ANALYSIS OF MICRO-STRUCTURE AND CRACK PROPAGATION IN CORTICAL BONE

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Summary: The project aims to create a semi-automatic method for segmentation of micro-CT images of cortical bone in order to obtain the internal microstructures, i.e. hollow canals that are circumvented by highly mineralized structures known as osteons, all of which are embedded in a bone matrix and play an important role in the mechanics of crack propagation through the bone.

1. INTRODUCTION

Cortical bone has a complex hierarchical structure. At sub-nano scale the structure is made up of soft collagen molecules interwoven by stiff mineral crystals [1]. On the micro scale, the tissue structure includes hollow canals, through which blood vessels permeate the bone, encircled by structures called osteons. These structures are mechanically interesting since the osteons increase the material toughness by causing crack deflection. Previous studies (e.g. [2]) have used manual segmentation to quantify and analyse the micro-structures. As the canals are hollow and the osteons have a lower mineral content than the surrounding bone matrix it is possible to differentiate between the structures by analysing images captured using x-ray tomography since the structures will yield different pixel intensities due to different amount of absorption of the x-rays. The project aims to develop the tools needed to accomplish this in a semi-automatic manner.

2. EXPERIMENTAL METHOD

Bone samples from bovine femurs (sample size 40x20x1 mm), were imaged with a 3D X-ray microscope (Zeiss Xradia XRM 520) located at the 4D Imaging Lab at LTH, Lund. A voltage of 80 kV was used and the resulting images had a field of view of approximately 20 mm and an isotropic voxel size of 9.25 µm. The samples were produced in a previous study where they were tested in tension until failure with surface strains simultaneously measured using Digital Image Correlation (DIC).
The microstructure were segmented using k-mean clustering. Different number of clusters (3-9) were initially explored and the resulting segmentations were compared to a manual threshold-based segmentation in order to determine the optimal number of clusters. The segmentations were then used to analyse and visualize the microstructures of the bone samples in order to quantify them in terms of orientation, distribution and size, and to improve the interpretation of the findings from the tensile tests. The thickness and spread of the canals were analysed using the BoneJ [3] plugin in ImageJ [4]. The orientation of the canals was analysed in Matlab using principal component analysis.

3. RESULTS

Segmentation of a µCT image stack from one representative sample can be seen in Fig. 1. The segmentation clearly enables visualization of the orientation and distribution of microstructures in the sample. During clustering, a higher number of clusters led to more detailed information in the segmentations, which yielded a better correlation with the manual threshold-based segmentation, and hence the optimum number of clusters was determined as 9. A yet higher number was not implemented since the comparisons were deemed good enough and the time consumption for segmenting full samples was already high (up to six hours).

When analysing the thickness and separation of the canals in the sample shown in Fig. 1 (Tab. 1), it was observed that

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the variability both within and between samples were large. The mean thickness of the canals in the samples are quite different but, as seen in Fig. 1, the canals vary in thickness throughout the sample.

	Mean thickness (μm)	Standard dev. thickness (μm)	Mean separation (μm)	Standard dev. separation (μm)
Complete sample (Fig. 1 (e))	33	10	1609	443
Sub-sample (Fig. 1 (c))	72	16	300	62

Table 1: Mean and standard deviation of thickness and spacing for the microstructures shown in Fig. 1c and e.

As can be seen in Fig. 1d-e the orientation of the microstructures differ in different parts of the sample. In the previous study, the area inside the red box in Fig. 1a showed higher surface strains, than the rest of the sample during the tensile testing. In general, there seem to be a correlation between orientation and strain when looking at the orientation of the microstructures (e.g. Fig. 1e), since the microstructures inside the red box appear to be oriented parallel to the applied load whereas in other parts of the sample they appear more or less perpendicularly oriented. This supports the hypothesis that the orientation and distribution of the microstructures affect the mechanical strength of the bone and hence the crack propagation in the samples. By calculating orientation vectors for the microstructures at different regions of the samples and comparing these vectors to the measured strain we hope to validate this hypothesis further.

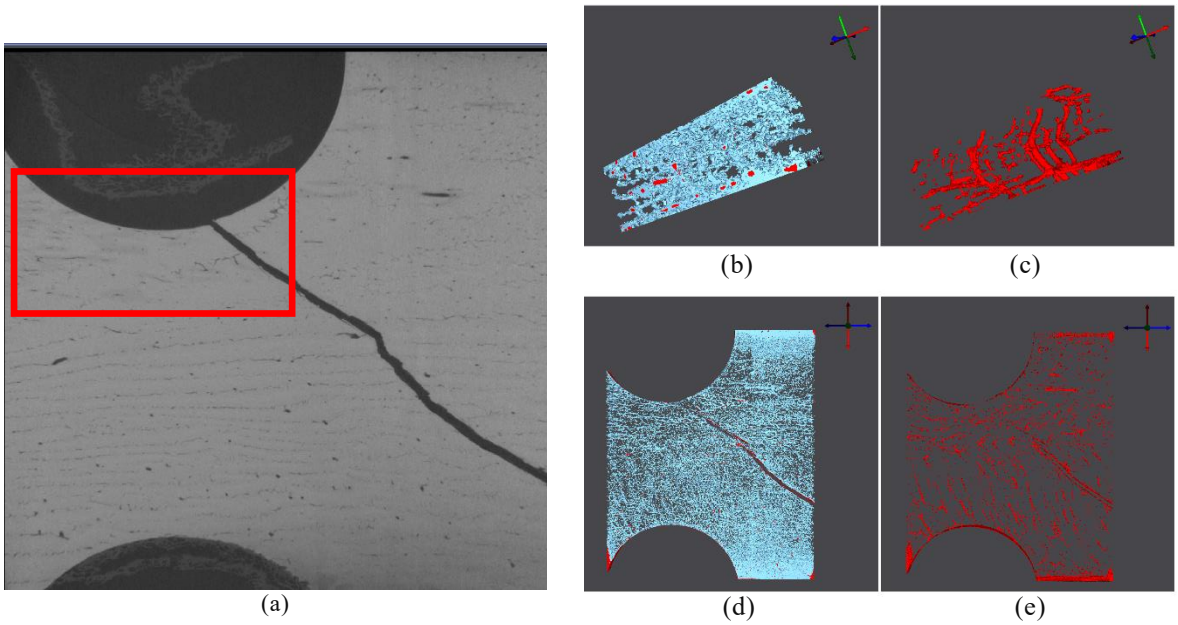

Figure 1: (a) Coronal view of an image slice of one of the bone samples. (b-e) Segmentation of microstructures in the sample shown in (a). (b) Shows the canals (red) and osteons (blue) in a small sub-sample. (c) Shows only the canals from the segmentation shown in (b). (d) Shows the canals (red) and osteons (blue) in the whole sample. (e) Shows only the canals from the segmentation shown in (d). The orientation and distribution of the micro-structures are evident from the segmentations.

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