

AUTOMATED HISTOMORPHOMETRIC ANALYSIS OF JOINT DAMAGE IN A MOUSE MODEL OF OSTEOARTHRITIS



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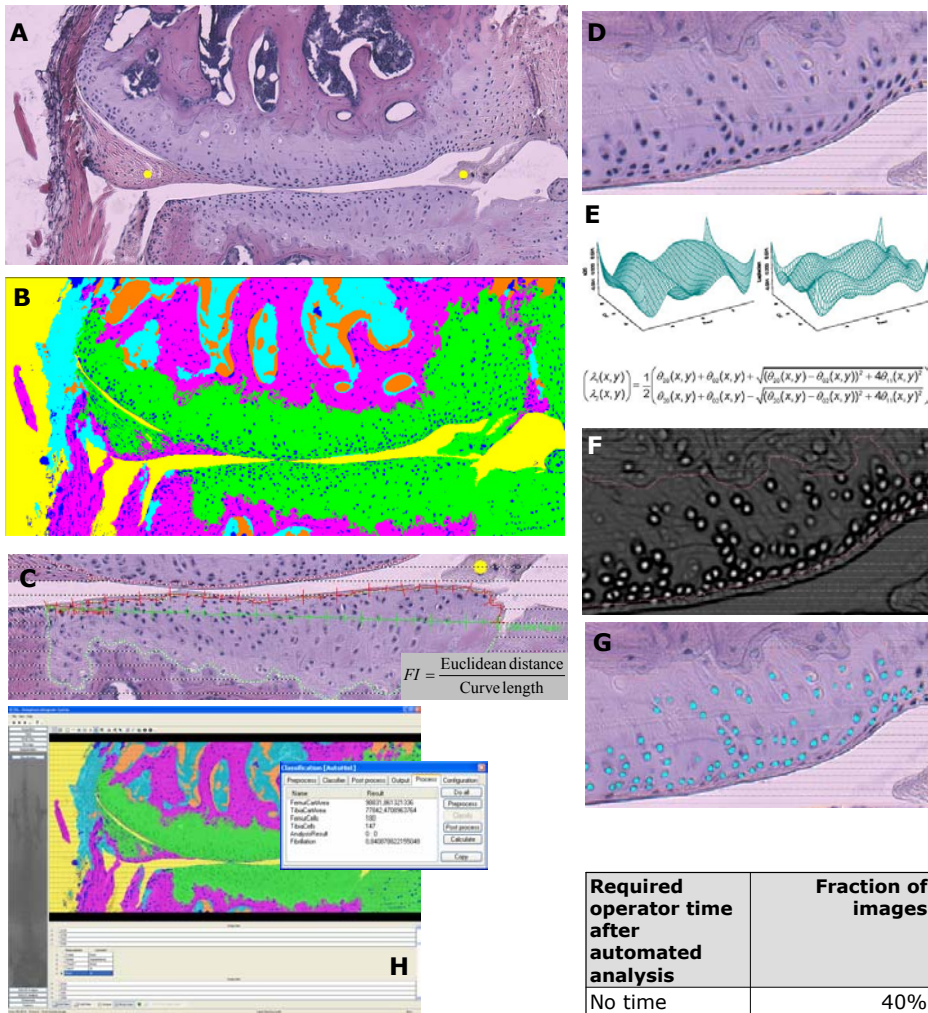
Materials and Methods

Preparation and imaging: 50 male STR/1N mice, developing spontaneous osteoarthritis, were sacrificed with CO₂ inhalation at 12 weeks of age. Their left knee joints were removed and decalcified for 3 days with formic acid (decalcifier, Shandon, Frankfurt/M., Germany), fixed in formalin and embedded in paraffin. Four sequential coronal sections of the complete knee joints (7 μm, 0.1 mm apart) were stained with hematoxyline-eosine. A total of 220 24 bit digital color images of size 2600x2060 pixels were obtained from the sections using a Zeiss light-microscope with a digital Camera (Zeiss Axiocam HRC) at resolution.

Image-processing and analysis: The first step is image segmentation, which must be capable of reliably separating the image into cartilage, bone, chondrocytes, lacuna, and background, as shown in Figure B. The background, potential chondrocytes, and lacuna are easily determined based on their appearance in an intensity histogram. Based on prior knowledge about the anatomy, it is possible to determine "certain" areas on bone and cartilage, that can be used for training of the Bayesian pixel classifier, making the algorithm robust towards varying staining intensity and imaging parameters. Various post-processing steps are used to remove isolated clusters of "bone" pixels in the cartilage region and vice-versa. Finally, prior knowledge about the shape of the cartilage regions can be used to exactly delineate the cartilage regions of relevance to further analysis, as seen in Figure C. This allows for a quantification of the regularity of the cartilage surface, using the fibrillation index defined by Pastoureau et. al.

Aim of the Study

Morphometric analysis of histological sections of the knee is essential for quantifying the degree of joint damage and drug efficacy in animal models of osteoarthritis (OA). It is of particular interest to measure the number of cells and the area of the cartilage lesions as they are manifested in the histological sections. Manual counting of cells is very time consuming, and scoring of cartilage loss is qualitative and subject to grader variability. The aim of the work presented here was to develop image analysis software capable of performing a fully automated segmentation of Hematoxylin/Eosin (H E) stained histological knee sections, thus allowing for measuring various morphometric variables, including cellularity and lesion area.



Screen dump of the VIS User Interface, illustrating how individual sections are segmented, and how results are computed. The computed results are stored in the system with the images, and can be invoked for further analysis through a built-in Excel mode.

The number of chondrocytes within the cartilage regions of the tibia and the femur must be counted. This cannot be done based on color alone, as adjacent chondrocytes might be counted as one instead of two or more. Therefore, the local bi-directional curvature can be measured in each pixel location in the image. It can be shown that the coefficients of an approximating spatial polynomial can be obtained as simple filter-kernels. Therefore, a simple filtering operation can be used for obtaining the second-order derivatives of the approximating polynomial (Figure E). Hence, the eigenvalues of the Hessian-matrix provides an entity related to the local bi-directional curvature, that clearly enhances chondrocytes (Figure F). Thresholding of these values in areas colored as chondrocytes, efficiently provides the number of chondrocyte profiles seen in the tissue section (Figure G).

Results

The algorithm described above was implemented in the VIS system, capable of automatically processing large batches of images. The database provides a full integration of image analysis with data management. The various drawing tools provide an easy way of editing and correcting those cases where the algorithm failed to provide a perfect result. The performance of the developed system is measured through the average time it takes an operator to complete the analysis of one image. With 100% manual demarcation, it took an operator an average of 15-30minutes to annotate one image. With the research tool developed here, the average time is ~30 seconds per image as illustrated in the table below.

Required operator time after automated analysis	Fraction of images
No time	40%
~ 30 Seconds	30%
~ 2 minutes	30%

Conclusion

If the promising first results of this automated image analysis method can be verified on a larger test set, a new and powerful research tool will be available to scientists for performing histomorphometric measurements much faster, and most likely with a higher degree of reproducibility. This will render histomorphometric measures as cellularity and joint-surface irregularity (fibrillation) feasible to consider in large-scale pre-clinical studies.