

Visualising and Quantifying Hydroxyapatite in Bone Using Confocal Fluorescence Microscopy and OsteoImage™

Dui Lan Sun, Joel Turner

Faculty of Medical Sciences, University College London (UCL), 74 Huntley Street, London WC1E 6BT, United Kingdom

1 Introduction

- **Hydroxyapatite (HA):** the mineral component of bone; strong, osteoconductive, and osteointegrative; widely used in grafts, implant coatings, and regenerative scaffolds [1].
- **Gold-standard marker of bone mineralisation;** clinically relevant due to the growing burden of fractures and osteoporosis [3].
- **Limitations of current assays:** often non-specific, destructive, costly, or restricted to late-stage mineralisation [7,9].
- **Confocal fluorescence microscopy with OsteoImage™:** a specific, non-destructive, cost-effective method for visualising and quantifying hydroxyapatite in 2D and 3D cultures.

2 Materials and Methods

- **Cell cultures:**
 - 2D cultures fixed on days 7, 14, and 21 (low glucose) and day 21 (medium glucose).
 - 2D cultures with bioactive glass (45S5, CoSiBG) fixed on day 21 (low and medium glucose).
 - 3D cultures fixed on day 21 (low glucose).
- **Fluorescent labelling:** Hydroxyapatite visualised with OsteoImage™; nuclei labelled with DAPI.
- **Confocal microscopy (Leica TCS SP8):** Pinhole 3.09 AU; lenses ×5 (overview) and ×40 (cell-level detail), ×10 excluded; optical zoom 0.75× (low magnification), 1.28× (high magnification); z-stacks captured for 3D reconstruction.

3 Results

Time Course

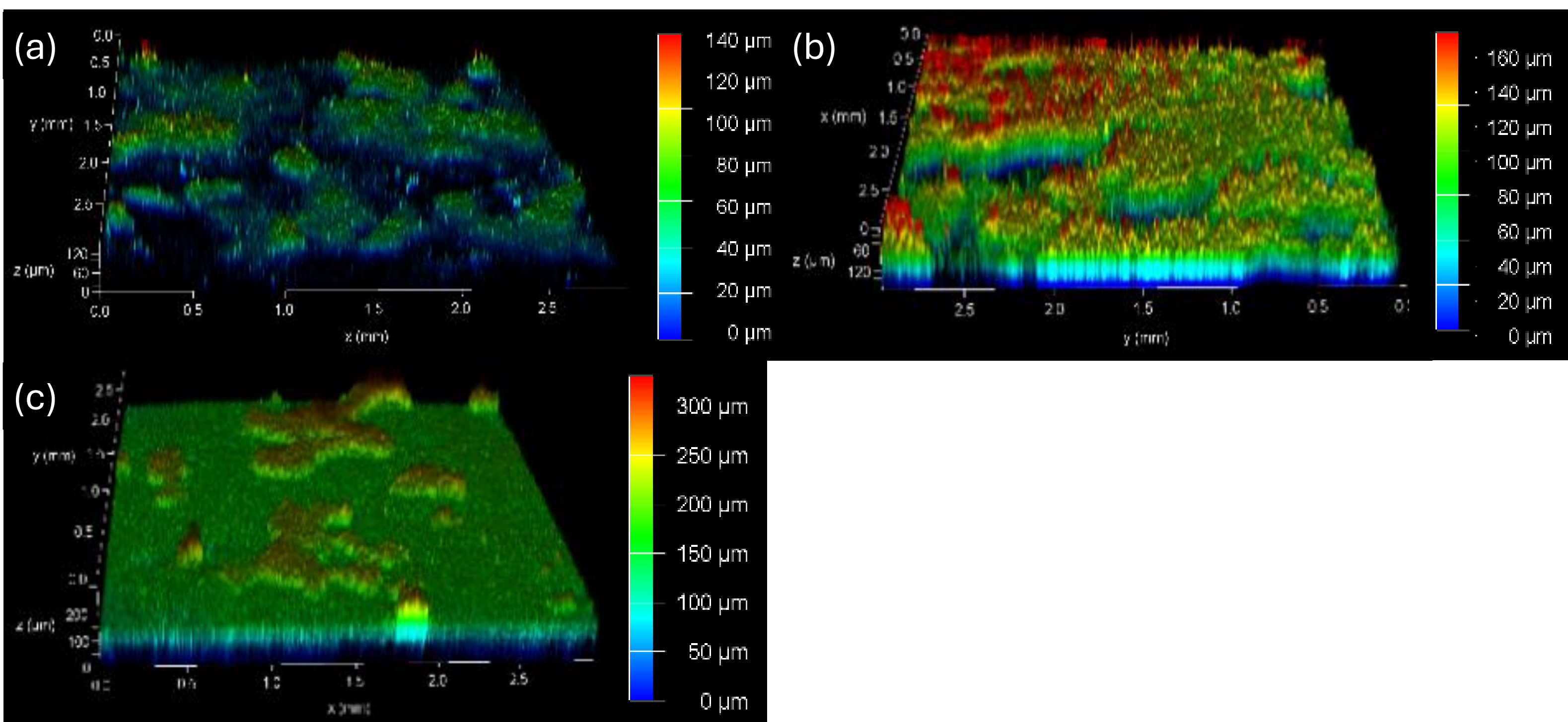


Figure 4. Hydroxyapatite with ×5 lens: (a) day 7, (b) day 14, and (c) day 21. Mineralisation increased over time.

Metabolic Environment

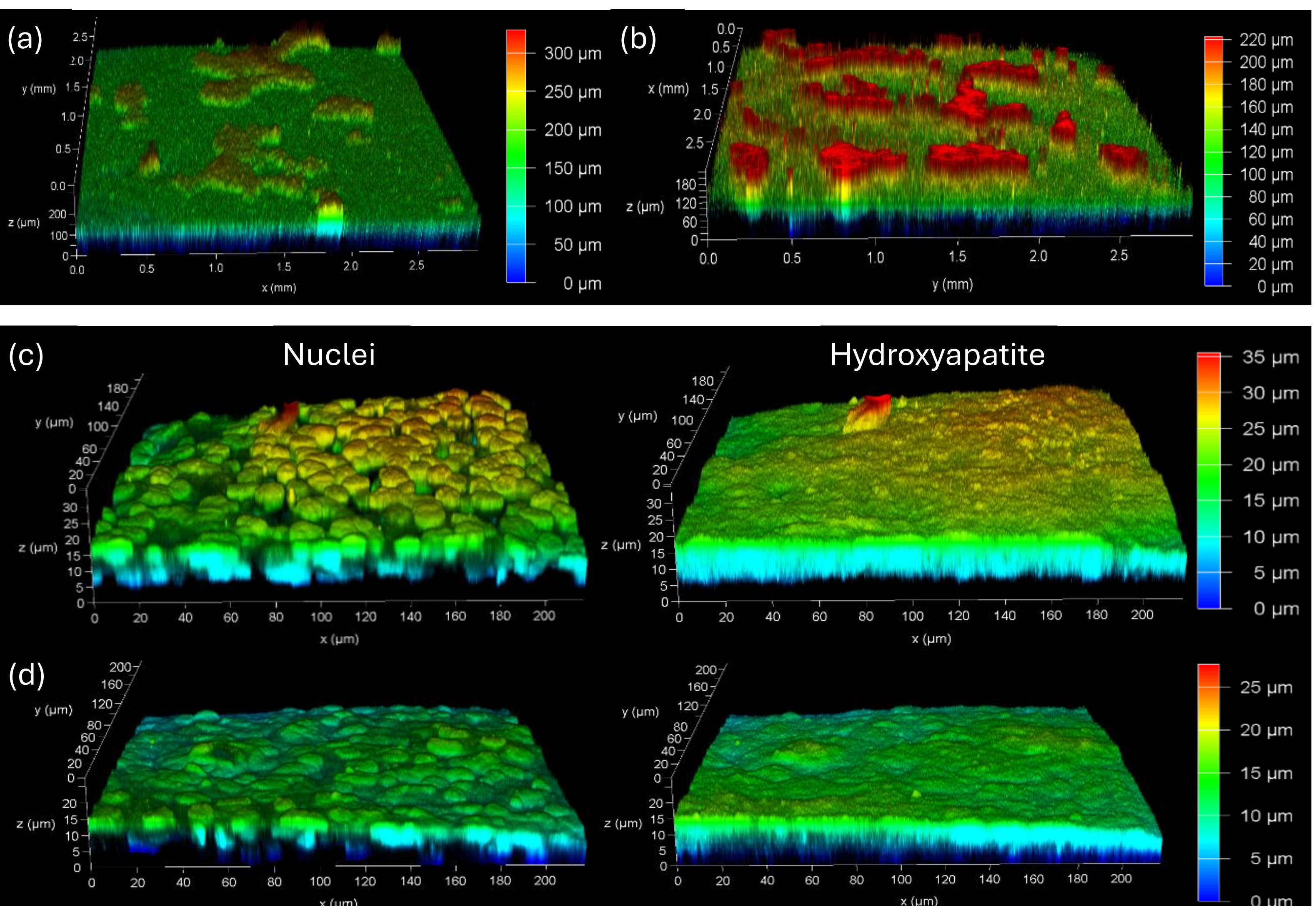


Figure 2. Hydroxyapatite at day 21: ×5 lens — (a) low glucose, (b) medium glucose; nuclei and hydroxyapatite: ×40 lens — (c) low glucose, (d) medium glucose. Greater mineralisation was observed under low-glucose conditions.

Biomaterial Composition

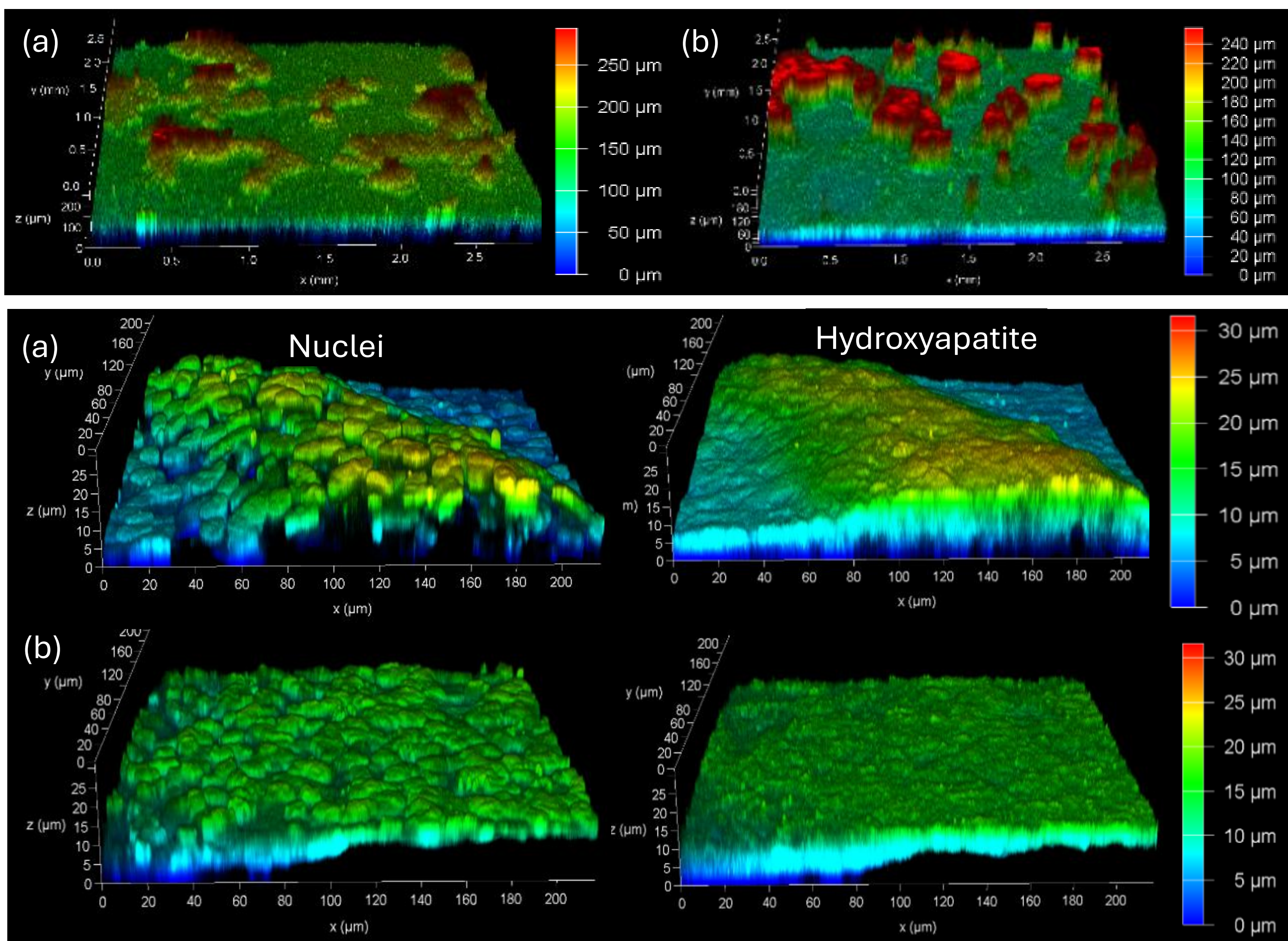


Figure 3. Low-glucose cultures at day 21: hydroxyapatite with ×5 lens — (a) 45S5 bioactive glass, (b) CoSiBG; nuclei and hydroxyapatite with ×40 lens — (c) 45S5 bioactive glass, (d) CoSiBG. Greater mineralisation was observed with 45S5 bioactive glass.

Culture Dimensionality

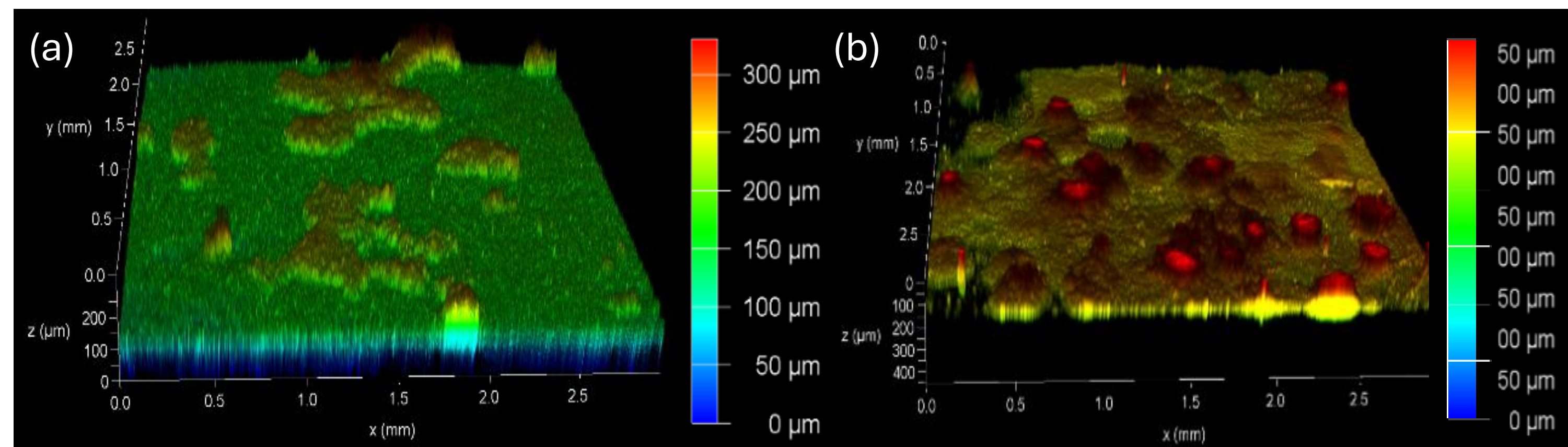


Figure 1. Hydroxyapatite with the ×5 lens at day 21: (a) 2-dimensional, (b) 3-dimensional. Greater mineralisation was observed in the 3-dimensional culture.

4 Discussion

- **Technique (Confocal + OsteoImage™):** Specific for hydroxyapatite, non-destructive, cost-effective, captures bulk and cell-level detail, scans <1 h/sample. **Limitations:** Background noise at z-stack edges; human error in defining the start and end of nodules.
- **Culture dimensionality:** 3D > 2D; better mimics bone microenvironment, enhancing osteoblast differentiation and matrix deposition.
- **Metabolic environment:** Low glucose supported higher mineralisation; medium glucose impaired mineralisation (ROS, AGEs, bone resorption).
- **Biomaterial composition:** 45S5 BG slightly increased mineralisation (rapid Ca/PO₄ release); CoSiBG modulated mineralisation via cobalt (HIF-1α, oxidative stress); controls had highest mineralisation under low glucose as osteoblasts function optimally in physiological conditions.
- **Time course:** Hydroxyapatite depth increased days 7 → 14 → 21 (normal osteogenesis).

5 Conclusion

Confocal fluorescence microscopy with OsteoImage™ provides a specific, reliable, non-destructive, and cost-effective method to visualise and quantify hydroxyapatite in 2D and 3D cultures, enabling evaluation of biomaterials, drug effects, and osteogenic processes.

References:

- [1] O.A. Osuchukwu, A. Salihi, I. Abdullahi, B. Abdulkareem, C.S. Nwannah, Synthesis techniques, characterization and mechanical properties of natural derived hydroxyapatite scaffolds for bone implants: a review, SN Applied Sciences 2021 3:10 3 (2021) 1–23. <https://doi.org/10.1007/S42452-021-04795-Y>.
- [2] H. Shi, Z. Zhou, W. Li, Y. Fan, Z. Li, J. Wei, Hydroxyapatite Based Materials for Bone Tissue Engineering: A Brief and Comprehensive Introduction, Crystals 2021, Vol. 11, Page 149 11 (2021) 149. <https://doi.org/10.3390/CRYST11020149>.
- [3] J. Scheinplug, M. Pfeiffenberger, A. Damerau, F. Schwarz, M. Textor, A. Lang, F. Schulze, Journey into Bone Models: A Review, Genes 2018, Vol. 9, Page 247 9 (2018) 247. <https://doi.org/10.3390/GENES9050247>.
- [4] S.E. Doyle, D. Winrow, F. Buckley, E. Pernevik, M. Johnson, K. Thompson, L. Howard, C.M. Coleman, Optimization of 3D Extrusion-Printed Particle-Containing Hydrogels for Osteogenic Differentiation, ACS Omega 10 (2025) 15036–15051. https://doi.org/10.1021/ACSOMEGA.4C10515/ASSET/IMAGES/LARGE/AO4C10515_0008.JPEG.