Cuanto: A Tool for Quantification of Bone Tissue in the Proximity of Implants

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***Abstract*—Quantitative analysis of histological images of bone-implant samples is a important step in the evaluation of bone-implant integration. However, the quantification is a tedious task when carried out manually in the light microscope. To automatize the quantification, Cuanto, a software for measurements of bone area and estimation of bone-implant contact length in histological images of bone-implant samples has been developed. The quantification result of the software is compared to manual measurements; area measurements correspond well with the manual quantification whereas significant differences in the length estimation is observed. The possibility of zooming in down to cell-level when quantifying manually is believed to render the discrepancy.**

I. Introduction

Researchers involved in bone-implant integration studies often evaluate implants based on images of sectioned samples retrieved from guinea pigs (see Fig. 1). The amount of bone tissue in the proximity of the implant, especially at the immediate bone-implant interface, is an important indicator of the degree of bone-implant integration. The sections are histologically stained and are analyzed qualitatively as well as quantitatively in a light microscope. This step is time consuming and subjective, hence it is of great interest to replace this manual measuring process by an objective automatic method. Image analysis methods aiming to automatize the quantification have been developed (discussed in Sect. II). However, for these methods to be utilized by the biomedical researchers, easy to use software is needed.

There are a number of image analysis software that can be used for automatized quantification of histological images (e.g. ImageJ/NIH Image [1], Biopix [2], Bioquant [3], etc). However, these software have a general approach and cover analysis of images of various samples. Hence, the software does not predefine any regions of interest and these have to be selected manually, leading to a more time-consuming preparation for an automatic quantification.

In this work, *Cuanto*, a semi-automatic quantification application, tailored to quantify 2D histological images of bone-samples is presented. It has a specific approach which enables a simple and fast work flow. With this application, we aim to provide a tool for an objective quantification and reduce the time needed for the evaluation step and thereby allow the biomedical experts to focus on their main research.

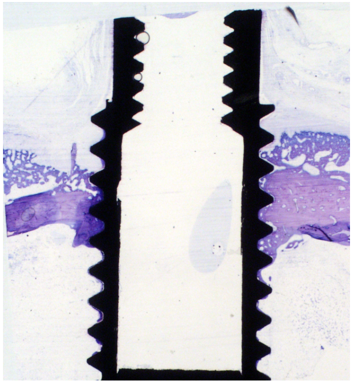

Fig. 1. Histologically stained section with the screw-shaped implant (black) and the surrounding tissues: bone (purple), and soft tissue (light blue).

II. Background

Automatic quantification of screw-shaped bone-implant samples is approached in [4], where an automatic (after an initial training step) method for measurement of bone area and estimation of bone-implant contact length is presented. Discriminant analysis is used to segment the bone tissue, soft tissue and implant. A comparison to the manual method shows that the area measurements are lower than the variation in the material and hence within acceptable error limits. However, the length estimations, on the other hand, differ significantly. This is due to misclassification of pixels in the interfacial region, where the intensity values of the implant and bone tissue are similar.

The segmentation method in [4] is intensity-based and does not include any spatial information. A method that makes use of the information about the spatial proximity of the pixels is iterative relative fuzzy connectedness (IRFC) [5]. Lindblad et al. introduce a two step segmentation of the mentioned images in [6]. The discriminant analysis of [4] is used to generate seed points for a subsequent IRFC-segmentation. The authors show improved results (both for area measurements and length

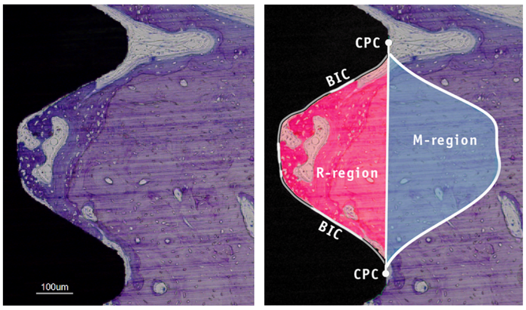

Fig. 2. Left: Images showing one thread of the implant. Right: marked ROIs: the gulf between two center points of the external thread crests (*CPC*) denoted *R* (reference area); the flipped R about the line connecting the two CPCs, denoted *M* (mirrored area) and regions where the bone is in contact with the screw, denoted *BIC*.

estimations) using the two step segmentation. However, the discrepancy between manual and automatic measurements, although reduced, still exists suggesting manual interaction is required to improve the results further.

III. Material and Methods

Screw-shaped implants of commercially pure titanium are retrieved from rabbit bone after 6 weeks of integration. This study is approved by the local animal committee at Göteborg University, Sweden. The screws with surrounding bone are processed in the laboratories according to internal standards and guide-lines [7], resulting in 10μm un-decalcified cut and ground sections. The sections are histologically stained prior to light microscopical investigations. The histological staining method used on these sections, i.e. Toluidine blue mixed with pyronin G, results in various shades of purple stained bone tissue: old bone light purple and young bone dark purple. The osteoid rims and osteoblast are stained in blue-gray tones. The soft tissue (for example muscle tissue) stains blue and in the soft tissue cavities in the bone the various cells occupying these areas are stained both light- and darker blue (Fig. 2). The images are acquired by a camera connected to a Nikon Eclipse 80i light microscope. The images used in the evaulation in this work have a pixel resolution of 2.2μm.

A. Region of Interests and Features

The following features for the quantitative analysis of 2D histological sections are presented in [8]: bone area ratio in the reference region, R, bone area ratio in the mirrored reference region, M, and bone-implant contact length ratio, BIC. Fig. 2b shows the regions of interest (ROIs) in a histological section. R is measured as the percentage of bone tissue area in the gap between two Center Points of the thread Crests (CPC) and M is measured as the bone tissue percentage in the out-folded region. (BIC) is expressed in percentage of the total length from one thread peak to another.

B. Software Description

Cuanto implements the quantification method described in [4] as a Microsoft Windows application.

Additional functionalities are added to the software, an important one being the result manipulation. The automatic quantification methods proposed in [4] and [6] are shown to overestimate the length estimation (BIC length) compared to the manual quantification [4] due to incorrect segmentation. To reduce the overestimation, the possibility of manually modify the segmentation result is provided. Another functionality that has been included allows the user to expand the implant and create an virtual implant interface, see Fig. 3. Furthermore, tools for manually marking regions of interests have been implemented. The functionallities enables extraction of additional information about the bone-implant integration

C. Graphics User Interface

A graphical user interface (GUI) has been designed using the guidelines presented in [9]. The GUI is developed with respect to usability principles and theories within human computer interaction. Usability tests (using usability inspection tools) are conducted on users with varied degree of computer experience. Test results are used to achieve a easy to use interface. The user interface framework provided by QT [10] is used as a GUI platform. It features modules for a number of common interaction tools, such as buttons, tabs, frames, etc, as well as geometrical shapes and displaying images. Additionally, it also offers non-GUI modules for e.g., file-handling, networking and XML parsing.

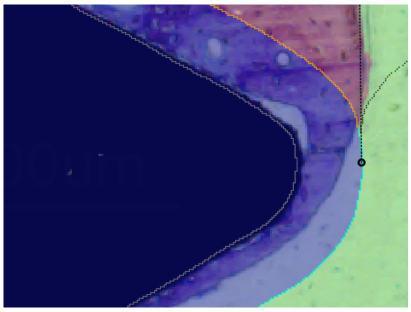

Fig. 3. Segmentation result superimposed with an opacity of 50%. The user has chosen to mark the implant as blue, the soft tissue as green and bone tissue as red. The implant has been expanded with 20 pixels, i.e., the implant is dilated, generating a virtual interface-line (orange and cyan showing bone tissue contact and non-contact respectively)

D. Work Flow

The quantification consists of four steps:

1) Image import. Libraries for loading common microscopy formats (such as ZVI, LIFF, OIF and CS2) have been developed in order to facilitate the number of steps from acquisition to analysis.
2) Training. Small regions belonging to different tissue types are marked in one of the images in the set.
3) Quantification. The images to be quantified are selected. A few parameters can be tuned in this step (optional).
4) Result manipulation. Incorrect classified regions can be marked in the segmented image and corrected. The segmented image, with the segmented regions

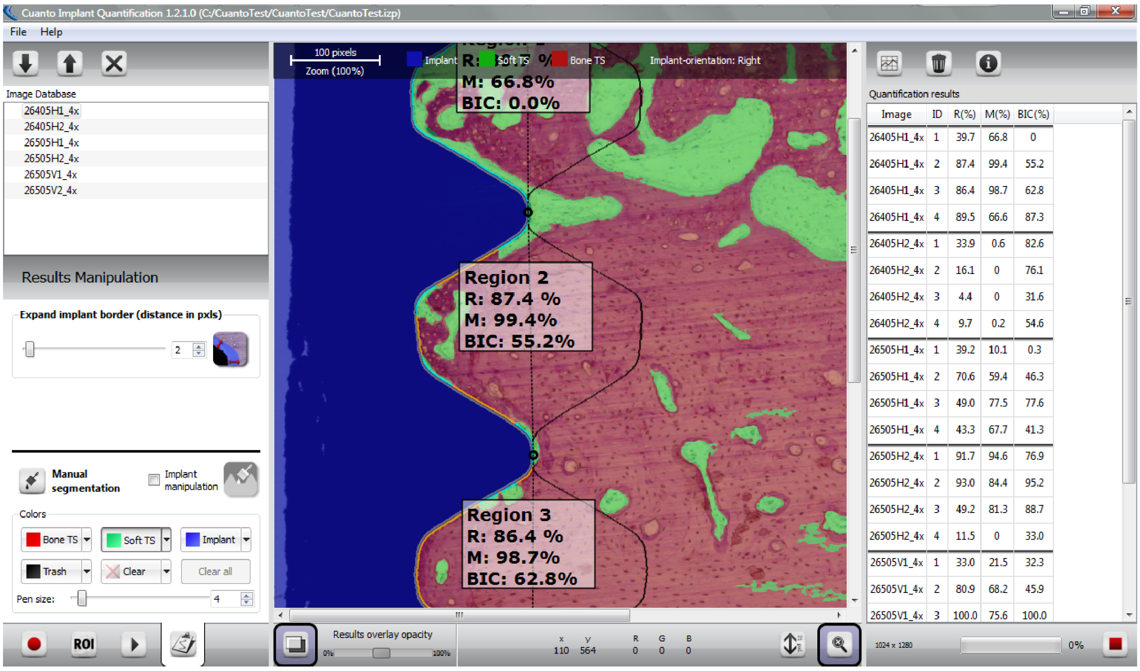

Fig. 4. Snapshot of Cuanto after the quantification and result manipulation step. The segmentation result is superimposed on the original with an opacity of 50%. The user has chosen to mark the implant as blue, the soft tissue as green and bone tissue as red (the desired colors can be chosen from the "Colors toolbar" on the left side). The implant is expanded with 2 pixels. The quantification result is presented in the table in the right frame.

shown in colors chosen by the user, is superimposed on the original image. The opacity of the superimposed segmented image is changeable which facilitates the identification of misclassified regions. The manipulated result is saved so the user does not need to modify the segmentation again next time the same image is quantified.

After the analysis, the quantification result is shown as a table which can be exported to a spread sheet application for further presentation.

IV. Evaluation and Results

Three sections (8 threads each) have been quantified using Cuanto (semi-automatic quantification with manual correction of the segmentation) and the method proposed by Lindblad et al [6] (automatic quantification) as well as manually by an expert. The results, shown in Fig. 5 , are illustrated by scatter plots and the correlation coefficients ρ between the respective method and the manual classification, as well as the coefficient of determination R^2 for the features. Furthermore, Table I summarizes the averaged absolute difference of the results between the semi-automatic and manual quantification and the averaged result of them.

The following timing observation is made: the training takes about 1 minute for a set and the quantification takes 10 seconds for each section on a computer with a Intel Core 2 Duo 1.74 GHz processor and 2 GB of RAM. The modification of the segmentation result is performed by a non-expert and takes about 2 minutes per sections for BIC-correction and about 0.5 minute per section for area correction.

TABLE I

THE AVERAGED ABSOLUTE DIFFERENCE OF THE RESULTS BETWEEN THE METHODS AND THE AVERAGED RESULT OF THE TWO METHODS FOR THREE SCREW SECTIONS (EIGHT THREADS/SECTION) FOR FEATURE f. f_{Δ} IS CALCULATED AS $\frac{1}{n}\sum_{i=1}^{n}|f_{Ci}-f_{Mi}|, n=8$, WHERE f_C AND f_M IS THE FEATURES QUANTIFIED MANUALLY AND BY CUANTO RESPECTIVELY

f	f_{Δ}	$\overline{f_C}$	$\overline{f_M}$
BIC	12.8	59.6	47.7
R	3.1	49.2	44.2
M	1.9	44.2	43.2

V. Discussion

The results show that the semi-automatic area measurements (features R and M) is highly correlated to the manual quantification. However, the BIC-length estimation is consistently overestimated. We believe that this dissimilarity is due to misclassification of interfacial regions where there is a implant loosening and the tissue is very darkly stained. These regions can easily be mistaken to have bone-implant contact with the implant, both by the automatic method and the user modifying the results. When analyzing the same region in the microscope, where there is a possibility of zooming in closer, it is possible to determine whether there is contact or not.

Availability

A demo version of the software is available at `http://www.izolde.se`.

Acknowledgment

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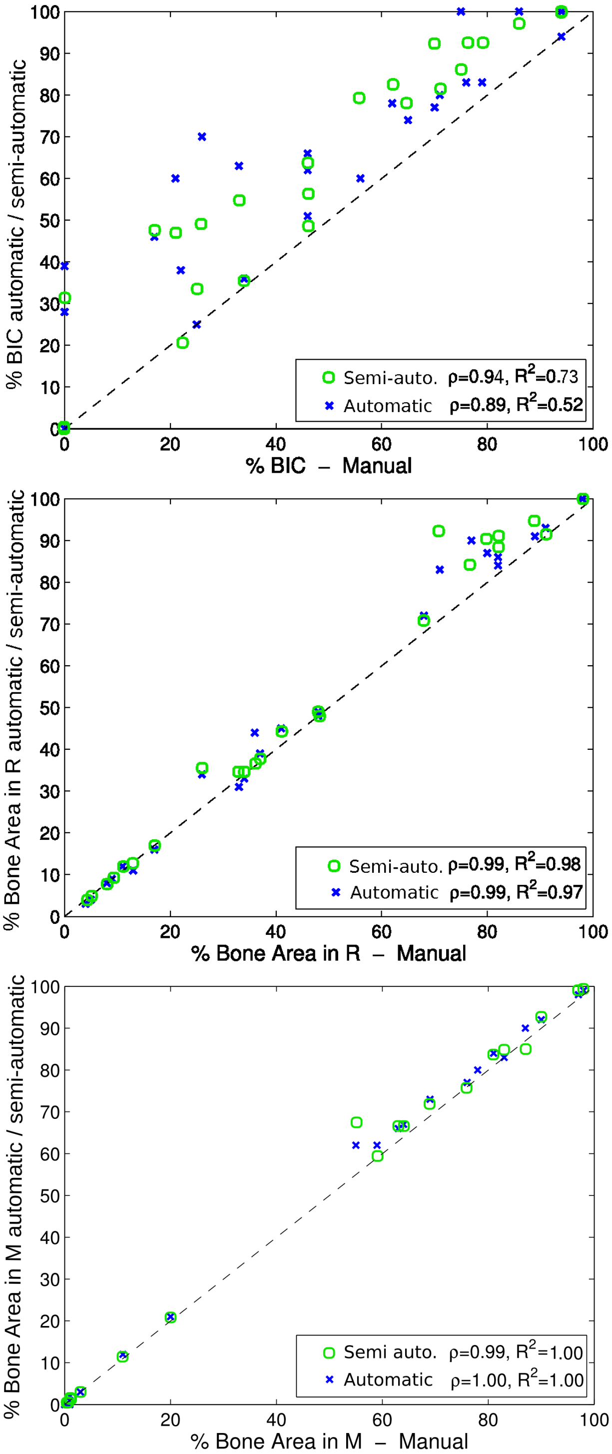

Fig. 5. Comparison of the BIC, R and M feature

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