

Combining confocal and BSE SEM imaging for bone and implant interfaces.A. Boyde^{1,2}, L. Lovicar^{1,3} and J. Zamecnik^{1,3}¹*Anatomy Dept., University College London, ²Centre for Oral Growth and Development, Barts & The London School of Medicine & Dentistry, University of London UK*³*Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic*

INTRODUCTION: Due to structural damage in cutting, no form of sectioning can be envisaged in the investigation of calcified tissues if we wish to study cells and hard matrices in undisturbed relationships. Study of tissue remaining in a block face, discarding the damaged tissue fragments as polishing or micromilling swarf, has to be the basic approach. We therefore need to concern ourselves with means of cutting back close to the layer(s) to be studied whilst creating minimal disturbance to the remaining tissue, and choosing and optimising microscopic observation modes. Compositional mode BSE SEM of plastic embedded tissue is of key value in demonstrating differences in the degree of mineralisation of hard tissue matrices at sub-micron resolution. Sample preparation technique is critical for the success of this approach, which assumes and requires that superficial topographic relief is minimal. The same requirement is met for optimal confocal light microscopy, which has revolutionised block face microscopy in hard tissues. Here, excellent structural information is obtained in reflection-backscattering mode, albeit that such contrast is minimised in well embedded tissue and that modern commercial CSLMs are not good in this mode. However, there is usually sufficient auto-fluorescence signal in CSLM to read general histology and identify and map cell types and matrix structure. This is more so in formaldehyde and glutaraldehyde preserved material. If this is not enough, a general purpose fluorescent stain is brilliant sulphaflavine. Of course we can also utilise intra-vital mineralisation-front labels. The present studies consider practical approaches to correlating qualitative and quantitative BSE SEM imaging with confocal imaging modes and will use examples from bones embedded in PMMA, +/- tetracycline, alizarin and calcein labels. We have developed a software package tailored to the demands of our problem area. The SEM has a proper digital scan generator: we leave the BSE image unchanged, and match the CSLM image to it, rather than the reverse, because the CSLM scan mechanism is not digital, though the signal is

digitised. Our overlapping program uses a linear transformation matrix which projects one system to the other, calculated by finding three corresponding points in BSE and FCSLM pictures. BSE images are empty where cells and osteoid are present. CSLM fills in these gaps. The combination images enhance our understanding of what is going on - and re-establish the need for good cellular preservation.