Correlative 3D structured illumination and single molecule localization microscopy of fenestrations in liver sinusoidal endothelial cells

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Liver sinusoidal endothelial cells (LSEC) are an important class of endothelial cells facilitating the translocation of lipoproteins and small molecules between the liver and blood. A number of clinical conditions, especially metabolic and aging-related disorders, are implicated by improper function of LSECs. The disruption of these cells due to e.g. paracetamol poisoning underlines their biomedical relevance. Their loss leads to acute liver failure and death.

Despite their importance, research into these cells is limited because the primary ultrastructures involved in their function are transcellular pores, called fenestrations, with diameters in a size range between 50–200 nm, i.e. well below the optical diffraction limit. Here, we show that we are able to resolve fenestrations with a spatial resolution of about 20 nm by direct stochastic optical reconstruction microscopy (dSTORM).

We compare the higher degree of structural detail that dSTORM provides to results obtained by 3D structured illumination microscopy (3D-SIM), where 3D-SIM and dSTORM was performed at the same microscope setup. Here, 3D-SIM is used on multi-color stained LSECs to rapidly acquire an overview of a large sample areas with approx. 100 nm resolution. Subsequently locations of interest can be revisited and imaged with approx. 20 nm resolution by dSTORM.

We show how a combination of the best of two superresolution microscopy techniques helps to maximize the information obtained from imaging these physiologically important cells in vitro.


Figure 1: Conventional widefield microscopy image (A) compared to the corresponding dSTORM reconstruction (B). [1]