

## Author's Reply to the Review Report (Reviewer 2)

We are immensely grateful to the reviewer for their time in reviewing our work which we believe has resulted in an improved manuscript. We respond to each of their comments below and have tracked changes throughout the revised manuscript highlighted in green.

**Point 1:** Manual registration and ground-truth measurements. Were those measurements performed by single operator? How many repeats were done? Authors should address limitations of such manual measurements (intra-/inter-operator variance). Additionally, given the rigid alignment prior to DVC computation was done also manually over a single slice, that likely cause the high strain gradient from bottom to top of the specimen. Authors should discuss why a rigid registration over the entire volume was not performed and how that would overcome some of the encountered issues in the 3D strain distribution.

**Response 1:** Yes, both the manual registration and ground-truth measurements were performed by a single operator. In regards to manual registration there were  $n = 5$  repeat measurements per sample and for ground-truth measurements there were  $n = 9$  repeat measurements per sample with presented results averaged from these measurements. We have added clarification to the methods section for both the number of operators ( $n = 1$ ) and number of repeats ( $n = 5$  or  $n = 9$ ). For registration this now appears on lines 160 and 162-163, and for the ground-truth measurements on line 356. Using a single operator negated inter-operator variance but introduced potential intra-operator error and potential bias. Further work to explore the both intra and inter-operator errors and associated repeatability and reproducibility metrics for both measurements is encouraged. Text addressing the limitations of a single operator performing measurements has been added to the discussion on lines 815-819 with the addition of a reference to research on intra-/inter-operator variance and the repeatability and reproducibility of measurements for imaging (Hudson et al.).

An oversight missing from the text previously was that between the manual registration and DVC calculation, the optional rigid body movement correction module in DaVis was utilised to provide volumetric registration. Additionally, post-DVC the “subtract vector at reference position” process was utilised to restore displacements to the original orientation. We apologise for not clarifying this part of the methodology sooner. Line 160 has been amended to make it clear that the manual registration was merely an initial approximation prior to further registration within DaVis. On lines 202-205 the DaVis registration and vector subtraction method was clarified as mentioned.

**Point 2:** Inhomogeneous deformation of the scaffolds.

Scaffolds seem to compact at both ends (Figure 4), suggesting plastic deformation and an inefficient load transfer throughout the volume. How do authors think this issue could be overcome during the design and manufacturing process? What would be the implications in regenerative applications? In particular, for cartilage tissue engineering applications, which type of local strain environment do authors consider appropriate for successful regeneration? How does that relate to the finding of this study?

**Response 2:** The reviewer is correct that peak deformation did occur close to the ends of each sample (Figure 5, Supplementary S3 and S4) though these figures and in particular the magnified micro-CT slices (inlayed Figure 4) demonstrate that load was transferred

throughout the scaffold volume. We expect that the strain concentrations at the ends of the scaffolds are caused by the uneven sample height creating during manufacture and is common with regenerative medicine scaffolds. Further optimisation of the sample manufacturing process to consolidate sample height could reduce this effect. For example by employing higher resolution 3-D printing or post-printing sample processing such as polishing to standardise sample height. Text covering these points have been added to the discussion on lines 638-640.