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Title: 3D Microrheology for Microstructural Analysis of Extracellular Matrix Based 3D Hydrogels

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Abstract

Spatial mapping of hydrogel microstructure enables discoveries on the formation of complex biomaterials and cell-hydrogel interactions. Passive microrheology with multiple particle tracking (MPT) using tracer particles enables hydrogel characterization in high spatial resolution and in 3D, enabling analysis of viscoelastic micromechanics and porosity. In MPT, a hydrogel is filled with fluorescence particles and imaged as time series, from which Brownian motion of the particles is tracked. These motion patterns reveal rigid, viscoelastic, and fluid-like areas in the hydrogel.

We used Nikon Eclipse Ti2 microscope, to image hydrogels filled with 200 nm diameter fluorescent particles (Bangs Laboratories). In our proof-of-concept research, we used Geltrex™ and collagen type I from rat-tail, with human fibroblasts in 3D. For data analysis and visualization, we developed MuRheo software with Unreal Engine 5. MuRheo does particle detection and tracking based on optimized nearest neighbour algorithm. When 3D data is used, different levels are processed in parallel for faster computations. From amounts of similarly moving particles, we get a statistical overview of hydrogel microstructure. From 3D mapping we can detect and study more closely areas where particles move freely, viscoelastic areas, and rigid areas, defined by adjustable mean squared displacement (MSD) thresholds. Our data shows that fibroblasts modify their environment for example by degrading material, and pore volume can increase even 20% from total volume during 4-day culture in same locations. We have also observed that pore volumes of Geltrex™ samples can be over 10% higher than in collagen I samples at d0.