Hard-tissue histology using CT guided laser ablation sectioning

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Introduction:

Histological analysis of hard-tissue is labor-intensive, destructive, and does not allow for the accurate targeting of hidden regions of interest (ROI). By applying our CT and laser microtome[1]-based sectioning workflow, we expect to reduce the time needed to process the tissue while achieving a better selection and extraction of the predefined ROI with limited loss of material.

Problem:

The classical histological workflow is prone for error and introduces physical strain to the specimen resulting in possible lesion, loss of material and distortions during the sectioning process. Here specimen are cut with a diamond blade and further refined using the cut-grinding method. Precise isolation of ROIs is not accounted for.

Proposed Solution:

Utilizing the spatial and anatomical information gained from an a-priori microCT scan we propose to extend the histological routine based upon our future work[2] with an additional digital planning phase consisting of i.)definition of an ROI and ii.) estimation of an optimal cutting plane. Following a priming cut by a diamond blade, the specimen are then prepared for histological analysis using a contactless laser microtome approach in place of the strain introducing cut-grinding process.

Methods:

Bone specimens with a dimension of about one square centimeter were embedded in resin and scanned with the in-vivo microCT (QuantumFX, Perkin Elmer) at an isotropic voxel resolution of 40 µm. The cutting plane was planned using this CT-data, the specimen was cut with a diamond saw at the predefined plane (Cut Grinder Primus, Messner) and subsequently glued to microscope slides. Using the LASER microtome (TissueSurgeon, LLS Rowiak) 30 µm thick sections were obtained and stained applying a Sanderson Rapid Stain protocol. Images of stained sections were acquired with a slide scanner (Epson Perfection V800). The three-dimensional representation of the tissue containing its structural features was obtained using the microCT and processed by VGStudioMAX (Volume Graphics).

Results / Discussion :

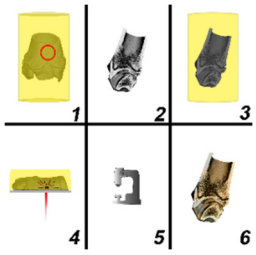

Fig. 1: Concept of CT guided approach integrated into the routine histological workflow

We developed a workflow for CT-guided hard-tissue histology, composed, as shown in Figure 1, of six steps: (1) embedding, (2) imaging with microCT, (3) cutting with a diamond saw, (4) sectioning with a LASER microtome, (5) staining and microscopic analysis, and finally (6) registration of corresponding CT-planes and histological images.

Using our method, we were able to identify an analogous CT-plane of a given histological slide as shown in Fig. 2. With the introduction of lase microtomy the severity of deformation introduced to the tissue during preprocessing was limited preserving its geometrical integrity. In the future we aim estimate the optimal transformation allowing to align both structures using registration algorithms.

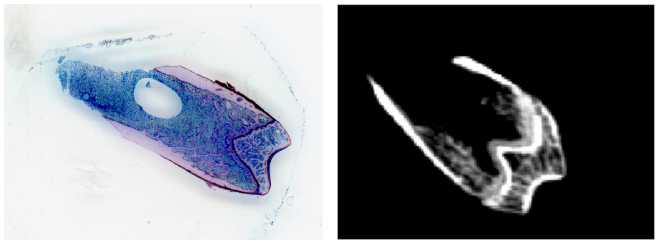

Fig 2: Comparison between histology and CT scan of a rat's femur. Structural features in both modalities can be observed and are planned to matched using image registration techniques

While a qualitative comparison between both modalities can be established we aim to express this relationship through a computational transformation of the histological slide in order to validate the accuracy of our workflow. Due to a low magnitude of deformation achieved by laser ablation sectioning we expect the complexity of the to be implemented registration algorithms to be restricted to non-elastic approaches.

Conclusion:

Our CT guided workflow enables the identification and preparation of a ROI for analysis within hard specimen which has been impossible so far, due to a lack of structural information gained by e.g., visual inspection. Moreover, this approach allows spatially correlating histological findings with 3D features. In the future, the CT scan will be registered to enrich the analysis in 2D with 3D structural features of the microenvironment.

References:

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