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## 3D reconstruction of neural networks in human brain organoids

Worldwide, there are estimated to be fifty million people with neurodegenerative diseases. This number is expected to double every twenty years as the population ages. The powerful combination of light-based techniques and human in vitro models has recently opened unprecedented opportunities for studying disease pathogenesis and performing drug screening. Organoids are 3D in vitro models derived from human induced pluripotent stem cells (hiPSCs) that self-organize into complex and functional mini organs, including specific brain regions, such as cortex and midbrain. In this work, we performed functional and morphological investigation in midbrain organoids (MBOs) using biophysical and molecular biology techniques. As for the morphological study of MBOs, we explored the possibility to fully reconstruct in 3D the neural network of a whole organoid (having 2-3 mm diameter) by combining advanced optical microscopy and clarification techniques to overcome the imaging limitations of confocal and 2-photon microscopes. The breakthrough of clarification techniques is dehydrating the sample and refilling it with a solvent that matches the refraction index of the imaging medium. The nearly full transparency of the clarified sample permits to reconstruct it up to several mm or even cm depth. Light sheet microscopy was used to visualize MBOs, allowing faster acquisition of large fields of view at sub-cellular resolution for final 3D reconstruction.

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