Hippocampal sclerosis (HS) is observed in 65% of patients suffering from temporal lobe epilepsy. It is characterized by profound cytological changes and altered tissue architecture. Those include segmental loss of pyramidal neurons, abnormalities of the dentate gyrus granule cell layer and reactive gliosis. The thickness of the hippocampal layers is altered and the hippocampal volume is reduced. Multiphoton microscopy enables the visualization of tissue properties and structure without any labeling or tissue preparation. Therefore, we investigated the ability of this technique to detect pathological changes in human hippocampal brain tissue. Human brain tissue samples of hippocampus sclerosis (n=14) were obtained during routine surgeries for treatment of epilepsy. Multiphoton microscopy was used to investigate fresh, bulk samples as well as tissue cryosections. Second harmonic generation (SHG) addressing collagen, two photon excited fluorescence (TPEF) visualizing endogenous fluorophores and coherent anti-Stokes Raman scattering (CARS), tuned to probe C-H vibrations at 2850 cm⁻¹ and visualizing mainly the tissue lipid content, were combined in multimodal images. For reference standard H&E and immunohistochemistry were performed. Spectroscopy mapping was performed as additional biochemical reference to prove the nature of tissue structures.

Multimodal multiphoton microscopy displayed the hippocampal layering and micromorphological details in both bulk samples and cryosections: CARS identified white and gray matter layers and the high lateral resolution of the technique allowed the assessment of axonal structures. SHG revealed the size and location of blood vessels based on adventitial collagen. Additionally, SHG-active corpora amylacea (CA) were found in the majority of samples with varying densities up to 2500/ mm². They were predominantly located in close relationship to blood vessels and in areas with marked neuronal loss. Pyramidal neuronal cell bodies were characterized by intense cytoplasmatic endogenous TPEF that might be assigned to intracellular accumulation of lipofuscin. The fluorescence of granular and non-neuronal cells was less pronounced. The assessment of the location of pyramidal cell loss allowed determining the type of HS.

We present an optical approach that reveals the main pathological aspects of HS on native tissue without the application of any labels or staining. Most importantly, hippocampal layering, pyramidal cells loss and CA indicative of sclerosis can be identified. In conclusion, multimodal multiphoton microscopy holds prospect for in vivo assessment of histopathological changes of the hippocampal formation in animal models or during epilepsy surgery. The intraoperative recognition of the subtype and extent of HS may lead to a refinement of the surgical approach to improve surgical outcome.