A METHOD FOR MEASURING VOLUME AND SURFACE-RELATED PARAMETERS ON MICROPHOTOGRAPHS BY USING FREE AND OPEN-SOURCE IMAGE PROCESSING SOFTWARE

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Introduction

There are different ways of obtaining volume and surface-related parameters for describing the micromorphology of different tissues, including cancellous bone. Histomorphometry and micro-computed tomography can be too costly for small laboratories and institutions, especially when proprietary software or expensive equipment is needed. Free and open-source software alternatives are available today. Some of these can be used for histomorphometry. Here we demonstrate how image processing and cancellous bone morphometry can be done by using freely available software.

Method description

This method was inspired by the paper by Egan et al. [1], however, we further replaced all of the proprietary and expensive software by using free and open-source alternatives. For measurements of the cancellous bone, images were first processed using open-source and free imaging software GIMP (GNU Image Manipulation Program) and measurements were performed by using FIJI software (FIJI is Just ImageJ), a distribution of ImageJ open-source image processing software [2, 3]. The measured parameters were: bone area (B.Ar), trabecular tissue total area (T.Ar) and trabecular bone perimeter (B.Pm). Using several mathematical formulas, we calculated the following parameters describing cancellous bone histomorphometry: trabecular bone volume (BV/TV, in %), trabecular bone surface (BS/TV, in /mm), trabecular thickness (Tb.Th, in mm), trabecular number (Tb.N, in /mm) and trabecular separation (Tb.Sp, in mm). These parameters are important in studies of bone development and quality. Also, simple measurements such as growth-plate thickness could be easily measured with FIJI (Fig. A). Using GIMP, a black and white mask was created on photographs of cancellous bone (Fig. B). A separate layer overlaying the original image was used, all the trabeculae were selected using the free select tool, and then tissues were colored differently using paint bucket tool (trabeculae colored black, all other tissues colored white area and artifacts, if present, colored red and later excluded from measurement). This black and white mask was opened in FIJI and the black areas were selected using the Wand (tracing) tool. After the desired parameters for measurement are selected in FIJI and pixel/micrometer ratio was set, trabecular perimeter and area were easily measured (Fig. C). For detailed protocol, see [4].

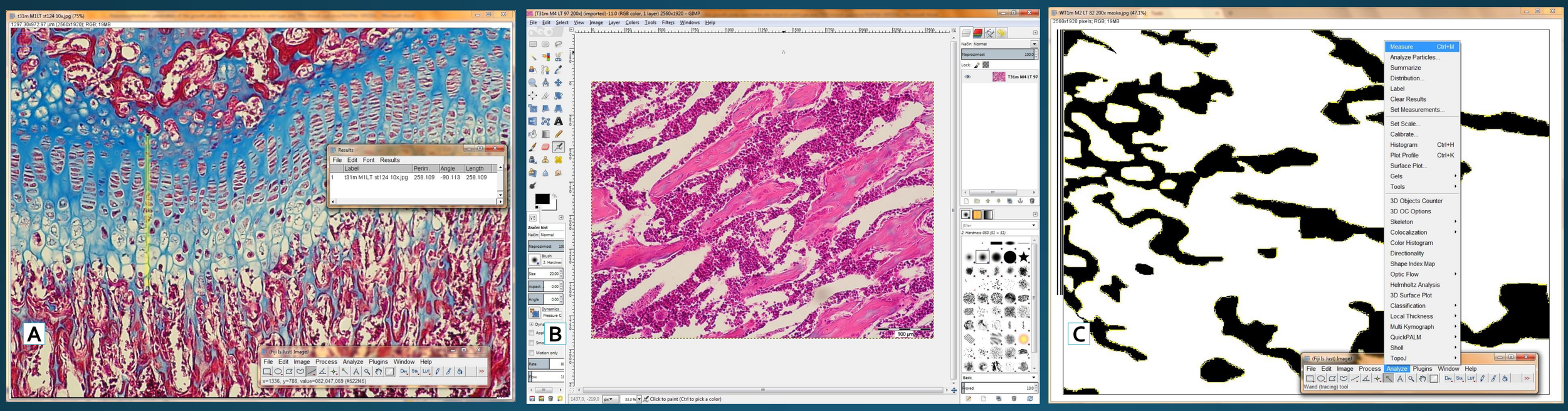

Figure A. Using FIJI to measure growth plate thickness. Figure B. Using GIMP for creating black and white masks from HE-stained tissue image. Figure C. Using FIJI to measure the cancellous bone parameters from a black and white mask created with GIMP.

Results and Conclusions

The abovementioned software has been successfully used to obtain and calculate the wanted parameters. There are many benefits of using freely available and open-source software for morphological research in life sciences. Some of them are the low cost, worldwide availability and transparency, involvement of research-field specialists in the development of such software and more. This work supports the notion that quality morphological studies can be performed using open-source and freely available software.

References

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