Validation of X-ray Micro-CT as Screening Tool for Bone Tissue Engineering Scaffolds.

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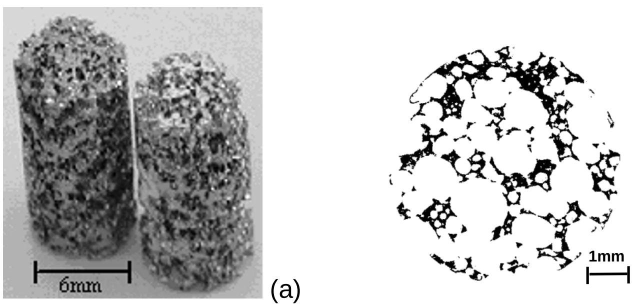
INTRODUCTION

Bone scaffolds, based on biodegradable ceramics, polymers, composites of both or bio-inert metals, form an innovative solution for repairing large bone defects. To initiate the growth of bone tissue inside such structures, they need to meet certain demands, such as biocompatibility, favourable cell-material interactions, optimal pore structure and pore dimensions, mechanical properties equivalent to bone, etc. Thus, the success of bone scaffolds in bone tissue engineering largely depends on their design. Microfocus X-ray computed tomography (Feldkamp 1984; Ruegsegger 1996; Sasov 1987) (micro-CT) provides a means to acquire a complete 3D image of the structure, visualizing the internal architecture at the microscopic level in a non-destructive way. Therefore, it is a suitable tool for screening the structure of bone scaffolds. It is recently also put forward as a non-destructive, 3D imaging tool to quantify bone formation in and around scaffolds and implants.
However, since it is known that artefacts are inherently present in micro-CT images and since segmentation possibly causes errors in the structural analysis, the accuracy and reliability of the technique needs to be assessed. This study provides a protocol for 2D comparison between micro-CT and microscopy and assesses the effect of the sample material and threshold on the accuracy and reliability of the binarized micro-CT data.

MATERIALS AND METHODS

Materials

In this study, cylindrical porous titanium (Ti) structures with thin struts (figure 1) are selected as a first material to investigate the validation protocol. Two other types of scaffolds are at present still under investigation: biodegradable polyester-based scaffolds containing alpha-tricalcium-phosphate particles (PH-αTCP) and biodegradable hydroxyapatite (HA) scaffolds. The Ti cylinders have a mean radius of 3.00 ± 0.05 mm and a mean height of 10.0 ± 0.1 mm [supplied by VITO (Mol, Belgium)]. They are produced by gel casting (Impens 2005; Snijkers 2005), resulting in porous structures with struts ranging from 20 to 100 µm, pores ranging from 50 to 500 µm and a global porosity of about 80 %. The broad range in strut thickness and pore size is preferred to address structural elements much larger than the attainable spatial resolution together with the smallest possible structural elements to be visualized by our micro-CT device in the same sample.



(b)

Figure 1. a) A typical cylindrical porous titanium structure with a mean radius of 3.00 ± 0.05 mm, a mean height of 10.0 ± 0.1 mm and a global porosity of about 80 %, and b) an optical light microscopy image of a typical cross-section of such a sample.

Validation protocol

In 2D both the amount of material as well as its spatial distribution is verified by matching interpolated micro-CT images of the scaffolds to the corresponding microscopic sections. The validation protocol can be divided into six parts as shown in figure 2, namely (1) acquisition of a set of micro-CT images of the full sample, (2) metallographic preparation of the sample and digitizing by microscopy, (3) fitting a micro-CT image to the microscopic image, followed by the reconstruction of an interpolated micro-CT image when required, (4) registration of the microscopic to the interpolated micro-CT image, (5) binarization of the interpolated micro-CT image and (6) matching the interpolated micro-CT to the microscopic image. The visualization and binarization error in the micro-CT images is defined by the percentage overlap, overestimation and underestimation with respect to reality which are determined from the overlay images.

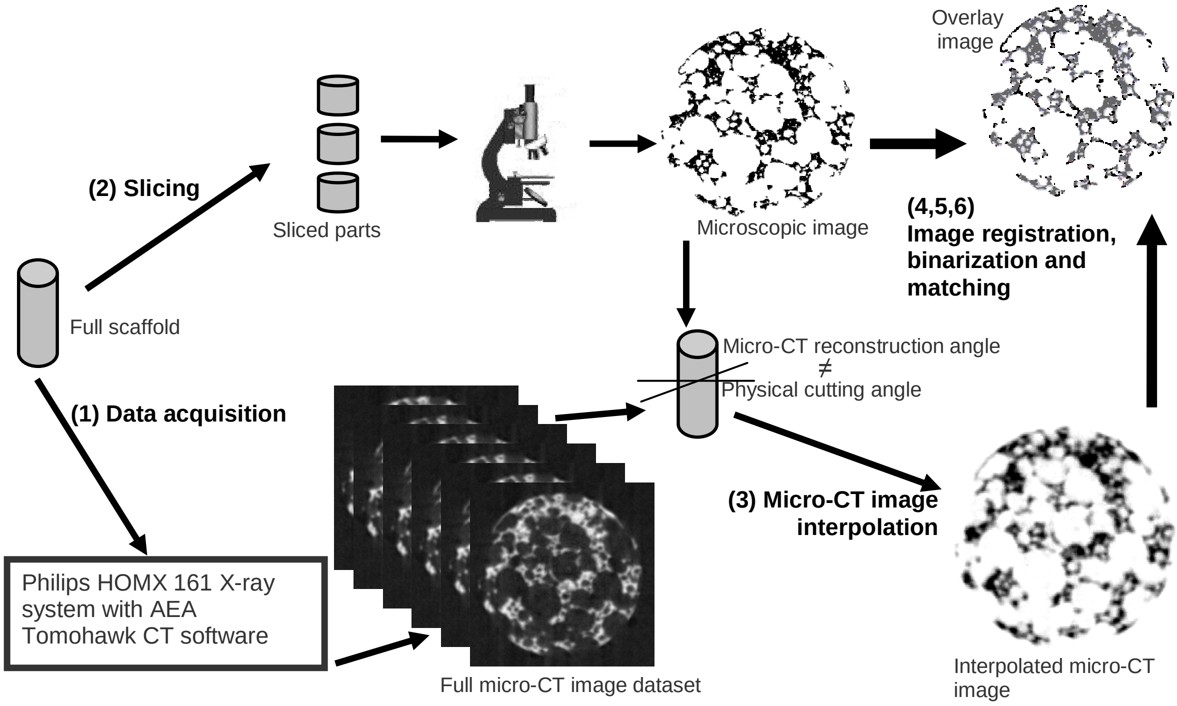


Figure 2. Schematic overview of the validation protocol consisting of six parts.

Thresholding method

In this study, a novel thresholding method is developed based on matching microscopic with their corresponding, interpolated micro-CT images. Microscopic images are 2D entities consisting of pixels (picture elements). Micro-CT images on the other hand are 3D entities expressed in voxels (volume elements) with a certain thickness. In this research the thickness of the micro-CT images is kept minimal which results in cubic voxels, thus no averaging is made over the thickness. To prevent confusion, from now on the pixels in the microscopic images and the voxels in the micro-CT images are referred to as ‘elements’.

When overlaying both the microscopic and their corresponding, interpolated micro-CT images, the optimum is represented by a maximum in coinciding and a minimum in non-coinciding solid elements. By altering the threshold value for the micro-CT images, this optimum can be approximated. Per set of microscopic and corresponding, interpolated micro-CT image (= image set), the threshold approximating the optimum the closest, named the ‘best’ threshold, is determined. By averaging the ‘best’ threshold values over a significant amount of image sets, the ‘optimal’ threshold is defined. This ‘optimal’ threshold is only valid for the selected material, micro-CT device and acquisition parameters. If one of these settings changes significantly, a new ‘optimal’ threshold needs to be determined.

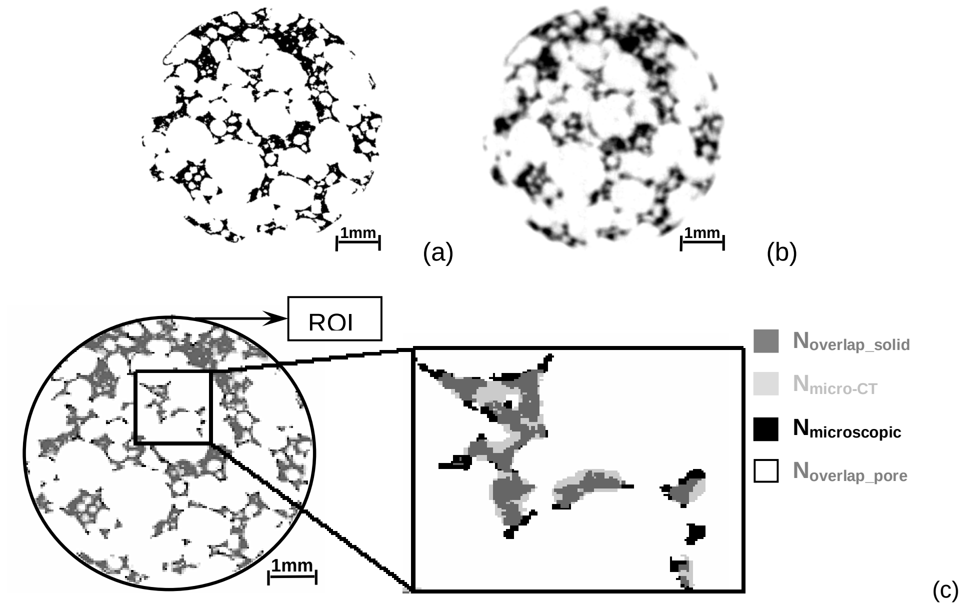


Figure 3. For a particular titanium porous sample (Φ 6mm): (a) the microscopic image of a metallographic slice, (b) the corresponding, interpolated micro-CT image and (c) the resulting match of both.

One of the major advantages of this novel thresholding method is that it accounts for closed pores, in contrast with the Archimedes method (Ding 1997, 1999). Also, since the physical visualization of the structure is taken as a reference, it results in binary images closely representing the real structure. It is a time consuming procedure, but once the 'optimal' threshold is determined for certain settings, it can be used for further research. Still, an important limitation of the method is that it is a global thresholding method and hence it does not locally account for the errors present in micro-CT images due to the limited spatial resolution, partial volume effect (PVE) etc. However, since this thresholding method is linked to the proposed validation protocol, this error can be quantified.

RESULTS AND DISCUSSION

In total 36 interpolated micro-CT images are matched to their corresponding microscopic images. Figure 3 shows an example of (a) a microscopic image of a metallographic section, (b) the corresponding, interpolated micro-CT image and (c) the resulting match of both where the overlapping pixels are displayed in dark grey, the non-coinciding microscopy image pixels in black and the non-coinciding micro-CT image pixels in light grey. White represents pore space. In this case, the ROI is defined as the circle with a radius of 3.00 mm surrounding the material present in both the microscopic and the corresponding, interpolated micro-CT image.

To get an overall view of the error made by micro-CT and binarization, all elements (solid and pore) within the ROI are accounted for. The 'optimal' threshold value is applied to binarize the 36 interpolated micro-CT images. For all 36 image sets the total overlap, overestimation, underestimation and total mismatch is determined and the results are summarized in table 1.

Table 1. Mean percentage in total overlap, total micro-CT mismatch, total microscopic mismatch and total mismatch.

Total overlap	89.1 ± 1.4 %
Overestimation micro-CT	7.8 ± 1.5 %
Underestimation micro-CT	2.8 ± 0.7 %
Total mismatch	10.9 ± 1.4 %

CONCLUSIONS

When using microscopy for characterizing porous structures, one is limited by its 2D character and a critical, time-consuming sample preparation. Therefore, micro-CT is put forward as the solution for 3D characterization of porous structures. However, to ensure and quantify the reliability and the accuracy of the micro-CT images, a validation protocol is developed by matching micro-CT tomograms to microscopic images. Microscopy is chosen as the 'golden standard' because of its physical character, its well-known procedure and its better resolution with respect to standard micro-CT. When high resolution micro-CT or nano-CT (standard or synchrotron) images are validated, SEM images can be applied to guarantee a superior resolution with respect to the CT images. The main advantages of the proposed validation protocol are first the opportunity to interpolate a micro-CT image under the same angle as the physical cutting angle of the microscopic sections, second the novel thresholding method and third the more precise and detailed quantification of the visualization and binarization error present in the micro-CT images.

In this study, as a proof of principle, the validation protocol is applied to porous titanium structures. Ceramic and polymeric scaffolds are at present under investigation. When scanning the samples on a Philips HOMX 161 X-ray system with AEA Tomohawk CT software and binarizing the micro-CT images with the determined 'optimal' threshold, a total overlap of about 89 % and a total mismatch of about 11 % are found. The total mismatch consists of about 8 % overestimation and about 3 % underestimation of the real structure. It has to be mentioned that, if only the solid fraction would be considered for validating micro-CT, one would conclude that micro-CT only overestimates the structure by about 5 %.

Thus, it is concluded that, despite the morphological complexity of the titanium samples and the variety in structural feature dimensions, a good match is found between microscopy and micro-CT. Additionally, the mismatch is quantified in a detailed manner providing the percentage of overestimation and of the structure not visualized by micro-CT. It is shown that metal porous structures can be analysed with sufficient accuracy by means of *standard* micro-CT. However, due to for example the limited spatial resolution and the high attenuating character of the metallic samples, a significant mismatch is found which needs to be accounted for when performing image-based structural analysis.

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