Label free, time-resolved, multisite observation of MT (de)polymerization in axons of mammalian neurons with wide field second harmonic microscopy

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Microtubules (MTs) are polar fibrillary structures that constitute one of the principal components of the cytoskeletal network of neuronal cells. They form a dynamic polarized structure and play an essential role during cell motility, morphogenesis or during the construction of cytoplasmic structures. Thus, to understand a variety of cellular processes knowledge of the regulation of MT dynamics is important, and a variety of drugs are available that can assist with this by interrupting the MT growth process. We used wide-field
Second Harmonic (SH) microscopy isa non-invasive label-free optical technique that can image axonal MTs in vitro [Ref to a dombeck paper]. The contrast of an SH microscope is determined by the presence, density and spatial organization of non-centrosymmetrical molecular structures, in this case MTs. A dense bundle of MTs with uniform organization will generate a strong SH signal whereas a non-uniform organization or less dense uniform bundle will have a faint SH signal.
Here, we perform high throughput wide field SH microscopy on MTs in living neurons to map drug induced spatio-temporal multisite fluctuations in the SH intensity. We used Nocodazole an anti-MT reversible drug that is used in neuro-degeneration research. When applied at nanomolar concentrations, it reduces growth and creates MT instability. Using our
Method, we mapped the dynamic instability and regrowth of MTs in axons of mammalian neurons that were kept 10 days in vitro. From the spatiotemporal intensity changes that originate from the MT network, we find that Nocodazole acts locally and heterogeneously, on the proximal part of the axon.