Title: Oxygen imaging inside 3D cell constructs

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Keywords:
biomedical engineering, cell and tissue models, 3D imaging, oxygen concentration, engineered heart tissues

Abstract
Within the last years, cell research and *in vitro* disease modelling have transitioned towards 3D systems from 2D cell cultures to create models which more accurately mimic physiological microenvironments, particularly with respect to locally varying parameters such as oxygen partial pressure (pO2). This increasing interest in 3D models has caused a demand to develop cell-friendly imaging technologies capable of imaging cellular structures and measuring pO2 in 3D. To address this need, we have developed a novel 3D luminescence microscopy platform combining luminescence lifetime microscopy (LLIM) and selective plane illumination microscopy (SPIM). The integration of LLIM with SPIM allows long-term luminescence lifetime imaging of 3D samples with minimal photobleaching and phototoxicity due to fast imaging and reduced light exposure. The system comprises three laser lines, which allows combining standard cellular fluorescence imaging with 3D pO2 measurements. In our preliminary experiments, pO2-sensing CPOx-beads were embedded within engineered heart tissues (EHTs), revealing a decreasing oxygen gradient from the tissue surface to its interior. Furthermore, large high cell-density EHTs typically displayed oxygen conditions nearing hypoxia, whereas oxygen levels in smaller EHTs were closer to ambient level. By imaging EHTs obtained from an mScarlet-expressing reporter cell line in our experiment, we successfully combined 3D fluorescence imaging with spatial pO2 measurement. These results indicate the sensitivity of the developed SPIM-LLIM system for 3D pO2 measurements and demonstrate its broad potential for many 3D cell culture applications where localized pO2 information is essential.