

## Challenges for correlating STED and synchrotron XRF nano-imaging to explore metal functions in synapses

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High spatial resolution correlative imaging approaches are needed to understand structure-function relationships in cell biology. These correlative approaches are particularly challenging for the study of biologically active metals in synapses due to (i) the labile binding of these elements, (ii) the nanoscale size of synaptic structures; (iii) the low concentrations of these elements. We correlated stimulated emission depletion (STED) microscopy of proteins and synchrotron X-ray fluorescence (XRF) imaging of metals, both performed at 40 nm spatial resolution, on primary rat hippocampal neurons. This correlative approach revealed the nanoscale co-localization of zinc and tubulin in dendrites, and the co-segregation of copper and F-actin in postsynaptic compartments. These results indicate new functions for zinc and copper in modulating the morphology of the neuronal cytoskeleton, a mechanism associated with synaptic structural plasticity. We will present and discuss the methodological workflow for correlative STED and synchrotron XRF imaging.

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