Electronic Supplementary Information (ESI)

Quantitative Temporal Interrogation in 3D of Bioengineered Human Cartilage using Multimodal Label-free Imaging

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Figure S1 Schematic of the custom-designed 2D imaging setup. This custom-designed setup was used for second harmonic generation (SHG) and two-photon excited autofluorescence (TPEAF) in 2D.



Figure S2 Schematic of the custom-designed multimodal imaging setup. This custom-designed image setup was used for 3D imaging with coherent anti-Stokes Raman scattering (CARS), second harmonic generation (SHG) and two-photon excited autofluorescence (TPEAF).



Figure S3 Raman spectrum of human fetal skeletal cells cultured in chondrogenic media for 21 days. Raman spectrum was obtained with a 633 nm laser (6 mW). The prominent C–H stretching mode peak at 2845 cm⁻¹ was targeted to image lipids using coherent anti-Stokes Raman scattering (CARS) microscopy.

Videos S1-S4: Temporal interrogation of 3D bioengineered cartilage using multimodal label-free imaging. Three label-free imaging modalities were used simultaneously in human fetal skeletal cells cultured in chondrogenic media for 4 (Video S1), 7 (Video S2), 14 (Video S3), and 21 (Video S4) days. Second harmonic generation (SHG) identifies collagen fibres (green), coherent anti-Stokes Raman scattering (CARS) detects lipid droplets within the cartilage pellet (red), and two-photon excited autofluorescence (TPEAF) distinguishes the chondrocytes using their intrinsic autofluorescence (blue). The videos show the acquired images as 3D volume rotations.