

ONi



Singe-Particle
Tracking

Nanoimager

Forging a path in particle dynamics, more than just live cell imaging.



Harnessing the power of single-particle tracking to measure cohesin interaction with chromatin

Introduction

Cohesin is essential for sister chromatid cohesion as well as DNA repair and regulating gene expression. Cohesin is a ring-shaped multi-protein complex comprised of antiparallel coiled coils that are bridged by SCC1/Rad21 (Figure 1). This tripartite structure traps DNA inside the ring¹, which is critical for its functions in connecting sister chromatids and organizing eukaryotic genomes into higher-ordered structures². During interphase, cohesin folds DNA into loops between gene regulatory elements. Cohesin mutations or dysregulation can lead to aneuploidy and severe developmental disorders². Therefore, understanding its interaction with chromatin is critical for determining the mechanism(s) by which cohesin mediates its DNA-regulating functions.

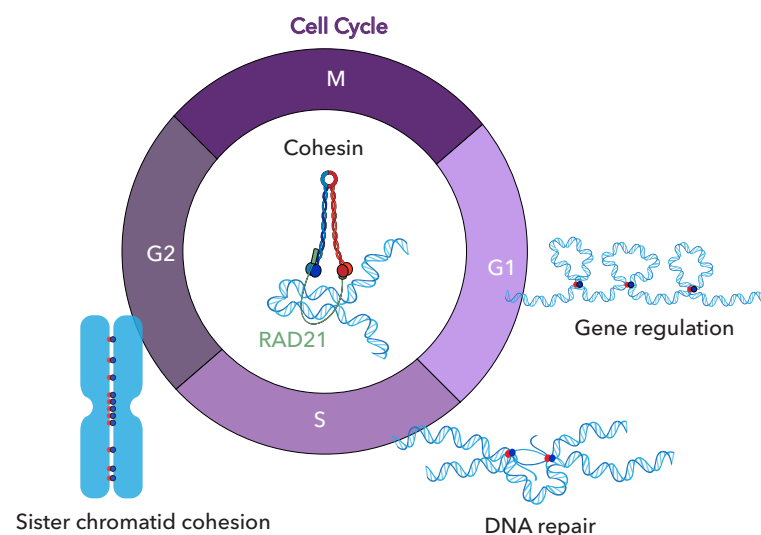


Figure 1 | Cohesin cell cycle functions.

Challenge

Measuring the dynamics of nuclear proteins has been fundamental in understanding their mechanisms in regulating gene expression, especially at the single molecule level. Populations of gene-regulating proteins can have heterogeneous behaviors, which are important for mediating chromatin structures. Using bulk methods like FRAP, the behavioral information of distinct populations is lost^{3,4}. Employing single-particle tracking (SPT) techniques by labeling individual cohesin complexes at a suitably low density allows for the identification of unique populations of cohesin that are either bound to chromatin or freely diffusing in the nucleus. Moreover, following the movement of these individual tracks can provide insight into how they interact with chromatin to mediate their DNA-regulating functions. NimOS, the image acquisition and analysis platform for the Nanoimager, is optimized for identifying single fluorescently-labeled particles and tracing their trajectories over time to extract displacement information and diffusion coefficients.

Summary

The Nanoimager is optimized for the visualization and quantification of diffusing nuclear protein complexes at the single protein level. Its built-in single-particle tracking (SPT) software tool further empowers this type of research and supports the identification and characterization of:

- Individual protein complexes such as DNA-regulatory proteins.
- The bound and unbound movement of single particles.
- The behavior of single protein populations and their interactions with other cellular structures including chromatin.

Results

To determine the behavior of cohesin complexes, real time imaging of individual cohesins was performed on the Nanoimager and the tracking data was analyzed in NimOS. U2OS cells expressing a HaloTag SCC1 CRISPR knockin⁵ were labeled with Janelia Fluor (JF) 549-HaloTag ligand (see⁶ for a list of fluorophores suitable for SPT). Imaging was performed using continuous illumination with 30 ms exposure time for a total of 5 min. Cohesin particles were identified by a diffraction-limited spot, which was localized with precision such that the resulting map of cohesin particles was below the diffraction limit. Applying user-defined parameters to analyze the time-lapse images in NimOS gained results indicating the positions, paths and movement speeds of single cohesin proteins. The spatiotemporal trajectories were then determined and categorized into a chromatin-bound or -unbound (diffusing) population based on a defined diffusion coefficient threshold of $\sim 0.1 \mu\text{m}^2/\text{s}$ (Figure 2C). When the bound fraction's tracks were overlaid with chromatin (Figure 2 A-B), the cohesin trajectories were confined to regions of dense chromatin signal whereas the tracks of the diffusing population were broader. Importantly, magnified images of the diffusing population (Figure 2D) showed that the trajectories of cohesin remained largely confined within chromatin regions as well, suggesting partial or transient chromatin-interactions in this diffusive population of cohesins and a touch-and-go search mechanism. The Nanoimager's precision imaging capabilities combined with the user-friendly tracking functionality of NimOS are ideally suited for examining the behavior of nuclear protein populations using SPT.

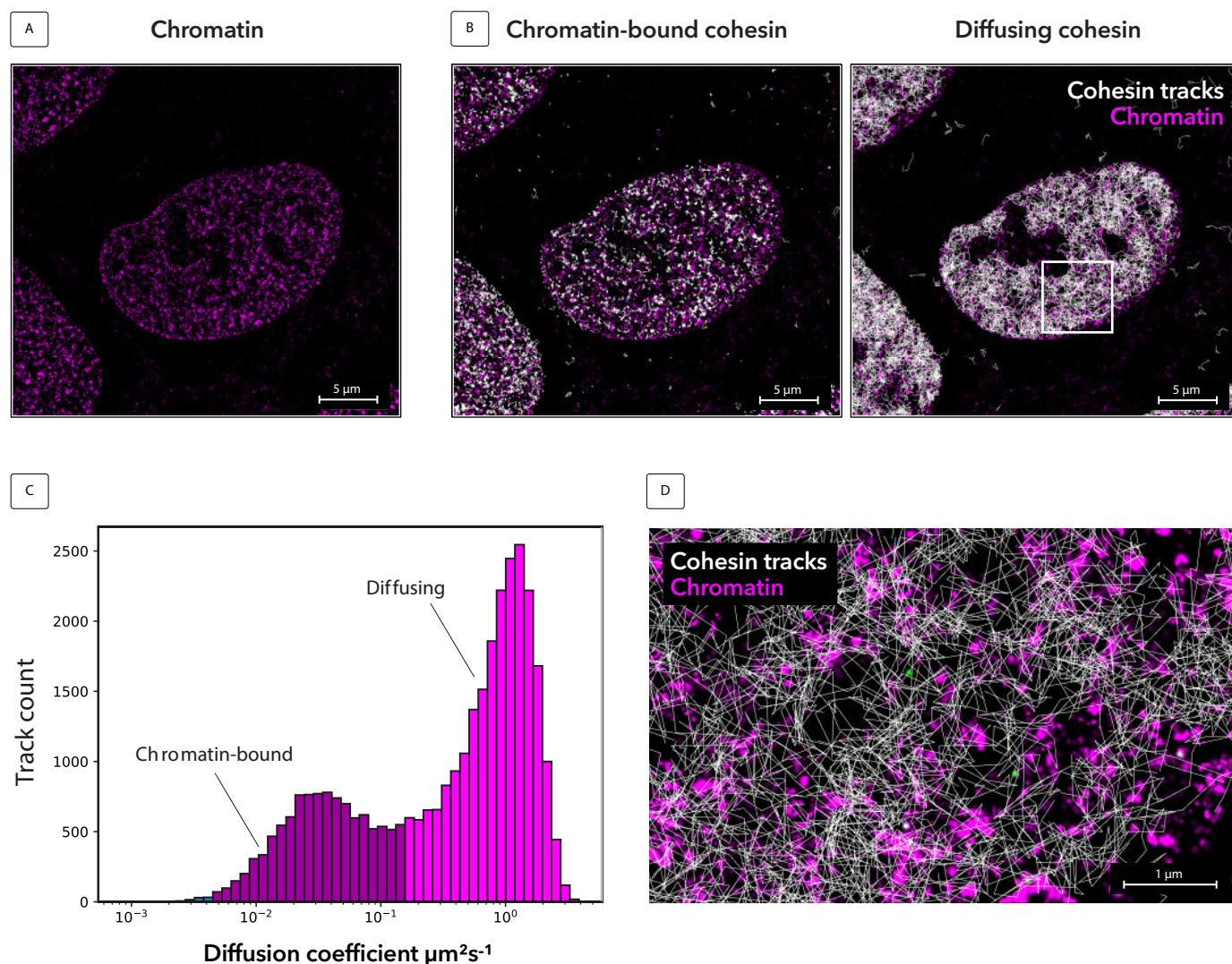


Figure 2 | (A-B) U2OS cells expressing Dy505-SNAP-H3 (chromatin) and SCC1/Rad21-HaloTag labeled with JF549-HaloTag ligand. Shown is a maximum time projection image of Dy505-SNAP-H3 captured at time 0 s overlaid with the cohesin tracks (shown in white) generated from the time lapse images in NimOS. (C) Graph shows the binned distribution of diffusion coefficients in the chromatin-bound and the diffusing populations, and the number tracks in each bin. (D) Magnified view of the white inset in (B). Scale bars: 5 μm in (A), (B), 1 μm in (D).

Solution with the Nanoimager

ONI is dedicated to advancing innovative tools that enable scientists to explore and learn the microscopic details of life. For more information on single-molecule localization microscopy and SPT in your research, please visit www.oni.bio

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