ONI Application note

Cell phenotyping

Investigating nuclear pore complexes at the single-molecule level

Challenge

The segregation of genetic material in the nucleus, away from the rest of the cellular machinery is vital for the protection, accurate replication and transcription of DNA. This means that constant import and export of molecular machinery and genetic material is necessary for many cellular functions, the most important of which is the export of RNA for translation, facilitated by the nuclear pore complex[1] (NPC). The NPC permits the transfer of material between the nucleus and cytoplasm, and is composed of ~30 different proteins that make a large and complex structure. Recently, cryo-EM, with its sub-atomic resolution has revealed the arrangement of the nucleoporins (NUPs) that make up the NPC[2]. However, cryo-EM suffers from a lack of molecular specificity, which makes it difficult to resolve single NUPs.

An alternative is super-resolution fluorescence imaging, using techniques such as dSTORM and PALM that overcome the diffraction-limit of light, and allows for NPCs and specific NUPs to be investigated at a resolution of 20 nm. Using this approach, NUPs can be immunolabeled or genetically tagged with SNAP® or Halo-tags™ with extremely high specificity and imaged on the Nanoimager, providing greater insight than cryo-EM[2,3].

Summary

The Nanoimager platform allows visualization and quantification of protein complexes at the single-molecule level.

This type of research supports assessment and characterization of:

- Macromolecular protein structures, such as nuclear pores
- Structural changes in protein complexes that result in disorders
- 3D dimensional complexes

Results

NUP96 is one of the 15 different types of proteins that forms the scaffold for nuclear pores. It exhibits 8-fold symmetry and forms two rings, one in the cytoplasm and one in the nucleus. With the individual NUP96 proteins spaced 12 nm apart and forming a ring with a radius of ~100 nm it is impossible to resolve the structure using diffraction-limited microscopy. However, dSTORM on the Nanoimager can accurately size the NUP96 ring and resolve the 8 subunits that compose the ring (Figure 1). In this particular example, the diameter of the nuclear pore is ~120 nm and the central channel, labeled with WGA-Alexa Fluor® 555 is ~30 nm. This finding is in line with values reported in the literature from both super-resolution and cryo-EM measurements.

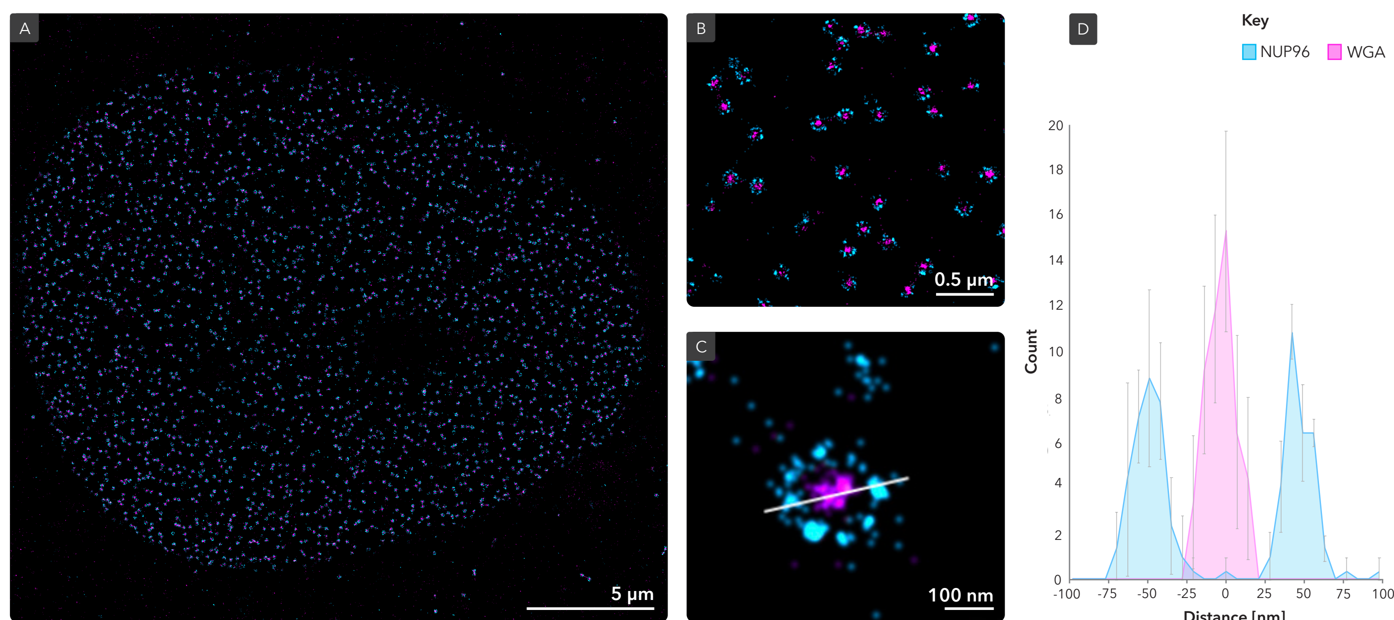


Figure 1 | dSTORM images of Cos7 cells genetically modified to express NUP96-SNAP-tag®. Cells were fixed and NUP96-SNAP tag® was labeled with BG-Alexa Fluor® 647 (cyan) with WGA-Alexa Fluor® 555 (purple). (D) Values are represented as means ± *S.D.*

@oniHQ in @ONI

ONI, Linacre House, Jordan Hill Road, Oxford, OX2 8TA, UK
ONI, ONI Inc. SPACES, 101 Jefferson Drive, Menlo Park, CA, 94025 USA

© 2020 ONI

t: +44 (0) 1865 951 820 | hi@oni.bio | www.oni.bio

Since the NPC is composed of an internal and external ring, many questions have arisen addressing potential structural variances between them, with recent evidence suggesting that differences in spatial configurations is important for mRNA export[4] (Figure 2). Gaining further insight into the intricate architecture can be achieved using the 3D imaging capabilities of the Nanoimager. When imaged using 3D dSTORM the curvature of the nucleus can be visualized, with the center of the nucleus closer to the coverslip (purple) and the edges curving away from the coverslip (red) (Figure 3). In addition, the two layers of NUP96 rings, one in the nucleus and one in the cytosol, can now be visualized, with the thickness of the nuclear pore along the z-direction ~300 nm.

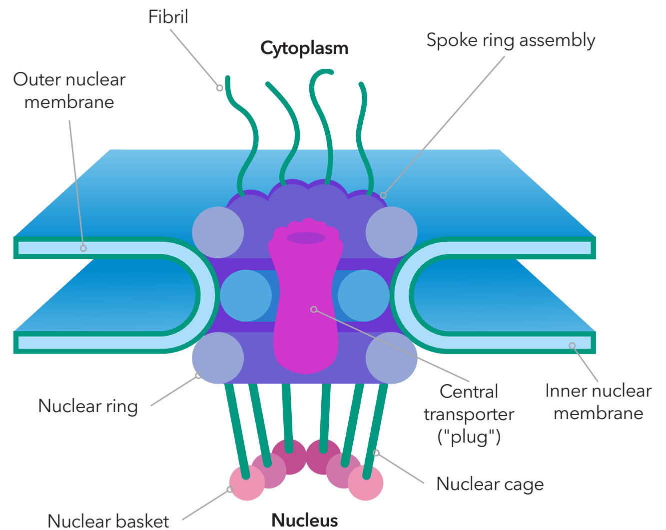


Figure 2 | Schematic representation of the NPC. The complex has a vertical symmetry, with a nuclear and cytoplasmic side. Structural differences between the two sides include fibrils that project outwards from the cytoplasmic spoke ring, and a nuclear basket within the nuclear side.

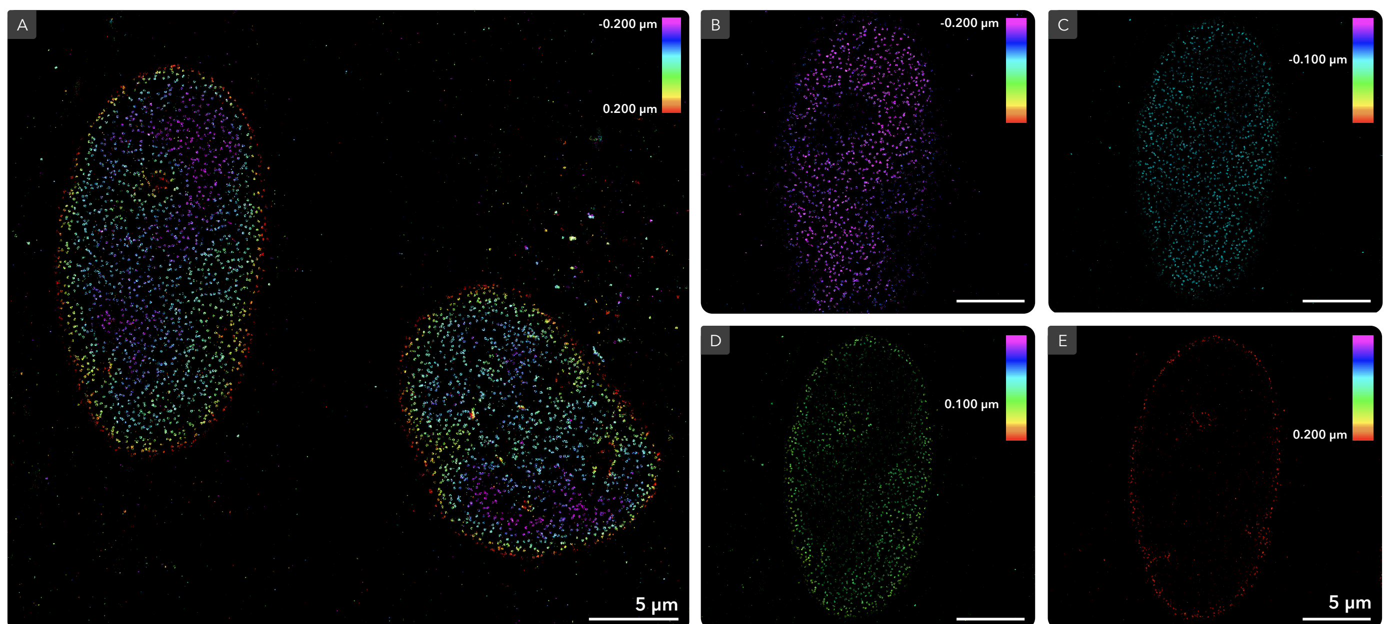
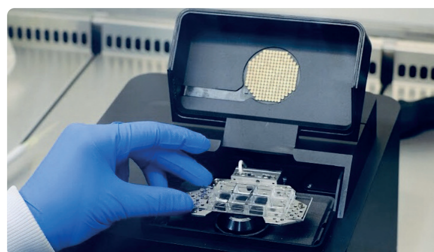


Figure 3 | A) 3D dSTORM images of Cos7 cells genetically modified to express NUP96-SNAP-tag®. Cells were fixed and NUP96-SNAP-tag® was labeled with BG-Alexa Fluor® 647 (cyan). (B-E) images of the same field of view, spaced at -200, -100, 100 and 200 nm in the z-plane.



Solution with the Nanoimager

The Nanoimager provides a comprehensive solution to characterize complex protein complexes and structures such as the NPC. With lateral resolution of 20 nm or better, axial resolution of 50 nm, nanoscale structures can be visualized and quantified.

To learn more about the microscope features and its different applications at ONI visit www.oni.bio

References

1. Thevathasan, J.V., Kahnwald, M., Cieśliński, K. et al. Nuclear pores as versatile reference standards for quantitative super resolution microscopy. Nat Methods 16, 1045–1053 (2019).
2. Von Appen, A., Kosinski, J., Sparks, L. et al. In situ structural analysis of the human nuclear pore complex. Nat 526, 140–143 (2015).
3. Löschberger A., van de Linde S., Dabauvalle M.C., et al. Super-resolution imaging visualizes the eightfold symmetry of gp210 proteins around the nuclear pore complex and resolves the central channel with nanometer resolution. J Cell Sci. 1;125 (2015).
4. Allegretti, M., Zimmerli, C.E., Rantos, V. et al. In-cell architecture of the nuclear pore and snapshots of its turnover. Nat 586, 796–800 (2020).

© 2020 ONI | t: +44 (0) 1865 951 820 | hi@oni.bio | www.oni.bio

ONI, Linacre House, Jordan Hill Road, Oxford, OX2 8TA, UK
ONI, ONI Inc. SPACES, 101 Jefferson Drive, Menlo Park, CA, 94025 USA