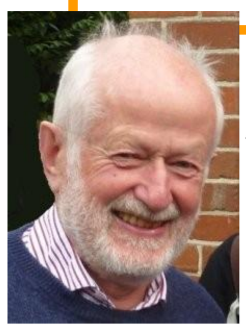
LB14 A new paradigm for bone formation in remarkable endosteal bone appositional rates in rat distal femur



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Objectives:

•To study details of bone growth rates and mechanisms in the distal femur of young rat using multiple combined 3D imaging methods.

Methods:

•80 male Lewis rats, final weight ~ 350 grams.
•Calcein at 12 days and tetracycline 3 days before termination.
•Right femurs: X-ray microtomography (microCT 8µm voxels).
•Transverse section at mid-shaft, distal portion cut longitudinally in the midline.
•*Lateral halves* PMMA embedded for block face microscopy, using confocal LM to read labels and for histology.
•20kV BSE SEM to image mineral content. More BSE imaging after tri-iodide or iodine vapour staining for histology.
•Finally, all bone was dissolved to produce 3D casts of marrow and capillary blood vessel canal space.
•*Medial halves* macerated for 3D BSE SEM.
•With knowledge gained from right-side data, we studied *left* femurs with 6 µm microCT, then cut them transversely to create serviette 'rings' at defined distances from the distal condyles. End faces used for imaging labels with confocal LM and the samples were then macerated for 3D SEM.
•All these types of image were cross correlated to produce composites.

Results and Conclusions:

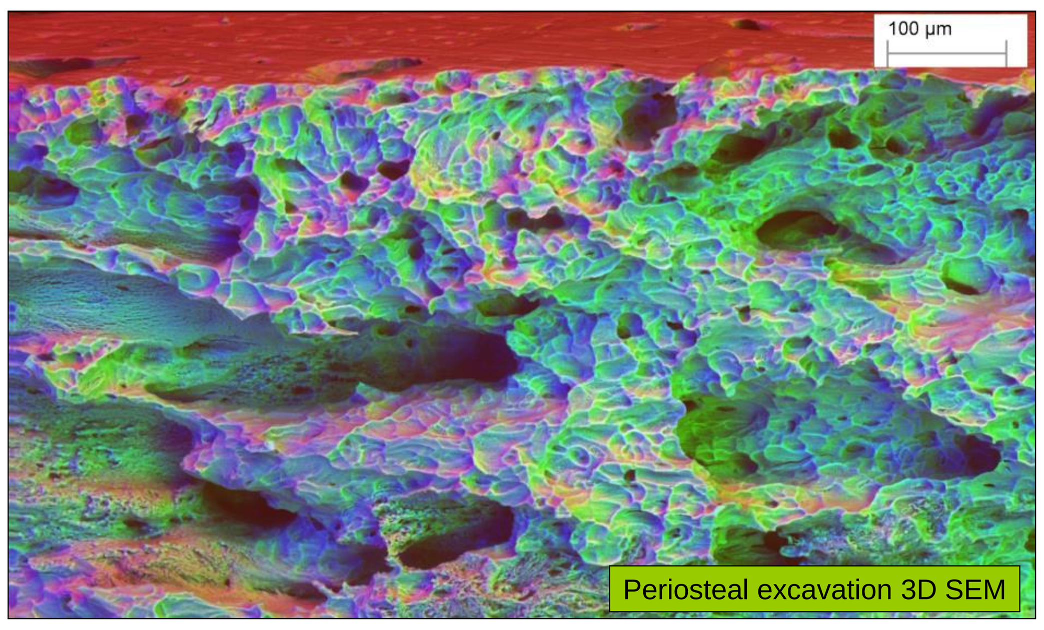
Remarkably high values for endosteal apposition were measured, with nearly matching high periosteal resorption rates to be assumed. In places, almost the entire 400µm thickness of the shaft was translocated in 15 days. This rapid endosteal bone growth is associated with the inclusion of capillary blood vessels which penetrate the osteoblastic layer at near normal incidence to the formative surface at ~50 micron spacing. This is a previously undescribed mode of compact cortical lamellar bone formation.

•Trabeculae drift centrally in parallel with cortical surfaces. Thus their double label intervals must be understood in a full 3D context.

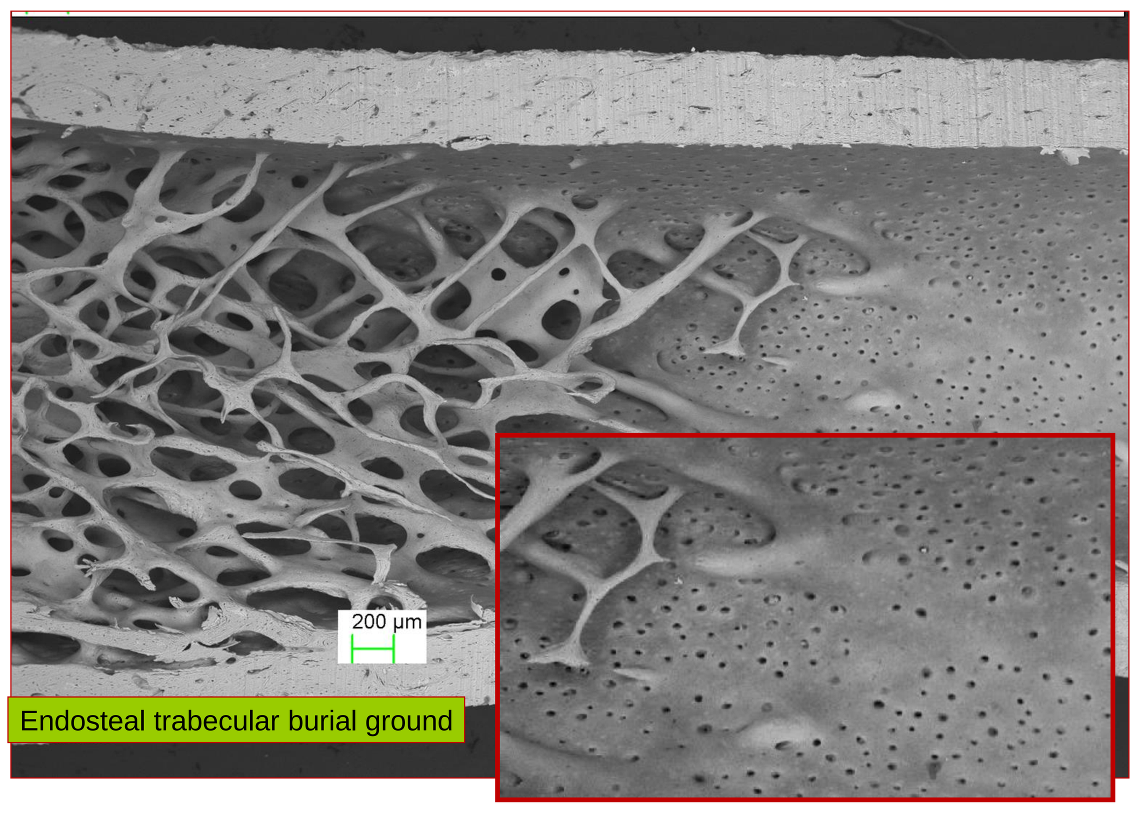
•MicroCT imaging 'loses' small trabeculae which are physically retained even in macerated SEM samples.

•Real loss of trabeculae occurs by their burial in compact bone at endosteal growth surfaces as well as through resorption.

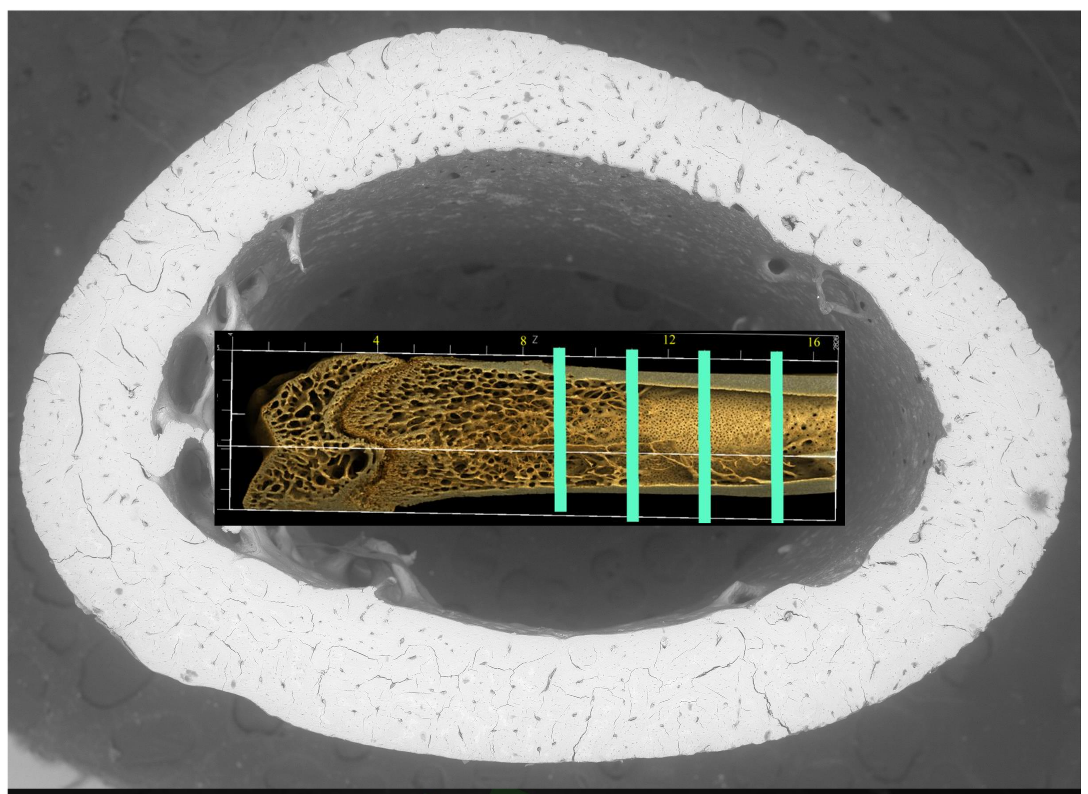
•The 'gold standard' must be held to be the combination several imaging methods

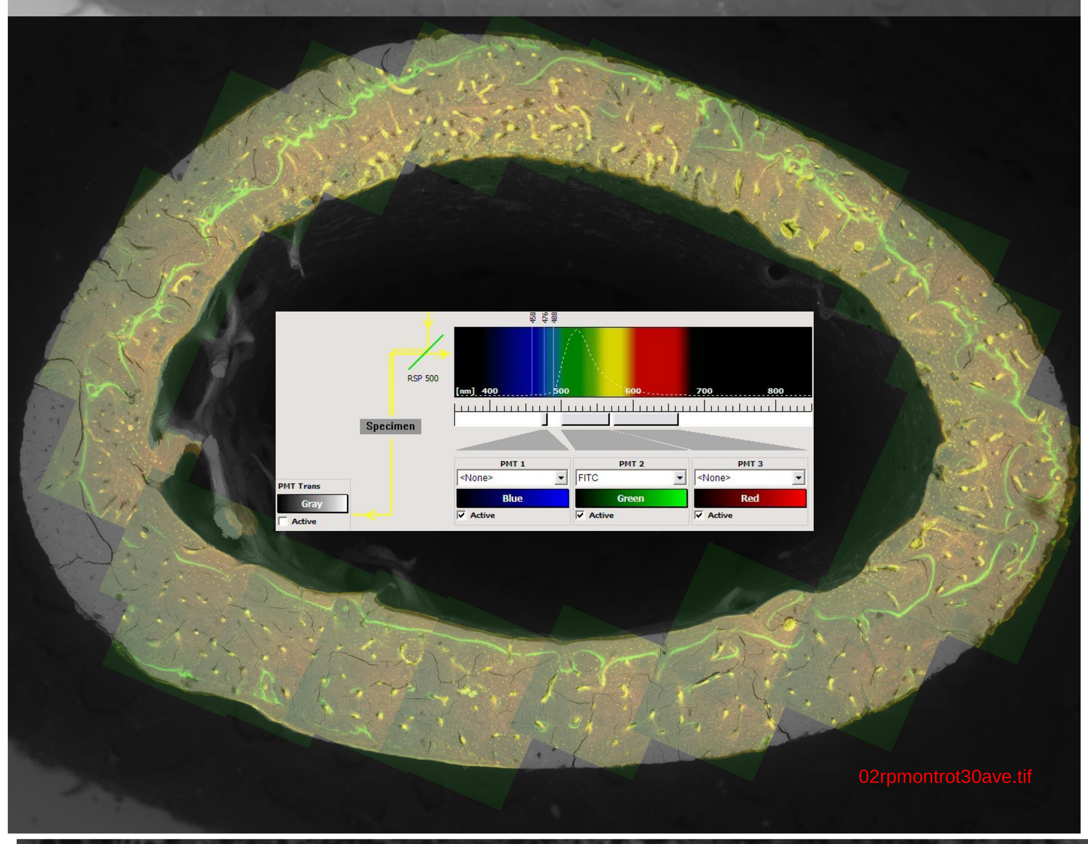


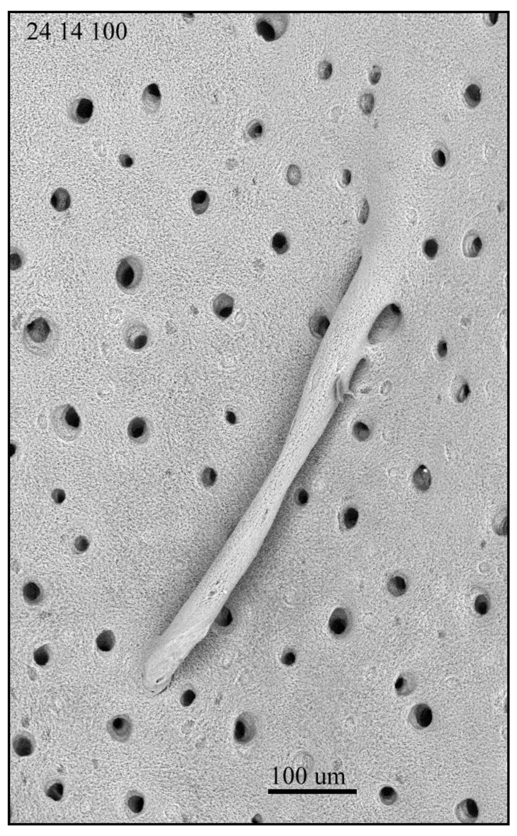
Periosteal excavation 3D SEM

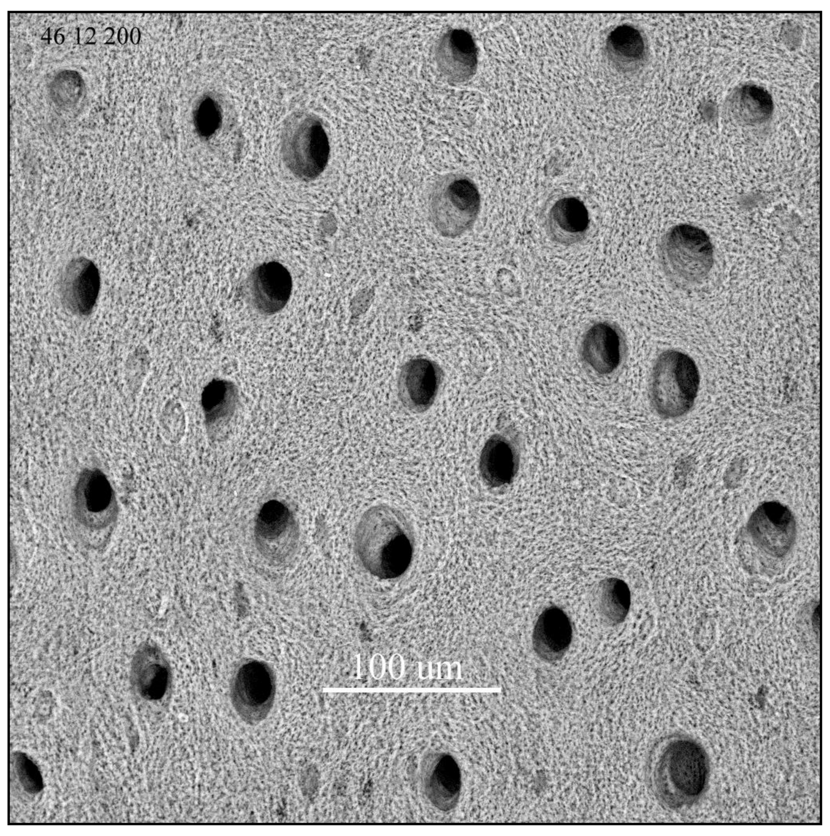


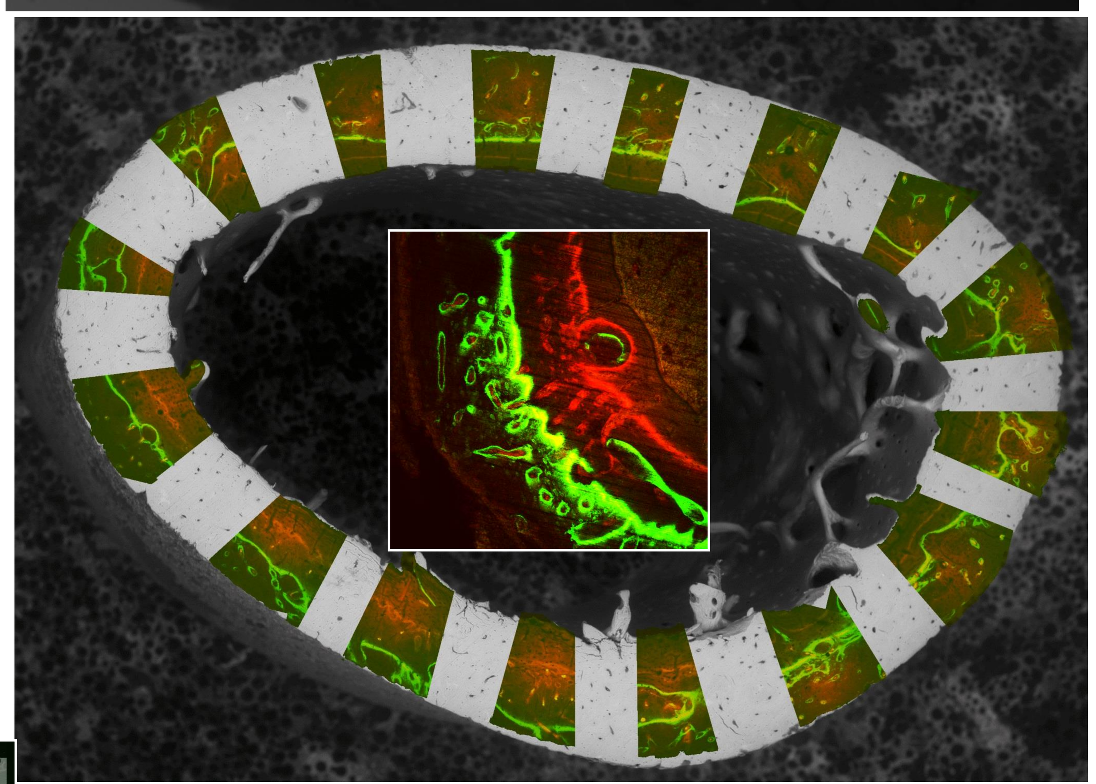
Endosteal trabecular burial ground

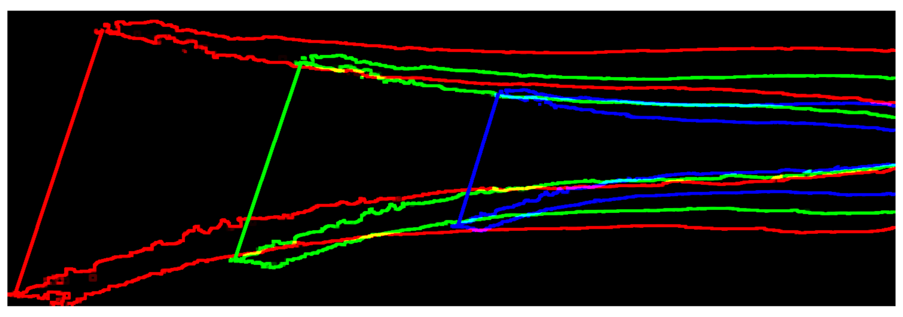


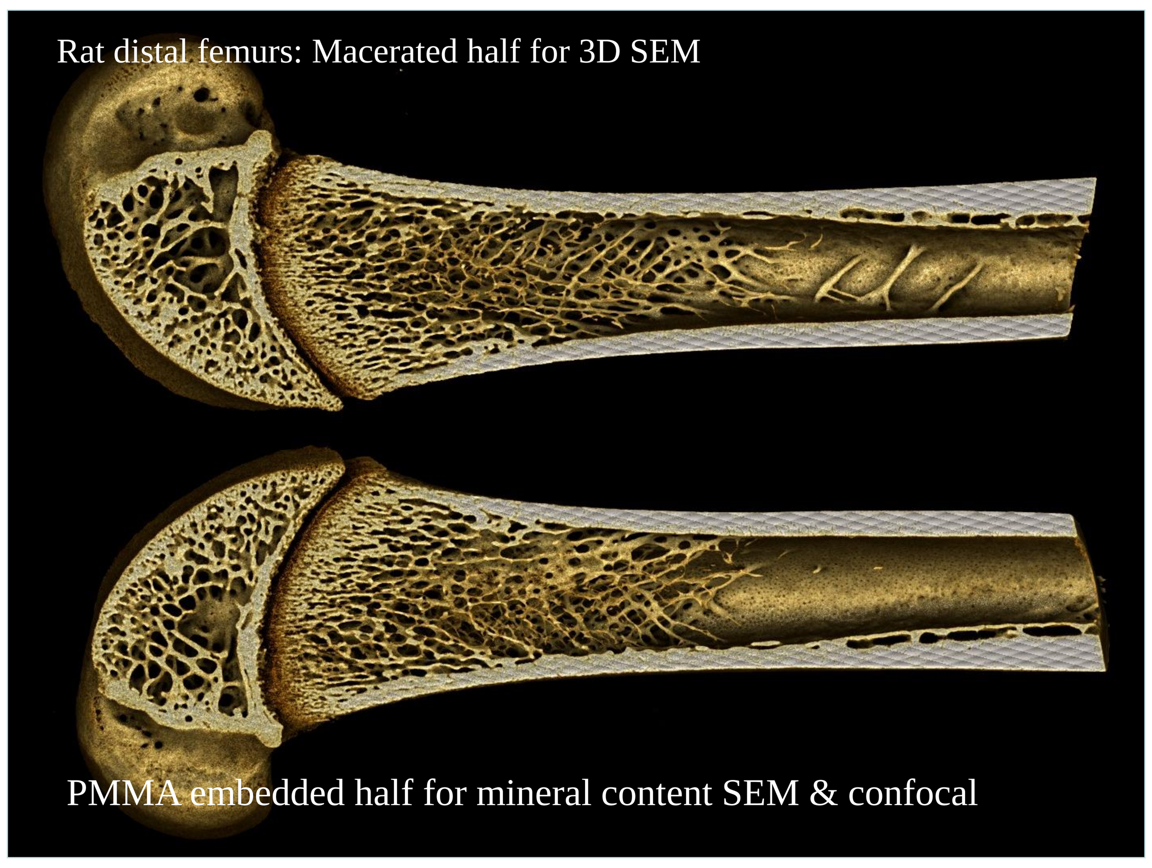


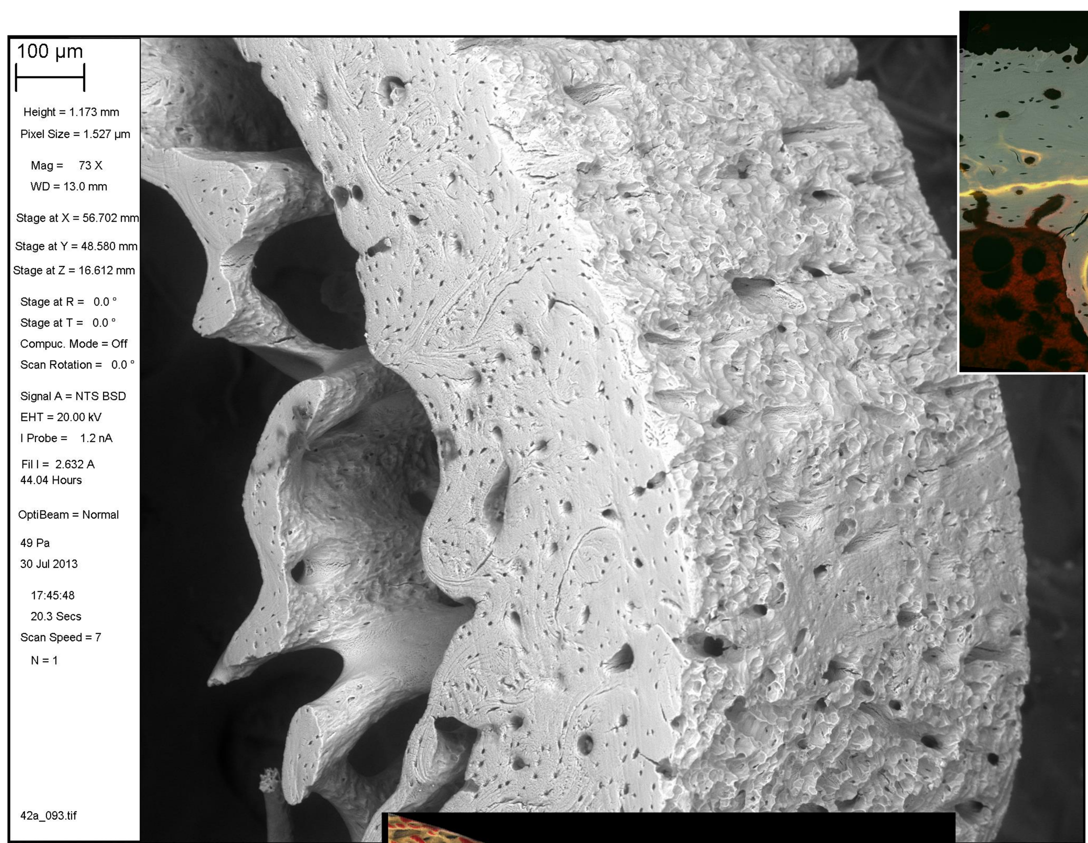


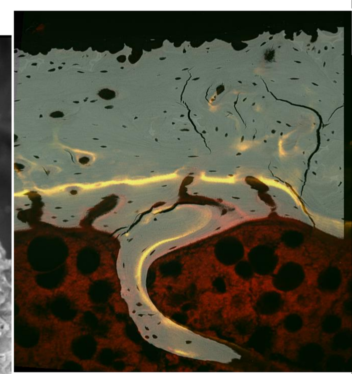


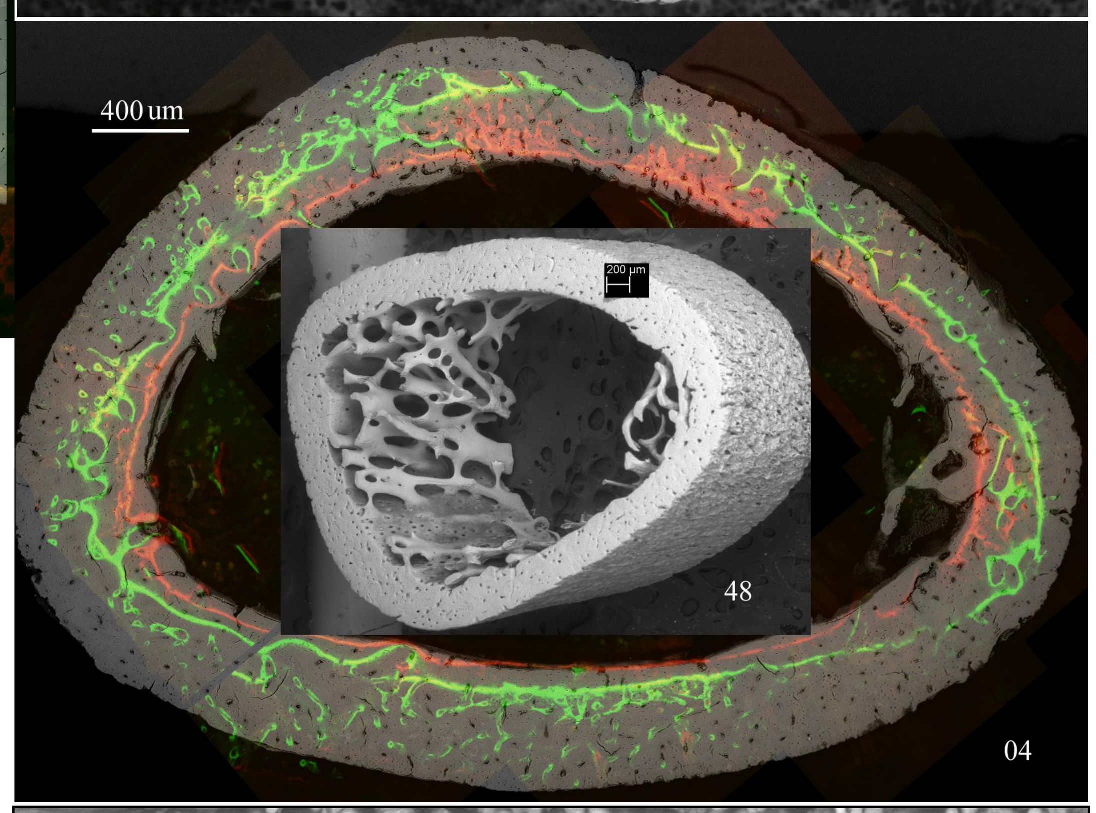


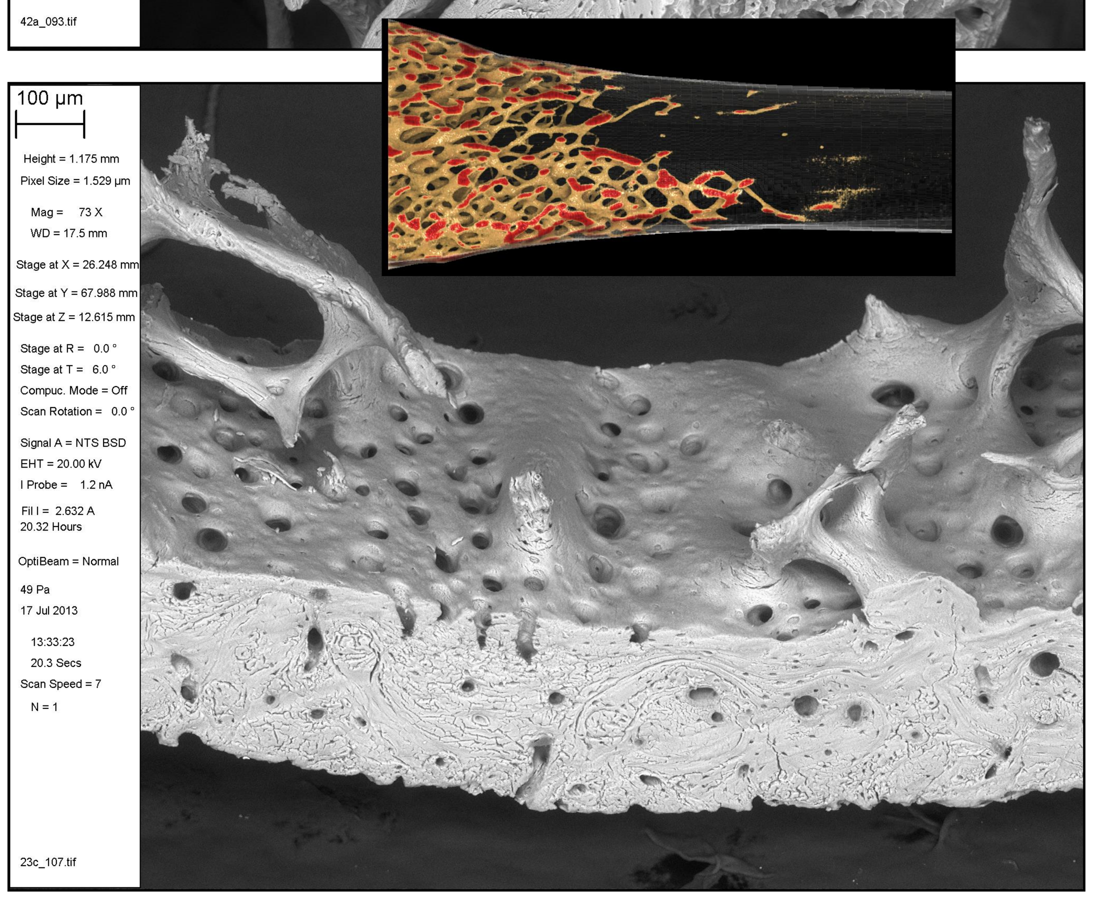


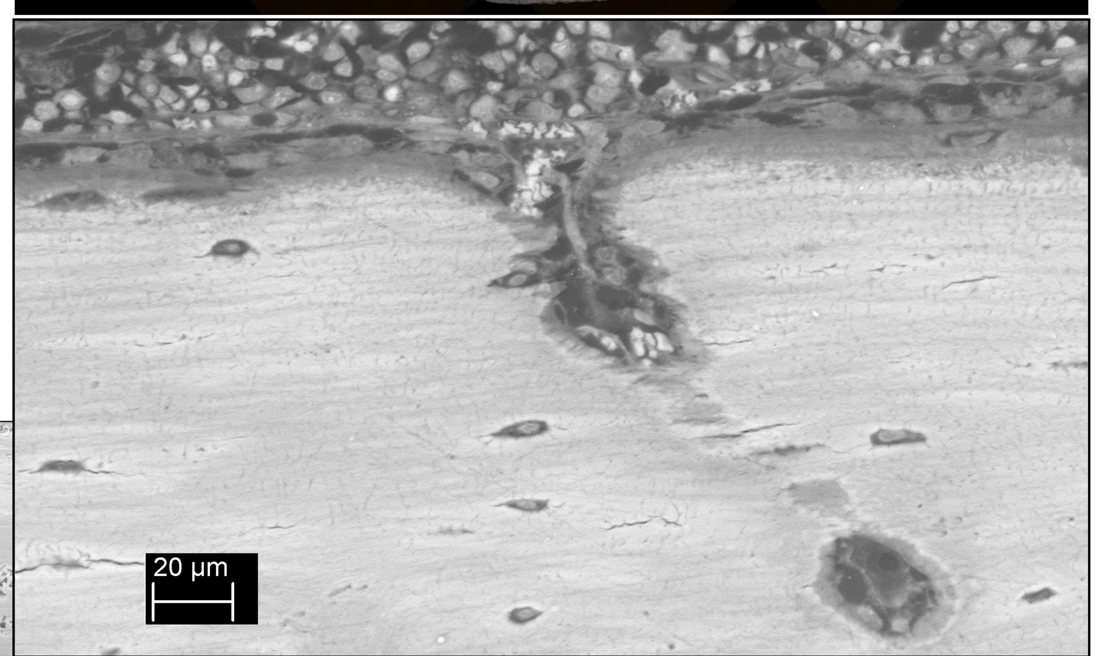


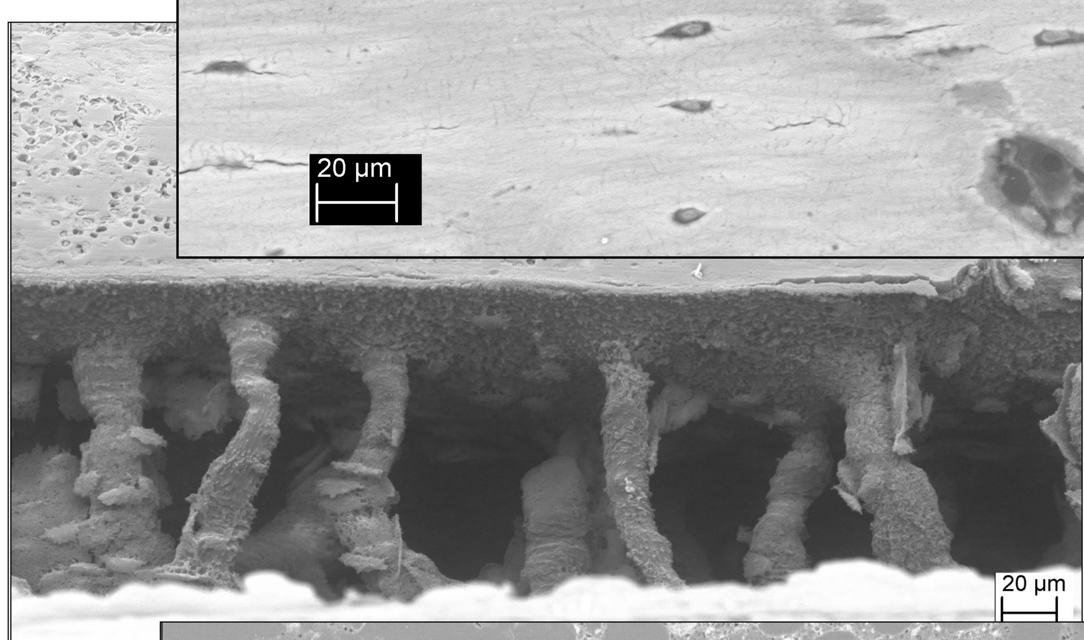












SEM of polished PMMA block NOT coated, permitting confocal optical microscopy