition time of multi-color super resolution DNA points accumulation in nanoscale topography (DNA-PAINT) while maintaining all its advantages: minimal color cross-talk, minimal photobleaching, maximal signal throughput, ability to maintain the fluorophore density per imaged color, and ability to use the full camera field of view. One outstanding biological question that will benefit from the development and application of advanced imaging technologies is the relationship between chromatin structure and gene activity. Chromatin is a complex of DNA and histone proteins, which helps compact and spatially organize the genetic code within the small space of the nucleus. Applying super resolution microscopy, previous work in the lab showed that nucleosomes within folded chromatin fibers are organized in heterogeneous groups named nucleosome clutches, unlike the textbook model that suggested a much more ordered and hierarchical folding of nucleosomes. Nucleosome clutches are smaller and less densely compacted in embryonic stem cells (ESCs) compared to neuronal progenitor cells (NPCs), in correlation with the more open chromatin state of ESCs. We applied modelling of chromatin and Single Molecule Tracking (SMT) to compare the structure of synthetic fibers and local nucleosome dynamics with the super resolution images of chromatin fiber in ESCs and NPCs. First, using coarse-grained modeling, we simulated the spatial arrangement of chromatin fibers corresponding to the pluripotency gene Oct4 in mouse ESCs (mESCs) and mouse NPCs (mNPCs), taking into account nucleosome positions from MNASE-Seq data, the ratio of linker histone H1 per nucleosome, and the amount of histone tail acetylation. The resulting folded fiber configurations showed higher compaction of the overall fiber and of the nucleosome clutches in mNPCs compared to mESCs, recapitulating the super resolution imaging data. We further use SMT both at short (15ms) and long (500ms) exposure times to show that nucleosome turn over and local dynamics within the chromatin fiber correlate with the structural features observed in super-resolution data and the polymer models.

Friday, April 24, 2020, 15:00. Online

Thesis Advisor: Dr Pia Cosma, CRG

Thesis Co-advisor: Dr Stefan Wieser

Hosted by: Dr Stefan Wieser

Pablo Gomez's Thesi Cover