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PERFORMANCE COMPARISON OF DEEP LEARNING SEGMENTATION MODELS ON HISTOLOGICAL SECTIONS IN A MURINE BONE ADAPTATION AND REGENERATION MODEL

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Summary: Histological analysis of bone samples from pre-clinical animal experiments is a well-established technique to investigate multiscale processes between tissue and cellular structures. Sections are stained for several bone metabolic and structural markers, such as Sclerostin and Safranin-O, supporting bone adaptation and regeneration studies. Nonetheless, the post-processing analysis is predominantly manual, limiting its throughput and integration into correlative multi-modal approaches that require segmented images to be registered to 3D micro-computed tomography (micro-CT) volumes of the same sample. Therefore, this work aimed to explore Deep Learning (DL) segmentation models to expedite the analysis of histological images of murine bone. A ground truth dataset of six Safranin-O and two Sclerostin stained histological sections (10µm thickness, with Fast Green counterstain) of a mouse femur was manually segmented using QuPath by two operators. All images were from the same mouse, obtained from a previous femur defect loading study [1]. Since only approximately 10% of the image pixels represented mineralised bone, a custom sampling algorithm was developed to generate a balanced training dataset. A total of 8640 patches (256x256) were extracted and zero-padded by 32 pixels. The Python package "segmentation-models" was used to compare four architectures (UNet, Linknet, PSPNet and FPN) and 35 backbones pre-trained on the ImageNet dataset. Four loss functions (Dice, Jaccard, binary focal and binary cross-entropy or binary CE) were available, and two evaluation metrics (intersection over union or IoU and F-score) were used for performance assessment. Linknet was selected to compare all backbones, given its speed and low resource usage, revealing a subset of the 12 best-performing options. Next, a robust loss function was determined by testing linear combinations of the four options available. A combination of Jaccard and binary CE achieved the most consistent results. Afterwards, a hyperparameter optimisation was performed on all architectures, combined with the 12 selected backbones and optimal loss function. A learning rate of 0.001, batch size of 16 and 200 steps per epoch were found to work best. An EarlyStopper callback was used to stop training when the loss function value remained constant for three consecutive epochs. UNet and Linknet were the best performing models, combined with seresnet101 and seresnet152 backbones, respectively, achieving an IoU score of 0.964 and 0.961. Furthermore, a model trained on Safranin-O images successfully segmented Sclerostin images, paving ways to transfer models trained on one type of marker to similar images. Overall, these results indicate that Deep Learning models can segment histological images from murine bone samples. Ongoing work will expand the models' performance assessment and the dataset's diversity to include images with other relevant bone markers. This segmentation model will enable a higher throughput of a correlative multi-modal approach under development that combines *in vivo* micro-CT , *ex vivo* histological sections, and spatial transcriptomics data. We believe this Local *in vivo* Environment (LivE) imaging approach will reveal novel multiscale mechanobiological pathways regulating bone adaptation and regeneration and advance our understanding of the causes of unbalanced bone mechanoregulation, with a particular focus on ageing, and potential ways to improve it.

References
[1] 10.1038/s41598-021-02368-y