Characterization of 3D oxygen tension profiles within hydrogels using oxygen sensitive fluorescence microbeads and widefield frequency domain fluorescence lifetime imaging microscopy (FD-FLIM)

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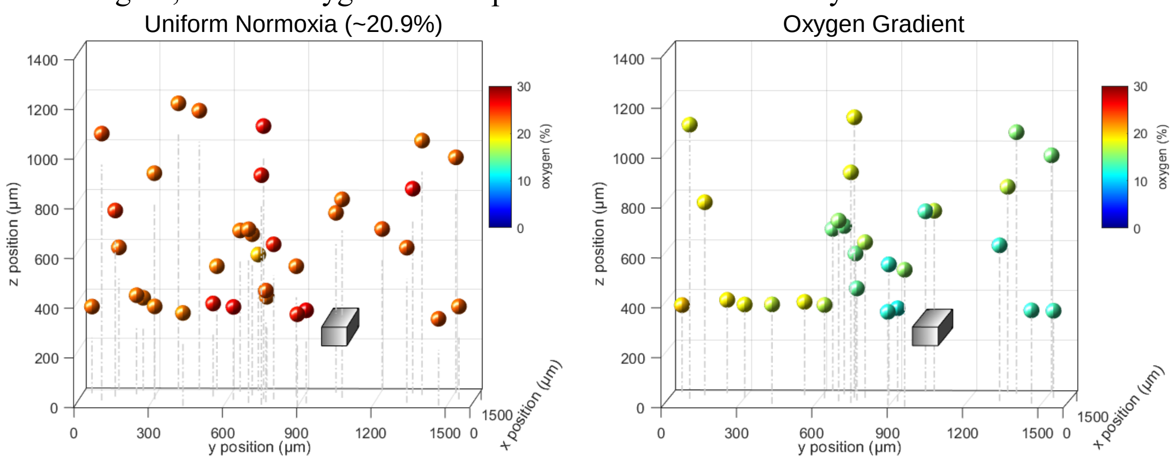
To mimic physiological conditions, it is desired to culture cells in 3D hydrogels under various oxygen gradients. Therefore, accurate characterization of the oxygen tension profiles in 3D is important. For oxygen tension measurement, oxygen sensitive fluorescence probes have been broadly used [1]. Oxygen, as a quencher, shortens the lifetime of the probe and thus decreases the fluorescence intensity. For 2D culture, measuring the fluorescence intensity gives adequate estimations of oxygen tensions. However, photobleaching caused by prolonged exposure and intensity variation resulted from different optical paths make 3D intensity-based oxygen tension measurement unreliable. In contrast, fluorescence lifetime-based oxygen tension characterization schemes overcome aforementioned challenges and thus are suitable for oxygen tension characterization in 3D [2]. In this research, we characterize the 3D oxygen tension profiles in hydrogels by embedding oxygen sensitive fluorescence microbeads and measure their lifetimes using widefield frequency domain fluorescence lifetime imaging microscopy (FD-FLIM) at different vertical positions. The oxygen tension profile within the hydrogels is controlled by a microfluidic device placing underneath the hydrogel using a spatially confined chemical reaction method [1]. To precisely and automatically determine the bead positions in 3D, we wrote a Matlab program to segment binary stacks into individual objects by k-mean clustering. The centroids of segmented objects then served as good estimation of bead positions. The 3D oxygen profile shows the perfusion of chemical reactants for oxygen scavenging in the channel decreases the local oxygen concentration around the channel region, and the oxygen tension profiles can be successfully characterized.



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