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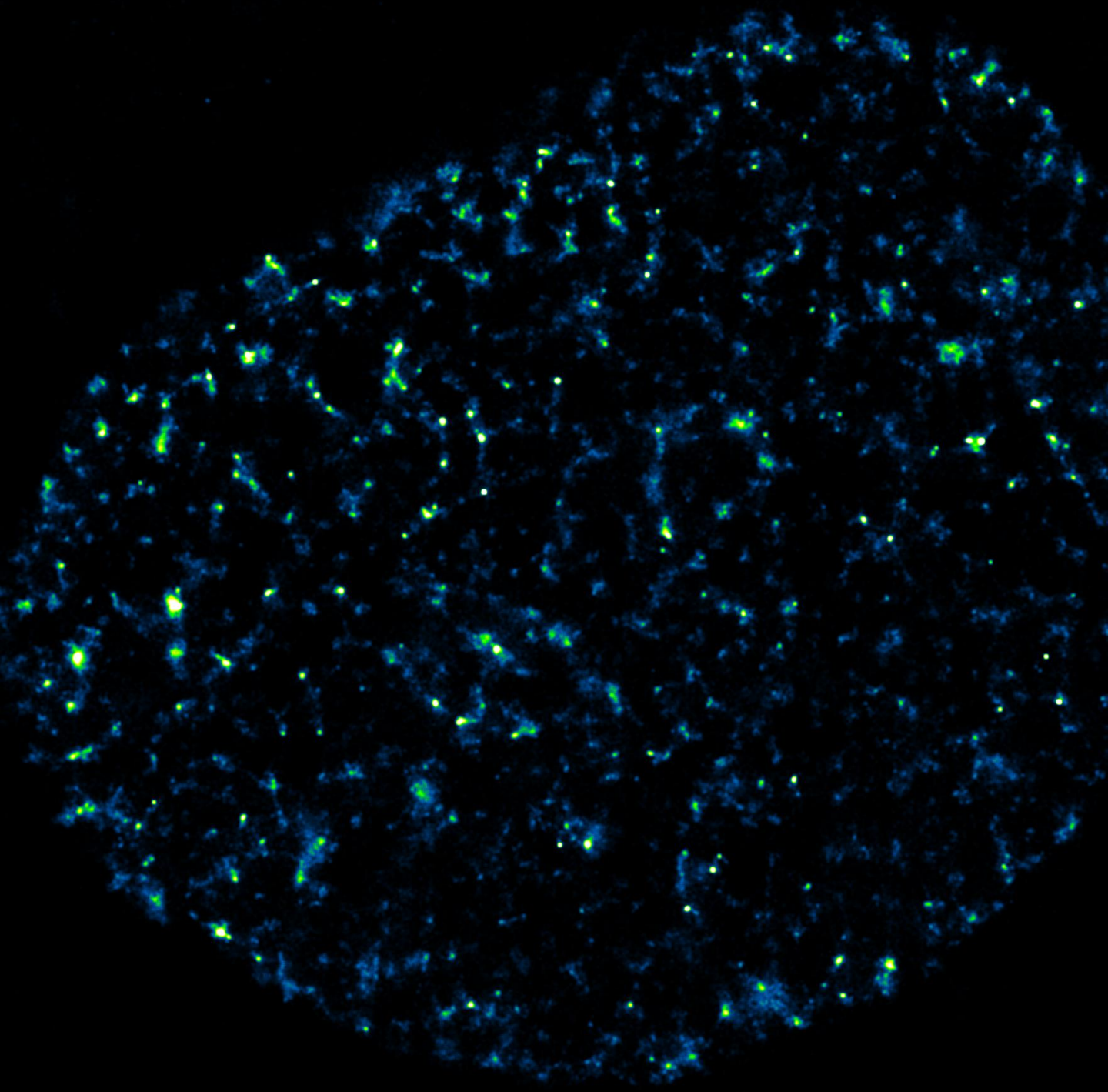
Imaging DNA replication sites at the nanoscale



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The genome is a highly organized and compartmentalized structure, which undergoes many dynamic events over the course of a cell cycle, including DNA replication. Standard microscopy techniques have enabled the visualization of replication sites, but with a diffraction-limited resolution of ~250 nm. Yet, the smallest units of chromatin are rather in the scale of a few nanometers. Although informative, standard microscopy images of the genome are only a blurred rendering of the actual biological structure. The emergence of super-resolution techniques in recent years has opened new avenue for the visualization of genomic events. In this note, we describe how single-molecule localization microscopy (SMLM) can be a powerful tool to study DNA replication sites.

What can I do with single-molecule imaging of DNA replication sites?

SMLM can achieve localization precisions of ~10 nm and can therefore reveal genomic structures with an unprecedented precision. SMLM can allow (i) the localization of DNA replication sites in the nucleus, and (ii) the quantification of the size and number of replication sites. These results can be used to study the effect of a given drug or mutant on replication sites, or to determine how replication may change depending on the timing of S phase or of the cell type. Additionally, when combined to a 2nd color staining, colocalization of a protein of interest and a replication site can be measured at the nanoscale.

In practice

What do I need to visualize replication sites in single-molecule?

- An EdU-AF647 labeling kit
- A specific imaging buffer for STORM imaging.
- A dedicated software for acquisition, processing and analysis

PREPARATION

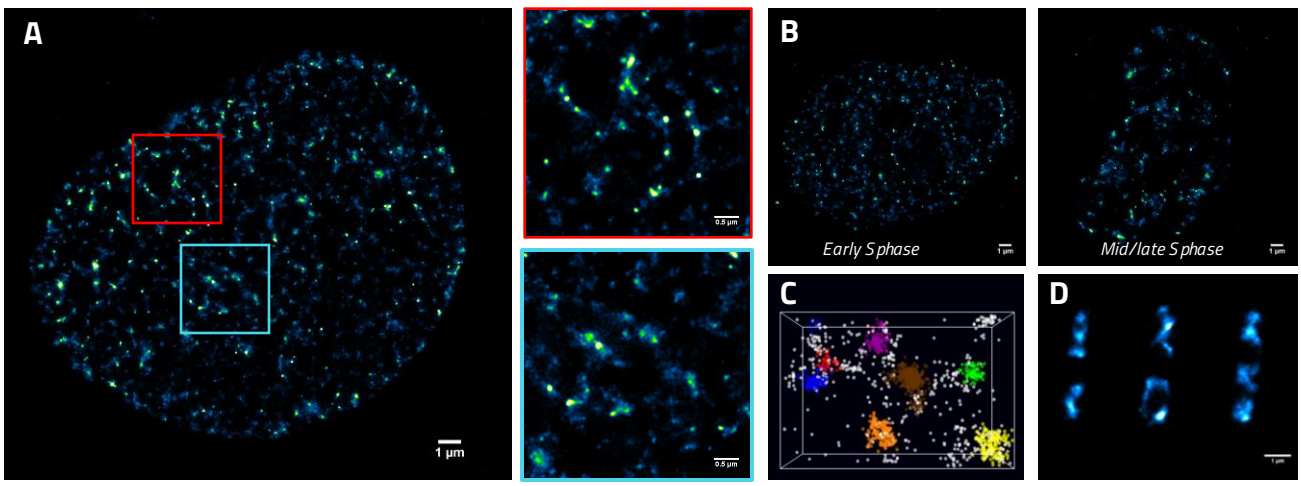
A commonly used biological tool to study and visualize replication sites is EdU. EdU can incorporate into replicating DNA (substituting for a nucleotide) when incubated with cells and can be coupled to a fluorophore post-fixation. Using an **EdU-AF647** combination, we can use **STORM (a SMLM technique)** to obtain highly-resolved images of replication sites.

IMAGING

Abbelight SAFe modules (**SAFe™ 180** and **SAFe ™ 360**) can both be used for single-molecule microscopy of DNA replication sites. These modules can adapt to any inverted microscope. In both cases, an astigmatic lens is recommended for **3D imaging**. In the case of **simultaneous multicolor imaging, SAFe ™ 360** dual-view system is recommended.

ANALYSIS

DNA replication sites form clusters in the nucleus. Several algorithms can be used to detect such clusters in the dataset, isolate them, and determine their position, size, and density. For this purpose, **Abbelight™ NEO software** offers several clustering algorithms, including **DBSCAN** and **Voronoi** (see our note on clustering analysis).



Images of replication sites visualized with single-molecule microscopy with the Abbelight setup. (A) STORM imaging of *COS7* cells DNA replication sites labeled with EdU-AF647. **(B)** STORM imaging of *COS7* cells DNA replication sites labeled with EdU-AF647 in (left) an early-replicating cell and (right) a mid/late-replicating cell. **(C)** Clustering analysis of DNA replication sites in *COS7* cells using DBSCAN algorithm in NEO Software. **(D)** Replication sites in dividing *E. coli* cells stained with EdU-AF647.

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