SUPER-RESOLUTION IMAGING OF THE INTERSTITIAL FLUID TO REVEAL THE NANO-ANATOMICAL ORGANIZATION OF LIVE BRAIN TISSUE

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All brain cells are surrounded by a narrow extracellular space (ECS), which is filled with interstitial fluid and the extracellular matrix. The ECS is a reticular structure that forms a reservoir for extracellular ions and corridor for nutrients from the blood stream. It is critical for brain homeostasis and metabolite clearance and serves as a communication channel for extrasynaptic volume transmission. Because its spatial structure is extremely dense and convoluted, the ECS has so far defied all attempts at visualization by conventional light microscopy, which makes it appear like a featureless and homogenous mass.

Here, we present a method to directly visualize the ECS and cellular structures in living brain tissue. It is based on a combination of 3D-STED microscopy and labeling of the interstitial fluid with a freely diffusible and membrane-impermeable fluorescent dye. This new approach, termed 'super-resolution shadow imaging' (SUSHI), allows visualization of dense biological tissue at sub-micron resolution and comes with minimal photobleaching and phototoxicity as inherent benefits. It hinges on the very high resolution of 3D-STED microscopy, which undercuts the volume resolution of 2-photon microscopy by about three orders of magnitude, breaking the 1 attoliter barrier.

We provide proof-of-concept of this method by applying it to living organotypic hippocampal brain slices. It yields strikingly detailed and rich images of the complex structure of the fluorescently labeled ECS, revealing the anatomical organization of the tissue and all its resident cellular structures in sharp relief. SUSHI provides quantitative data on sub-diffraction structures like axon shafts, spine necks and interstitial spaces and lends itself to imaging cell motility, revealing the physical interactions between migrating cells and the surrounding tissue. By adding a second color channel, positively labeled cells can be imaged in the context of their neuropil, allowing the anatomical identification of synaptic connections.

In summary, SUSHI represents a new paradigm for nanoscale imaging of dense biological tissue, which complements and extends traditional approaches based on intracellular labeling, potentially bridging the gap between brain connectomics approaches based on serial section electron microscopy and MRI-based diffusion-tensor imaging. As a versatile fluorescence imaging technique, it opens up many experimental opportunities to study the structure and mechanisms of brain ECS.