Title: Enabling Next Generation Functional Characterization of Human Neural Organoids

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Human organs, such as the brain, are challenging to study being inaccessible to direct optical observation and experimental manipulation. However, three-dimensional (3D) culture techniques enabled novel study of tissue- or stem cell-derived organoids, spheroids, and organotypic cultures resembling cell type diversity, developmental processes and function of mammalian organs.

The ability to measure the electrical activity of a self-organizing *in vitro* cellular model in real time, live and label-free can provide much needed insights into the complexity of its functional structure. High-density microelectrode arrays (HD-MEAs) provide unprecedented means for non-invasive *in-vitro* electrophysiological recordings, and can be used to acquire real time measurements from any cell with electrogenic properties, such as iPSC-derived neurons, retina and brain organoids, as well as tissue slices.

In this study, a HD-MEA platform featuring 26,400 electrodes per well (MaxWell Biosystems AG, Switzerland) was used to capture fast propagating extracellular action potentials in neural organoids at different scales, ranging from network through single-neuron with high spatio-temporal resolution and low noise. Metrics, such as firing rate, spike amplitude, network burst profile as well as synchronicity, were extrapolated in a parallelized manner. Furthermore, at the subcellular level, we tracked the propagating action potentials across axonal branches to compute and characterize the conduction velocity across multiple neurons within a network.

Our HD-MEA platforms and the extracted parameters highlighted in this study provide a powerful user-friendly approach for identifying and isolating active areas of a 3D culture in acute recordings or in longitudinal studies allowing long-term disease modelling and/or compound testing *in-vitro*.

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