



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

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Framework: <u>High throughput</u> screening of bone scaffolds





Materials

Naked scaffolds



Ti bone scaffolds

HA bone scaffolds



Composite bone scaffolds

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Materials

- Cylindrical or squared porous Ti structures (ø-h: 6-12 mm or w-h: 10 mm)
- Produced by gel casting
- 25 vol% Ti-powder + 3 wt% dispersion agent + foaming agent dispersed in distilled H₂0
- Pore diameter can be adjusted to obtain the desired pore size distribution by altering the composition of the slurry and the stirring time
- Altering the sintering temperature influences the final mechanical and structural scaffold properties





 \Rightarrow Pore size:50 - 500 μ m ; Strut size: 20 - 100 μ m ; Global porosity: 80%





Framework: <u>High throughput</u> screening of bone scaffolds





Protocol for morphological quantification

Step 1: Protocol for acquisition and reconstruction of micro-CT images for different material classes

Step 2: Validation protocol and coupled to this a novel global thresholding method to define the visualisation and binarization error in the binary images







Experimental protocol

Interpolation of the micro-CT image

 \rightarrow Finding the corresponding micro-CT image in the dataset







Matching

- Automatic image registration (Maes et al. 1997, 2003 KULeuven)
- Result = overlapping binarized images





Thresholding method

Overlap and mismatch: influence of threshold



120

100 80

> 60 40

20

Reference

threshold

Total Mismatch (%)



3D quantification of bone formation: flow chart





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Results: Overlap and total mismatch

Overlap, overestimation and underestimation for naked Ti scaffolds





Protocol for morphological quantification

Step 1: Protocol for acquisition and reconstruction of micro-CT images for different material classes

Step 2: Validation protocol and coupled to this a novel global thresholding method to define the visualisation and binarization error in the binary images

Step 3: What is the influence of the resolution on the novel thresholding method and what is the effect on the visualisation and binarization error ?????

Lower resolution is wanted/required:

- when the sample is larger

- when limited CPU and memory is available



Spatial resolution

= a measure of how closely details can be resolved in an image (Wikipedia)



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Partial Volume Effect (PVE)

= the effect wherein insufficient image resolution leads to a mixing of the different material types present in the image within a voxel









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Influence of the resolution

- If the resolution goes down, it is inherently known that the total mismatch increases
- Three options for binarizing images with lower resolution:
 - A) Use the same threshold as was determined 'optimal' for the highest resolution
 - B) Use the same technique as described previously (minimizing the total mismatch) for different resolutions
 - **C) Keep the overlap and underestimation constant**





Overlap and total mismatch for different thresholding methods



B) Use the same technique as described previously (minimizing the total mismatch) for different resolutions C) Keep the overlap and underestimation constant



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Results

Optimal threshold and mutual mismatch in function of the pixel size for the different thresholding methods



A) Use the same threshold as was determined 'optimal' for the highest resolution

B) Use the same technique as described previously (minimizing the total mismatch) for different resolutions C) Keep the overlap and underestimation constant



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Results

3D analysis and influence of the mismatch





Discussion and conclusions

- A validation protocol is developed by matching micro-CT tomograms to microscopic sections
- The validation protocol has 3 main advantages against existing protocols:
 - Opportunity for interpolation of the micro-CT images
 - A novel thresholding method was developed
 - A more detailed quantification of the visualization and binarization error
- Despite the morphological complexity of the Ti samples and the variety in structural feature dimensions → sufficiently accurate quantification via micro-CT
- The mismatch found needs to taken into account in the structural analysis





Discussion and conclusions

- When decreasing the spatial resolution, the threshold cannot be kept constant → linear decrease in threshold
- Decreasing resolution results in a larger mutual and total mismatch
- When looking at 3D structural analysis, the best method for thresholding is looking for the combination of a maximum in overlap and a minimum in total mismatch
- Of course: the less the resolution will be, the less accurate the images





Discussion and conclusions

Validation and thresholding protocol

Linear functions for threshold and mismatches in function of the spatial resolution

When acquiring images on other resolutions, you can determine the 'optimal' threshold and its corresponding mismatches





Thank you for your attention!!!



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