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Automated segmentation of synchrotron radiation micro-computed tomography biomedical images using Graph Cuts and neural networks

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ABSTRACT

Synchrotron Radiation (SR) X-ray micro-Computed Tomography (μCT) enables magnified images to be used as a non-invasive and non-destructive technique with a high space resolution for the qualitative and quantitative analyses of biomedical samples. The research on applications of segmentation algorithms to SR-μCT is an open problem, due to the interesting and well-known characteristics of SR images for visualization, such as the high resolution and the phase contrast effect. In this article, we describe and assess the application of the Energy Minimization via Graph Cuts (EMvGC) algorithm for the segmentation of SR-μCT biomedical images acquired at the Synchrotron Radiation for MEdical Physics (SYRMEP) beam line at the Elettra Laboratory (Trieste, Italy). We also propose a method using EMvGC with Artificial Neural Networks (EMANNs) for correcting misclassifications due to intensity variation of phase contrast, which are important effects and sometimes indispensable in certain biomedical applications, although they impair the segmentation provided by conventional techniques. Results demonstrate considerable success in the segmentation of SR-μCT biomedical images, with average Dice Similarity Coefficient 99.88% for bony tissue in Wistar Rats rib samples (EMvGC), as well as 98.95% and 98.02% for scans of *Rhodnius prolixus* insect samples (Chagas's disease vector) with EMANNs, in relation to manual segmentation. The techniques EMvGC and EMANNs cope with the task of performing segmentation in images with the intensity variation due to phase contrast effects, presenting a superior performance in comparison to conventional segmentation techniques based on thresholding and linear/nonlinear image filtering, which is also discussed in the present article.

1. Introduction

Synchrotron Radiation (SR) [1] facilities provide high brilliance X-rays with very high flux at small source size compared to tube X-rays, enabling investigations of samples in the micro- and even the sub-micrometer levels. Therefore micro-Computed Tomography (μCT) [2] obtained with SR X-ray conjugates several qualities for the investigation of biomedical structures such as high brilliance and high space resolution. Besides the characteristics resulting from the high coherence and monochromaticity of the beam, it is also possible to achieve the enhancing of contrast during imaging due to wavefield phase information, when absorption effects do not provide sufficient information to distinguish structures in, for example, media with low density, such as soft tissue or insects' biological structures. This interesting specificity of the SR, the *phase contrast* effect [3], is useful and important in many biomedical applications.

Thus, given the special characteristics of SR-μCT and the possibilities of the current third generation SR facilities, especially regarding their application in medicine and biology, image processing algorithms applied to SR-μCT segmentation are currently investigated. Traditional segmentation methods such as thresholding, possibly aided by morphological filters, may be highly sensitive to small parameter changes, and may cause loss of details; both phenomena are not desirable for analysis and/or quantification, and we also provide results that in fact corroborate this statement, showing that conventional methods based on thresholding and linear/nonlinear filtering do not cope with phase contrast intensity variation. In addition, the presence of a large number of 2D images (approximately 500 or 600 slices) in each SR-μCT scan, with also the large number of scans should be performed in biomedical research, would make the manual

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segmentation impractical, which leads the researches toward automated segmentation methods that cope with phase contrast intensity variation. Therefore, investigation on the application of state-of-the-art segmentation methods to μCT is an important research topic, due to the interesting characteristics and applications of that imaging modality.

As examples of the application of novel approaches to CT images, Krebs et al. [4] describe the segmentation of high resolution CT images using fuzzy approaches [5] for the assessment of trabecular distances. Meneses et al. [6,7] reported the application of Artificial Neural Networks (ANNs) [8,9] to the segmentation of SR-μCT biomedical images, and the proposal and the assessment of ANNs training strategies for segmentation with respect to SR-μCT bone images for histomorphometry applications [10].

Since the μCT imaging modality is only at its beginning [11], algorithms that yield high-quality results shall also be investigated and assessed. One of those state-of-the-art algorithms that yield remarkable results is the min-cut/max-flow algorithm [12–15], used for segmentation based on energy minimization. The energy minimization problem is relevant in many computer vision areas. As an example in medical imaging, Schneider et al. [16] use the min-cut/max-flow algorithm for energy minimization in their *annulus segmentation algorithm* for ultrasound imaging. The contributions of Boykov et al. [13], especially regarding the swap move and expansion move algorithms, represent a breakthrough in image segmentation research, yielding fast and accurate results in many image processing fields. For simplicity, we refer to the application of the min-cut/max-flow swap move algorithm for the minimization of energy as Energy Minimization via Graph Cuts (EMvGC).

For SR-μCT images, in the case of bone tissue segmentation, the results of EMvGC almost perfectly overlap manual segmentation, as expected and as we will discuss later. As an example of application, Fig. 1 depicts the 3D visualization of the sample E8D; Fig. 2 depicts the slice no. 305 (with normalization, the effects of the phase contrast effect become more pronounced). In Fig. 2, there exist basically three regions: bone, marrow (surrounded by the bony tissue region), and cartilage (outside the bone; seen in Fig. 1 as a sort of halo surrounding the bone).

For other types of images, such as those of biological soft tissues, phase contrast effects allow a better visualization of structures. Phase contrast effects cause variation in the intensity

Fig. 1. Three-dimensional visualization of dorsal portion of Wistar rat rib bone (sample E8D).

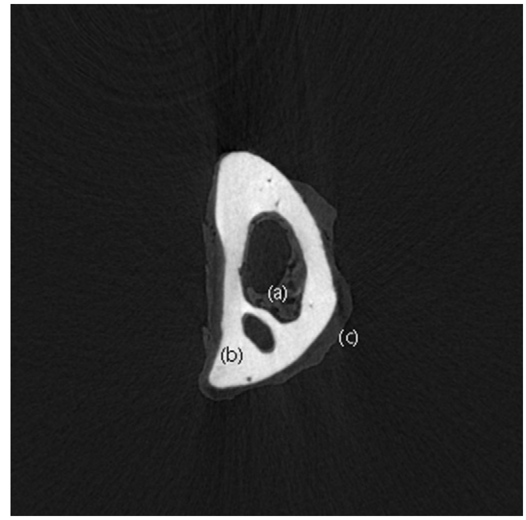

Fig. 2. Slice 305 of Wistar rat rib (bone from the dorsal portion; sample E8D): (a) marrow; (b) bone and (c) cartilage.

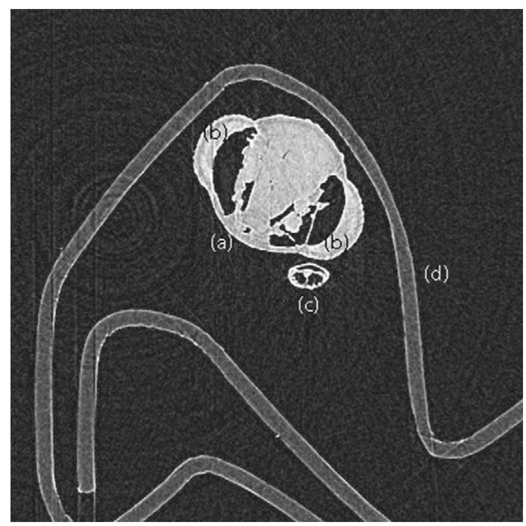

Fig. 3. Slice 183 of the insect *Rhodnius prolixus* (sample C4B): (a) head; (b) eyes; (c) proboscis (which was beside the body due to the preparation of the sample) and (d) plastic used in order to fasten the sample on the experimental setup.

at the edges of an object or region, basic characteristic for their great potential for analysis and visualization of biological microstructure [17,18]. In those cases, such as represented in Fig. 3, one slice of the μCT of the insect *Rhodnius prolixus* (also known as *kissing bug*), vector of the Chagas's disease (slice no. 183, sample C4B), the area of interest (light gray) has an intensity similar to the intermediate gray region (plastic used in order to fasten the sample on the experimental setup), or the insect's inner structures may have similar intensities. Notice the variation of intensity at the edges of the regions, with darker pixels in the background and lighter pixels on the insect's body and plastic regions.

Scans of bone samples, as the one depicted in Fig. 1, are relatively less complex for segmentation due to the contrast between the region of interest (bone) and the rest of the image. On the other hand, images such as the μCT scans of *Rhodnius prolixus* are more complex due to phase contrast effects, summed to the lack of contrast between regions, which in fact cause misclassifications during the segmentation performed by the EMvGC algorithm. Nevertheless it is possible to correct the results