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Imaging of 3D neuronal cell cultures in hydrogels under iSPIM with an electrically tunable lens

Three-dimensional in vitro neuronal networks could give better insights to the functioning of the brain, mimicking extracellular conditions more accurately than two-dimensional ones. Moreover, the development of genetically encoded calcium indicators together with fluorescence microscopy has facilitated the recording of neuronal activity as sharp calcium changes upon neuronal firing. Nonetheless, imaging such fast events, in three-dimensional cultures and at high resolution, requires instrumentation capable of acquiring wide fields of view along large depths and high frame rates.
In our study we image 3D neurons from rat primary cell cultures placed in hydrogels. We use an inverted SPIM configuration, allowing for the imaging of samples on standard petri dishes. In our iSPIM, the sample remains static while we scan the light sheet along it with a galvanometric mirror. By including an electrically tunable lens in the detection path of the microscope, we rapidly refocus on the illuminated section of the sample, avoiding the need to displace the detection objective and reducing vibrations on the sample. We present our observations of these three-dimensional cultures under our optical setup.

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