Long-term monitoring of oxygen tension in living cerebral organoids using frequency domain fluorescence lifetime imaging microscopy (FD-FLIM)

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Oxygen tension plays an important role in manipulation of neurogenesis during embryonic development. However, monitoring the oxygen tension during the neurogenesis has been seldom studied due to technical challenges. 3D cerebral organoids have been used to mimic the brain tissue-like structure *in vitro* (Lancaster et al., 2013); therefore, they are exploited in this research as a model system. Oxygen sensitive fluorescence beads (CPOx) are used as the oxygen sensors to be embedded into the organoids. For oxygen tension quantification within the organoids, fluorescence lifetimes of the beads are measure using a widefield frequency domain fluorescence lifetime imaging microscopy (FD-FLIM) setup. The FD-FLIM measurement provides desired advantages including less photobleaching and accurate quantification due to its widefield capability and insensitivity to environmental noise. The cerebral organoid with embedded CPOx beads is shown in Figure 1. In the experiments, the fluorescence lifetimes of the CPOx beads within the living organoids are monitored during culture from 2 to 9 weeks and confocal images are also captured for bead localization. The experimental results have demonstrated that the newly developed method in this research enables characterization of long-term oxygen tension in the cerebral organoids for the neurogenesis studies.

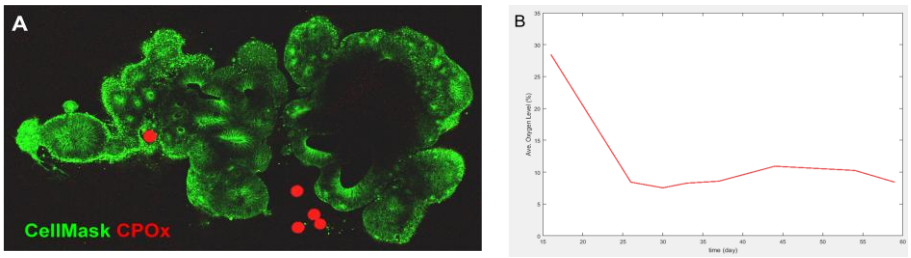

Figure 1. (A) Confocal image of cerebral organoids stained with CellMask (green) for localization of the oxygen sensitive CPOx (red) beads after 26-day culture. Scale bar: 100 mm. (B) Oxygen tension variation during the organoid culture.

Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., and Knoblich, J.A. (2013). Cerebral organoids model human brain development and microcephaly. Nature *501*, 373-379