

APPLICATION OF STED MICROSCOPY FOR NEUROFILAMENT STUDIES IN RE-DIFFERENTIATED HUMAN NEUROBLASTOMA

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Neurofilaments are major structural components of the neuronal cytoskeleton. By providing stability and flexibility to the axons, they play a crucial role in maintaining the neuronal network. Many neurodegenerative disorders like Alzheimer's Disease and Amyotrophic Lateral Sclerosis are related to incorrectly localized or assembled neurofilaments.

The axonal transport and assembly of neurofilament subunits is highly regulated by post-translational modifications (PTMs) such as phosphorylation and O-GlcNAcylation of Serine or Threonine residues. The phosphorylated neurofilament subunits are able to interact with each other while O-GlcNAcylation of the same sites prevents them from complex formation, thus enabling the subunits to be transported.

Using high resolution STED microscopy we could show the reciprocal relationship between these two modifications by using specific antibodies against the phosphorylated/non-phosphorylated epitopes and against the O-GlcNAcylated epitopes, respectively, of the middle and heavy subunit of neurofilaments. We could also observe a direct influence of a reduced cellular glucose uptake on the neurofilament's PTM state, causing neurodegeneration. Since post-translational modifications on neurofilaments take place on a scale below 50 nm, conventional light microscopy cannot be used to study them.

Therefore, we employed superresolution STED microscopy [1] to be able to image the changes in the structure of the neurofilaments. The employed custom-built instrument is designed around a single supercontinuum laser source and provides a spatial resolution down to ~20 nm.

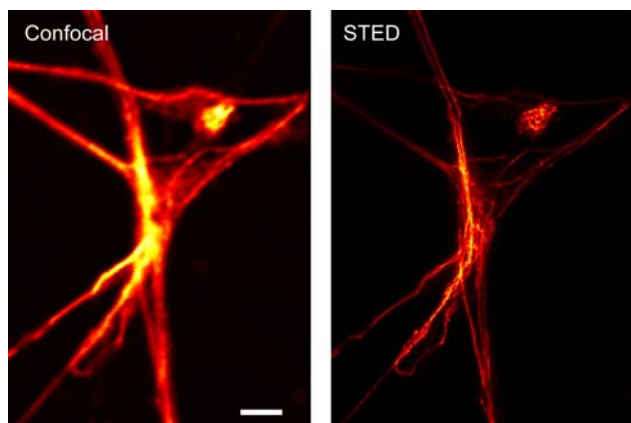


Figure: O-GlcNAcylated epitope of NFM in differentiated SH-SY5Y cells
Scale bar 2 μ m.

[1] D. Wildanger, E. Rittweger, L. Kastrup, S. W. Hell, "STED microscopy with a supercontinuum laser source", *Opt. Expr.* 16 (13), 9614 - 9621 (2008)