# Mind the gap: Super-resolution imaging of the extracellular space of the brain

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The advent of super-resolution microscopy has created unprecedented opportunities to study the anatomical complexity and structural mechanisms that underlie brain physiology and animal behavior. At the neuro-functional level, the brain is dominated by anatomical structures whose nanoscale dimensions critically influence their cardinal biophysical properties. I will present our recent methodological advances to improve the visualization of 1) the nanoscale morphology and turnover of dendritic spines in the hippocampus in vivo and 2) the topology and dynamics of the extracellular space of the brain.

We established chronic *in vivo* super-resolution imaging of dendritic spines in the hippocampus, based on an upright 2P-STED microscope equipped with a long working distance objective and 'hippocampal window' to reach this deeply embedded structure. We measured spine density on pyramidal neurons in the CA1 area and determined spine turnover by repetitive imaging. Spine density was two times higher than reported by conventional 2P microscopy, and around 40% of all spines turned over within 4 days, indicating a high level of structural remodeling.

We combined 3D-STED microscopy and fluorescent labeling of the extracellular fluid to develop super-resolution shadow imaging (SUSHI) of brain ECS in living brain slices. SUSHI enables quantitative analysis of ECS structure and produces sharp negative images of all cellular structures, providing an unbiased view of unlabeled brain cells with respect to their anatomical context in a live tissue setting. I will also present ongoing progress to apply SUSHI to the intact brain in vivo.

**References**

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# Biosketch

Valentin Nägerl has been a professor of neuroscience and bio-imaging at the University of Bordeaux since 2009. He leads a team of physicists and biologists who focus on the structural mechanisms of neural plasticity, developing and applying novel super-resolution microscopy approaches.

He studied physics in Göttingen and obtained his PhD in neuroscience with Istvan Mody at UCLA in 2000. He worked as a postdoc with Tobias Bonhoeffer at the Max Planck Institute of Neurobiology in Munich, received his habilitation under Arthur Konnerth at the Technical University of Munich and was also trained by the Nobel laureate Stefan W. Hell at the Max Planck Institute for Biophysical Chemistry.

Dr. Nägerl received the 'Equipe FRM' award (2016), became a member of the 'Institut Universitaire de France' (2017) and won the 'Great Advances in Biology' prize from the French Academy of Sciences (2018).