

## **Characterisation of the molecular determinants that allow the second harmonic generation signal of microtubules**

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Microtubules (MT) are a main component of the cell's cytoskeleton and are crucial for healthy cell functioning. Apart from providing a robust structure to a wide variety of cell morphologies, MT are involved in several cellular processes such as transport, motility and mitosis. Current imaging methods to study MT in living cells require fluorescent labelling of tubulin, interfering with intracellular processes. Second harmonic (SH) imaging microscopy (SHIM) is a label-free nonlinear optical technique that provides detailed information on the organisation and orientation of large biomolecules such as MT. The use of SHIM of MT is however limited as many elements contributing to the signal generation remain unclear. In this study, we aim to clarify these main determinants. We show that apart from MT polarity, MT number and organisation are important factors influencing the signal intensity. These are not sufficient to fully characterize the signal as they for instance do not clarify loss of the SH signal upon fixation. Our final results show that the SH signal mainly originates from the GTP-bound tubulin dimer conformation, a stable conformation found at the MT stabilizing cap that prevents depolymerisation. The SH signal positively correlates with GTP-tubulin staining intensities and increased intensities are measured in cells treated with paclitaxel, a MT stabilising drug that acts by changing the tubulin dimer conformation towards the GTP-bound state. Fixation of the MT network induces a conformational change in the dimer which explains the loss of the signal in fixed cells or tissue. Interestingly, axonal projections do not only contain GTP-bound tubulin at the stabilizing cap of the MT but also display multiple rescue sites consisting of GTP-bound tubulin along the lattice. The combination of these rescue sites along the densely packed, highly organised and polarized MT bundles in axons make the use of SHIM a powerful tool in neuroscience to gain insight in the axon's MT network.