ONI Application note

Neuroscience

Super-resolution characterization of oligomeric tau species in aggregates associated with neurodegeneration

Based on publication by Gyparaki, M. T. *et al*. (2020) Tau forms oligomeric complexes on microtubules that are distinct from pathological oligomers in disease. BioRxiv.

Introduction

Tau is a highly abundant neuronal protein, functionally linked to microtubule stability and assembly. Under pathological conditions, tau has a propensity to aggregate and form neurofibrillary tangles (NFTs), which are hallmarks of neurodegenerative diseases including Alzheimer's disease (Figure 1). Evidence has suggested however, that soluble tau oligomers that precede the appearance of NFTs, may be the more toxic species contributing to cellular defects and cognitive abnormalities[1]. Therefore, the ability to visualize and characterize oligomeric tau species in a cell model of neurodegeneration could contribute to our understanding of the mechanisms underpinning pathogenic tau oligomerization.

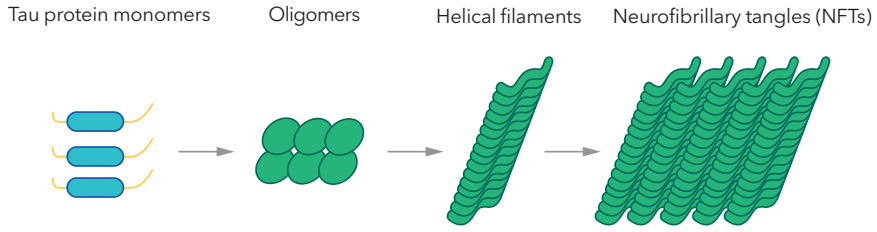

Figure 1 | Schematic representation of tau oligomerization. Adapted from Šimić G, *et al.* (2016)[2]

Summary

The Nanoimager allows for super-resolution imaging of protein aggregates and toxic oligomeric species in neurodegenerative disorders

SMLM techniques enable researchers to:

- Detect and characterize protein aggregates based on parameters such as size and shape
- Distinguish monomeric vs oligomeric protein species
- Investigate abnormalities in cellular organelles
- Accurately assess protein co-localization

As these small oligomeric tau complexes lie below the resolvable limit of conventional light microscopy techniques, it is challenging to accurately detect pathological changes in the nanoscale organization of tau. In this work, Gyparaki *et al.* used the Nanoimager to perform single-molecule localization microscopy (SMLM) and obtain high-resolution images of tau nano-clusters[3]. Subsequent analyses allowed them to determine the monomeric vs. oligomeric state of tau within these clusters, a method that would not be otherwise possible using conventional fluorescent imaging techniques.

Results

In engineered cell lines expressing either wild-type or mutant tau, and in hippocampal neurons, Gyparaki *et al.* labeled tau with Alexa Fluor® 647 and subsequently imaged cells by dSTORM, a super-resolution SMLM technique. The resulting images demonstrated that nano-clusters of tau protein existed under both physiological and disease conditions. By quantifying the number of localizations per cluster and applying a Distance Distribution Correction (DDC), it was possible to calculate the relative breakdown of tau monomers, dimers and trimers within the detected clusters.

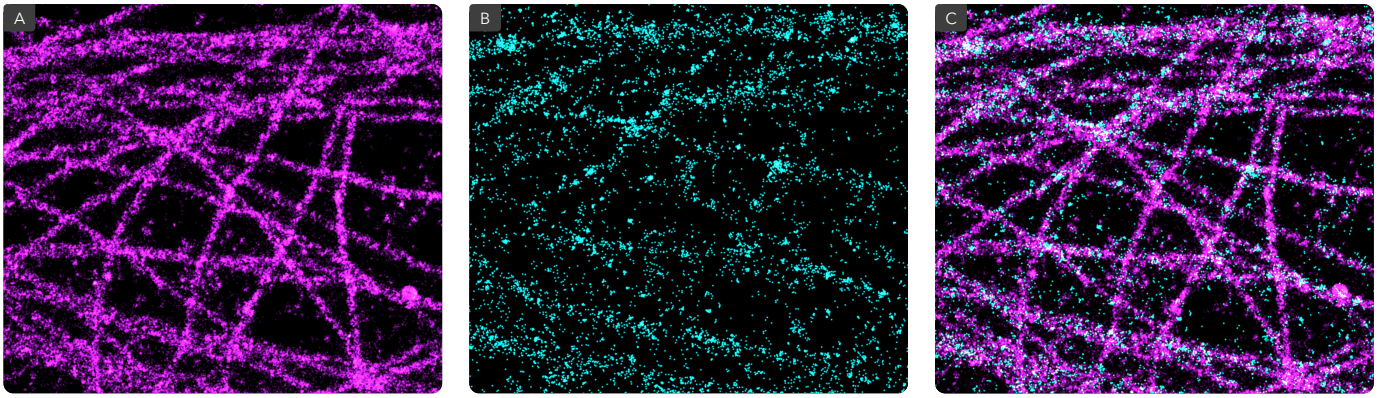

Figure 2 | Tau colocalizes with α-tubulin under physiological conditions. Two colour dSTORM imaging of α-tubulin (A) and total tau protein (B) showing a high degree of colocalization between the merged channels (C). Images adapted from data kindly provided by Gyparaki *et al*.

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

Next, two color SMLM imaging was performed to assess the percentage of tau protein that co-localized with microtubules or with a T22 antibody, that specifically labels oligomeric tau species. The results showed that tau is almost exclusively associated with microtubules and approximately 40% of tau clusters are comprised of dimeric/trimeric species under physiological conditions (Figure 2). In contrast, model cell lines for tau aggregation showed the presence of large cytosolic aggregates with only ~17% of tau co-localizing with the microtubules.

Finally, a robust classification of the diversity of tau aggregates was carried out by performing DBSCAN clustering and characterizing clusters based on parameters such as the size, shape and number of localizations per aggregate (Figure 3). This identified different classes of tau aggregates that may represent different stages of tau aggregation. Subsequent immunolabeling with an antibody against phosphorylated-tau allowed these classes of tau aggregates to be linked to different phosphorylation states.

These results clearly highlight the strengths of SMLM and the wealth of information that can be extracted by using these techniques. With diffraction-limited imaging, the resolution limit would have rendered it impossible to accurately quantify tau cluster number or assess true levels of co-localization with the microtubule network. In contrast, dSTORM imaging allowed for Gyparaki *et al.* to develop deep quantitative analyses, based on the localization data, to quantify proportions of monomeric and oligomeric tau species within the nano-clusters of tau.

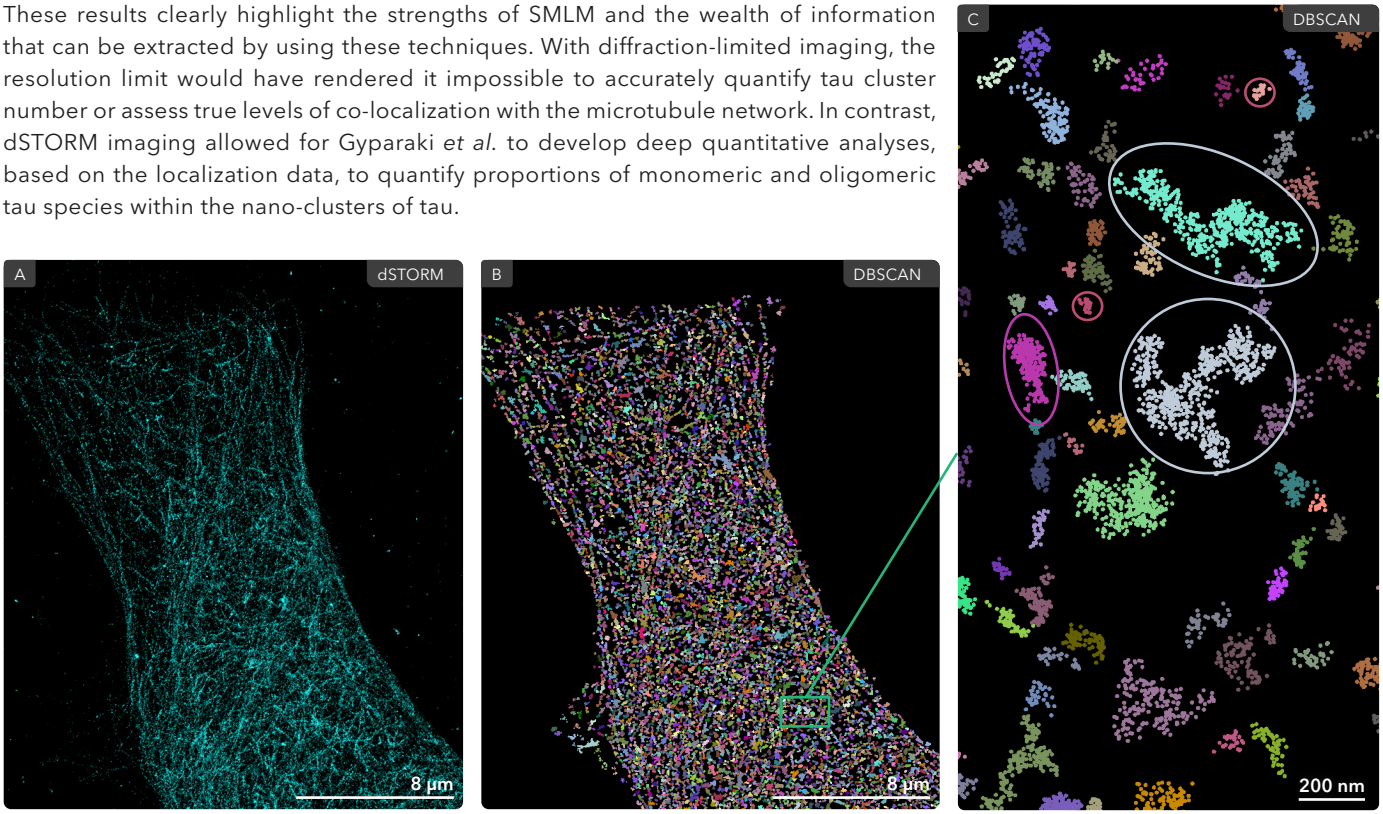

Figure 3 | Pathological tau forms discrete classes of aggregates. (A) dSTORM image of GFP-tau labelled with Alexa Fluor® 647 in a mutant tau cell line. (B) DBSCAN cluster analysis of SMLM image. (C) Zoomed area from boxed region of panel B showing tau nano-clusters (red circles), fibrillary structures (pink circle) and branched fibrils (grey circles). Images adapted from data kindly provided by Gyparaki *et al*.

Solution with the Nanoimager

The Nanoimager provides a comprehensive solution for high-resolution imaging and characterization of protein nano-clusters. The ability to visualize clusters with single-molecule sensitivity and probe their structural makeup via quantification of localization data provides insights into the role of aggregates in neuronal cell death. With four laser lines and dual-colour simultaneous imaging capabilities, accurate co-localization data can be obtained of protein aggregates with specific cell organelles to provide context on which cellular pathways may be perturbed in neurodegeneration.

References

1. Lasagna-Reeves, C. A. *et al*. (2011) Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. Mol. Neurodegener. 6.
2. Šimić G, *et al.* (2016) Tau protein hyperphosphorylation and aggregation in Alzheimer's disease and other tauopathies, and possible neuroprotective Strategies. Biomolecules. 6;6(1):6.
3. Gyparaki, M. T. *et al*. (2020) Tau forms oligomeric complexes on microtubules that are distinct from pathological oligomers in disease. BioRxiv.

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