

# Supplementary Materials

Marta Peña Fernández <sup>1</sup>, Enrico Dall'Ara <sup>2</sup>, Alexander P. Kao <sup>1</sup>, Andrew J. Bodey <sup>3</sup>, Aikaterina Karali <sup>1</sup>, Gordon W. Blunn <sup>4</sup>, Asa H. Barber <sup>1,5</sup> and Gianluca Tozzi <sup>1,\*</sup>

## Evaluation of 'baseline' strains

### 1. Methods

The evaluation of the baseline strains was performed in the first two consecutive datasets for the four specimens, where irradiation-induced damage was deemed as minimal. As the images were acquired in the same deformed state (i.e. 'zero-strain' repeated scans), null displacement and strain fields are expected. Therefore, any non-zero values of the measured displacement and derived strains using DVC were considered as error. Ten multi-pass schemes [1] with final sub-volume sizes ranging from 8 to 80, in steps of 8 voxels were investigated. For each sub-volume, three different parameters were computed.

- Random errors of the displacements: standard deviation of each displacement component, as in [2].
- Mean absolute strain value: average of the average of the absolute values of the six components of the differential strain, similar to MAER or "accuracy", as in [3].
- Standard deviation of the strain value: standard deviation of the average of the absolute values of the six components of the differential strain, similar to SDER or "precision", as in [3].

### 2. Results

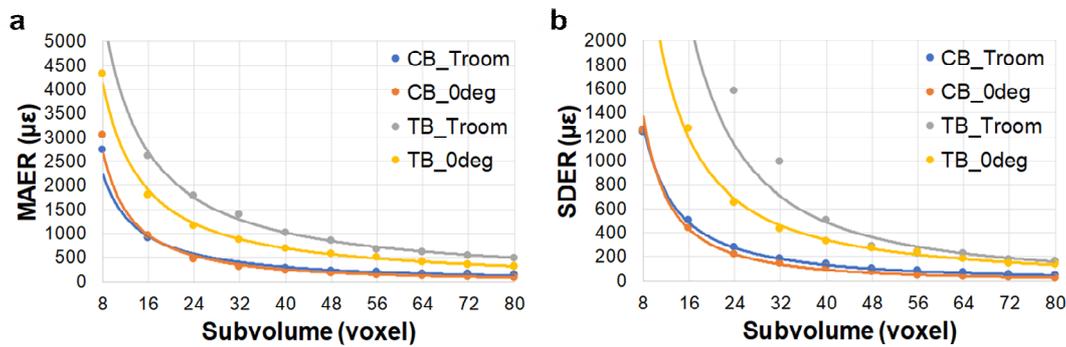
The random errors of each component of the displacement never exceeded 0.30  $\mu\text{m}$  for the compact bone specimens and 0.33  $\mu\text{m}$  for the trabecular bone specimens (Table S1). The errors obtained for the displacements in the compact bone were higher than those for the trabecular bone in x and y directions, but lower in z direction. A trend could be observed for both bone type specimens where the higher the sub-volume size, the lower the random errors.

**Table S1.** Random errors for the three displacement components for compact and trabecular bone specimens. Median values of the two specimens per group are shown.

Multi-pass scheme sub-volume sizes (voxels)	Displacement random errors ( $\mu\text{m}$ )					
	Compact bone			Trabecular bone		
	X	Y	Z	X	Y	Z
64-32-24-16-8	0.30	0.27	0.12	0.32	0.33	0.26
80-40-32-24-16	0.25	0.24	0.08	0.25	0.26	0.19
96-48-40-32-24	0.23	0.23	0.07	0.23	0.24	0.18
112-56-48-40-32	0.23	0.23	0.07	0.20	0.21	0.17
128-64-56-48-40	0.22	0.22	0.07	0.17	0.18	0.17
144-72-64-56-48	0.22	0.22	0.06	0.16	0.16	0.17
160-80-72-64-56	0.21	0.22	0.06	0.13	0.16	0.15
178-88-80-72-64	0.21	0.22	0.06	0.13	0.16	0.15
192-96-88-80-72	0.20	0.21	0.06	0.13	0.15	0.14
192-112-96-88-80	0.20	0.21	0.06	0.12	0.15	0.14

As expected from previous studies on bone [3,4], the strain uncertainties of the DVC had decreasing trends with respect to the sub-volume size, and the values of the mean value of the strain (MAER) were larger than the standard deviation (SDER) (Figure S1). The MAER ranged between 3000  $\mu\epsilon$  and 100  $\mu\epsilon$  for the compact bone samples and between 5500  $\mu\epsilon$  and 300  $\mu\epsilon$  for the trabecular

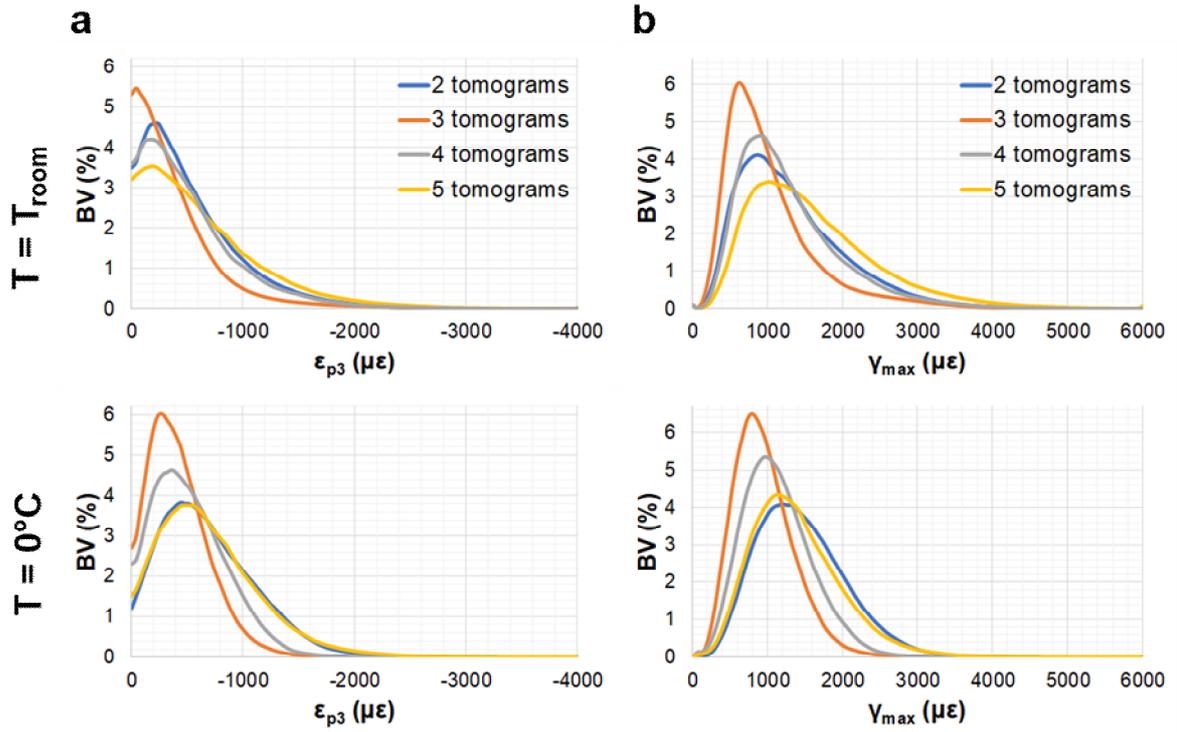
bone samples, in sub-volumes of 8 to 80 voxels (6.5 to 65  $\mu\text{m}$ ). The SDER ranged between 1250  $\mu\epsilon$  and 30  $\mu\epsilon$  for the compact bone and between 5000  $\mu\epsilon$  and 140  $\mu\epsilon$  for the trabecular bone, in the same sub-volumes.



**Figure S1.** Relationship between (a) MAER and (b) SDER with the sub-volume size for the four bone specimens.

## References

1. Peña Fernández, M.; Barber, A.H.; Blunn, G.W.; Tozzi, G. Optimisation of digital volume correlation computation in SR-microCT images of trabecular bone and bone-biomaterial systems. *J. Microsc.* **2018**, *00*, 1–16, doi: 10.1111/jmi.12745.
2. Palanca, M.; Tozzi, G.; Cristofolini, L.; Viceconti, M.; Dall'Ara, E. 3D Local Measurements of Bone Strain and Displacement: Comparison of Three Digital Volume Correlation Approaches. *J. Biomech. Eng.* **2015**, *137*, 1–14, doi:10.1115/1.4030174.
3. Palanca, M.; Bodey, A.J.; Giorgi, M.; Viceconti, M.; Lacroix, D.; Cristofolini, L.; Dall'Ara, E. Local displacement and strain uncertainties in different bone types by digital volume correlation of synchrotron microtomograms. *J. Biomech.* **2017**, *58*, 27–36, doi:10.1016/j.jbiomech.2017.04.007.
4. Dall'Ara, E.; Peña-Fernández, M.; Palanca, M.; Giorgi, M.; Cristofolini, L.; Tozzi, G. Precision of DVC approaches for strain analysis in bone imaged with  $\mu\text{CT}$  at different dimensional levels. *Front. Mater.* **2017**, *4*, 31, doi:10.3389/fmats.2017.00031.



**Figure S2.** Histograms of the residual strain distribution in compact bone tissue imaged at room temperature (top) and  $0^\circ\text{C}$  (bottom). (a) Third principal strains ( $\epsilon_{p3}$ ) and (b) maximum shear strains ( $\gamma_{\text{max}}$ ) after each acquired tomogram are shown.