IMAGING

Illuminating the brain's dark matter

SUSHI is a new method for imaging the extracellular space with high resolution in living brain tissue to reveal insights into the structure and dynamics of the extracellular space.

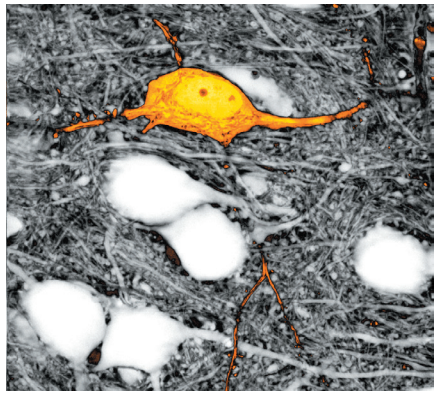
The extracellular space (ECS) is an important but often neglected part of tissues. It surrounds, for example, all brain cells and comprises extracellular fluid and molecules of the extracellular matrix. Although the ECS is important for brain homeostasis and clearance of brain metabolites, surprisingly little is known about its structure and dynamics. It is like the "dark matter of the brain," as Valentin Nägerl from the University of Bordeaux describes it. This gap in knowledge is due partly to stronger interest in the cell types that build the brain, but also to a lack of appropriate techniques. Because the space between cells is small (100–300 nm for most structures), the ECS cannot be imaged with conventional light microscopy approaches. Therefore, most data on the ECS have been obtained either with biophysical methods that provide only average numbers on the diffusive properties of the ECS or with electron microscopy, which involves tissue fixation, is prone to artifacts, and cannot be used to measure dynamic changes of the ECS in living brain tissue.

To analyze the dynamics of the ECS in living brain slices, Nägerl and his colleagues have developed SUSHI (super-resolution shadow imaging). The researchers discovered that they could label the ECS with a common fluorescent dye by simply perfusing organotypic brain slices with artificial cerebrospinal fluid in which the fluorescent dye was dissolved. The authors tested the dyes Alexa Fluor 488 and calcein, and both exclusively labeled the ECS when applied as described above. This resulted in brightly labeled ECS, while the cells remained dark, thereby providing a strong contrast between ECS and cells.

To image the ECS, the authors used the super-resolution technique 3D stimulated emission depletion (STED) microscopy. After optimizing the optics of their microscope, the authors were able to achieve a resolution in the range of tens of nanometers, which corresponds to a volume resolution of less than one attoliter. Unfortunately, STED microscopy requires high laser powers, which can cause bleaching of fluorophores and induce phototoxicity that harms the cells. SUSHI, however, is "completely immune to bleaching, because you have perpetual replenishment from the infinite pool of dye molecules in the bath," explains Nägerl. Another distinct advantage of having the dye only in the ECS and not within cells is that phototoxic compounds are diluted away immediately. This makes it possible to acquire hundreds of images with STED microscopy without damaging the cells.

The researchers demonstrated SUSHI's capability by measuring the reversible increase in ECS volume after a hyperosmotic challenge. In contrast, epileptiform discharges triggered by blockade of inhibitory GABA receptors caused the ECS volume to shrink. SUSHI is also sufficiently sensitive to detect local events, such as changes in ECS volume caused by the activity of a single synapse.

Another important aspect of SUSHI is that the ECS is the negative imprint of all cellular structures. This means that SUSHI is also a tool for imaging cellular structures, in an inverse way. And in contrast to labeling of cell populations with dyes or fluorescent proteins, SUSHI makes every single cell visible, rather than only a selected subpopulation of cells. "It is an unbiased way of imaging," says Nägerl, because the user does not have to choose the cell of interest before the experiment. The researchers used this approach to watch a putative microglial cell migrate to a lesion site. They were able to observe not only the migrating cell itself, but also the complete environment the cell had to squeeze through.



3D STED microscopy image of neurons in an organotypic brain slice acquired with SUSHI. Adapted with permission from Tønnesen *et al.* (2018).

SUSHI can also be combined with positive labeling of cell types, as the researchers demonstrated by, for example, using YFP-labeled neurons to visualize dendritic spines in combination with unlabeled presynaptic boutons.

There are three major perspectives for SUSHI. One is its application *in vivo* to analyze the ECS in living animals. Second, with further improvements in resolution and depth penetration, SUSHI will allow live-cell connectomics in the brain "with the benefit that it is live tissue and free of fixation artifacts and you can do longitudinal [studies]," explains Nägerl. Finally, it will be interesting to apply SUSHI to other organs as well and, for example, learn more about ECS changes in cancer tissues.

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RESEARCH PAPERS

Tønnesen, J. *et al.* Super-resolution imaging of the extracellular space in living brain tissue. *Cell* **172**, 1108–1121 (2018).