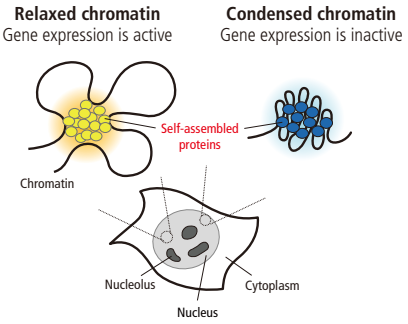
APPLICATION NOTE

N-STORM Super Resolution Microscope

# Localization analysis of RNA polymerase I using a super-resolution microscope and cluster analysis function

In recent years, the phenomenon of "phase separation," by which proteins or nucleic acids compartmentalize the cells by self-assembly and control various biological functions, has been reported and is attracting attention. Structural conversion, such as relaxation and condensation of chromatin in the nucleus, and the control mechanisms of nuclear functions including gene expression, are beginning to be explained by phase separation.
This application note introduces an experimental case conducted in collaboration with Ryu-Suke Nozawa, Ph.D., Life Sciences, Division of Experimental Pathology, The Cancer Institute, Japanese Foundation for Cancer Research. He is attempting to identify the mechanisms of life phenomena through analyses of chromatin structure, which serves as the foundation for a variety of nuclear functions, and the molecular mechanisms that control chromatin structure.




## Outline of the experiment

RNA polymerase I, which is one of the major constituent proteins of the nucleolus in the nucleus, transcribes ribosomal RNA from ribosomal DNA. In this experiment, the localization of RNA polymerase I was observed at a single molecule level by using the N-STORM, Nikon's super-resolution microscope (Figs. 1 and 2).
Then, actinomycin D treatment was performed to inhibit the function of RNA polymerase I and changes in the molecular distribution pattern caused by this chemical treatment were analyzed using the cluster analysis function (Fig. 3).

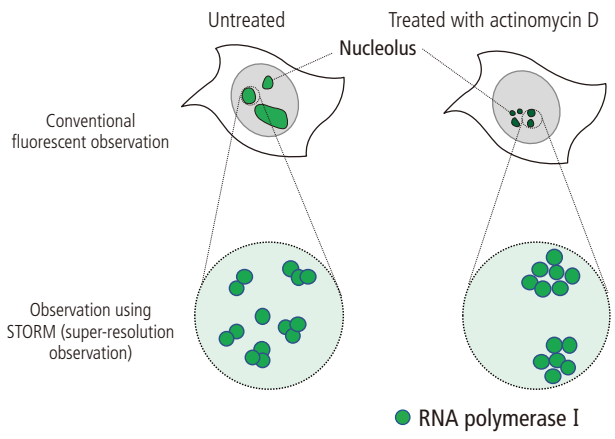



Figure 1. Image of RNA polymerase I localized in the nucleolus

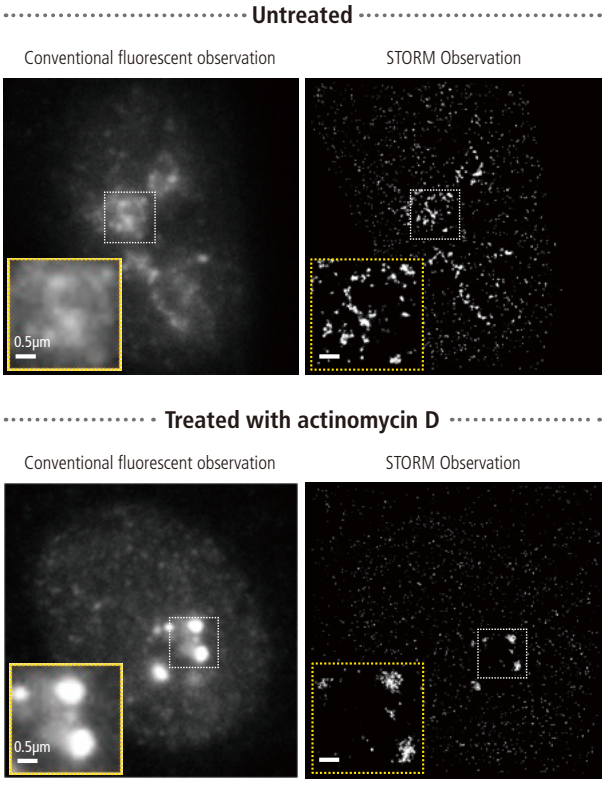



Figure 2. With conventional fluorescent observation, the RNA polymerase I signal appears to be a continuous large structure, whereas in STORM observation it is seen as an assembly of small clusters. In STORM observation after actinomycin D treatment, it was observed that RNA polymerase I forms larger clusters.
Samples: hTERT RPE1 cell (retinal pigment epithelial cell), primary antibody: RNA polymerase I (sc-48385), secondary antibody: antimouse IgG, Alexa647 labelled antibody (A21236)
Acquisition mode: 2D-STORM, excitation wavelength: 647nm, number of acquisitions: 10000 frames, exposure time: 20 msec
Objective lens: CFI SR HP Apochromat TIRF 100XC Oil
Data provided by Dr. Ryu-Suke Nozawa, The Cancer Institute of the Japanese Foundation for Cancer Research

The result of the cluster analysis showed that when the function of RNA polymerase I was inhibited by actinomycin D treatment, molecules of RNA polymerase I formed larger clusters compared to untreated RNA polymerase I (Fig. 3).
This suggests that single-molecule imaging using N-STORM with cluster analysis is useful as a new "phase separation" evaluation system, as it allows quantification of the degree of assembly and disassembly of proteins.

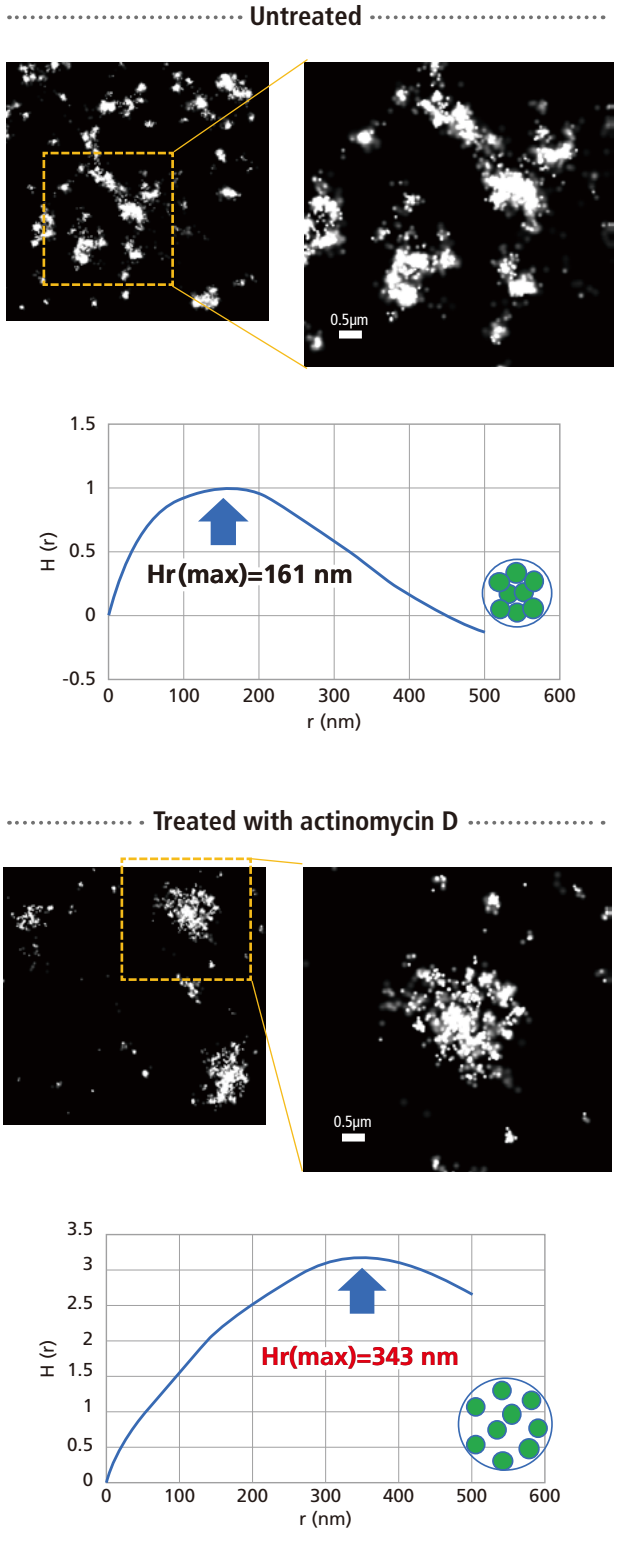



Figure 3. Cluster analysis was conducted for the ROI specified in the STORM image. The result showed that the size of a cluster formed by RNA polymerase I was 161 nm when untreated and 343 nm after actinomycin D treatment.
Data provided by Dr. Ryu-Suke Nozawa, The Cancer Institute of the Japanese Foundation for Cancer Research

## Benefits of N-STORM and contributions to research

- Observation using N-STORM enables acquisition of ultra-fine information that is not possible with conventional optical microscopes.
- In combination with cluster analysis, the degree of assembly of the target protein can be analyzed quantitatively at the single molecule level.
- Cluster analysis of the target protein at a single molecule level has been suggested as a useful new evaluation system for "phase separation."
- The series of operations from detection of the target to STORM image capture/analysis is organized into a flow that enables smooth execution.

STORM imaging is not only expected to be utilized as an evaluation system for "phase separation" of proteins or nucleic acids using cluster analysis, but also to be applied to visualization and structural analysis of chromatin using DNA-FISH (DNA Fluorescence *In Situ* Hybridization).

In addition, due to its multicolor imaging capability, N-STORM has the potential to be applied to analysis of chromatin changes accompanying stem cell differentiation and correlation analysis of factors involved in cell events such as transcription and DNA replication.

## Product Information

### N-STORM Super Resolution Microscope

The N-STORM utilizes a localization technique called STochastic Optical Reconstruction Microscopy (STORM) to achieve a resolution about ten times that of conventional light microscopes.
It allows imaging of the structure of cell organelles on a molecular level.
Lateral resolution: Approx. 20 nm
Axial resolution: Approx. 50 nm

### Cluster Analysis Function

Allows automatic analysis of the distribution patterns of fluorescent spots by specifying the target area with the ROI in the image captured by the super-resolution microscope. With quantitative evaluation added to image acquisition of nanoscale accuracy, the reliability of the information obtained from images is significantly increased.