Adaptive thresholding
in CTAn

Method note
Introduction

2D or 3D morphometric analysis of micro-CT images always requires binarization (also known as segmentation or thresholding) of the dataset as a first step: 8- or 16-bit (grey scale) images are transformed into 1-bit (black-white) images. Thresholding, besides image quality and resolution of course, will have a large impact on 2D or 3D analyses, and give false results when done improperly.

The most common thresholding algorithm is a global threshold, which is available in CTAn from the binary page as well as the custom processing page. However, global thresholding is not always sufficient for an adequate segmentation. Samples that contain both thick and thin structures of the same type of material are difficult to threshold due to the partial volume effect: thin structures appear to have a lower density compared to thick structures with the same chemical composition, especially at low resolution. This is for example the case in long bones where the thick surrounding structure represents the bone cortex, whereas the thin bony structures on the inside represent trabecular bone (see Figure 1). The same is true for example for foam structures where cell walls can be very thin, while the cell corners are much thicker. In such cases, a more advanced thresholding method has to be applied: adaptive thresholding.

This method note will illustrate how to perform a correct thresholding using bone cross-sectional images as an illustration. Figure 1 shows a cross-sectional image of a murine long bone scanned in vivo, showing both the thick cortical bone on the outside, and the fine trabecular bone on the inside. Global thresholding of this dataset is unfortunately not adequate. Setting a threshold from (in this case) 50 to 255, to include all fine trabecular bone, results in an overestimation of the thicker structures including the cortical bone. Setting a threshold from (in this case) 90 to 255 will more precisely threshold the thick bone structures, but underestimate the fine bone structures (Table 1). The blue arrows indicate either overestimation of thick structures (threshold 50-255) or underestimation of thin structures (threshold 90-255). As mentioned above, this problem is related to the partial volume effect.
Figure 1. Cross-sectional image of an *in vivo* scanned murine long bone.

Table 1. Global thresholding of a bone cross-section.

<table>
<thead>
<tr>
<th>Global thresholding (50-255)</th>
<th>Global thresholding (90-255)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Global thresholding (50-255)" /></td>
<td><img src="image2" alt="Global thresholding (90-255)" /></td>
</tr>
</tbody>
</table>
**Adaptive thresholding**

To circumvent the segmentation artefacts caused by global thresholding, an adaptive thresholding algorithm will be applied which is available from the thresholding plug-in in the custom processing tab (see screenshot below).

![Threshold settings](image)

You configure the adaptive thresholding by choosing to run the adaptive thresholding in 2d or 3d space, setting the correct background colour, choosing a pixel radius of image processing, and choosing a “constant” offset value.

Adaptive thresholding can be applied in 3D (analyses sphere around each voxel) or 2D (analyses circle around each pixel). The 3D method is more accurate but takes significantly more processing time. The radius defines the circle/sphere in which the threshold is calculated, using one of the three methods (median, mean, mean of min. and max.). The constant applies an offset to the minimum density contrast recognised by the thresholding. Increasing the constant can remove noise-associated structures.

Note that a pre-threshold can be applied. The pre-threshold effectively delineates a region of interest in which adaptive thresholding is applied. All pixels outside the pre-threshold white mask are excluded from the
adaptive thresholding and set as black (space). Note that when a pre-threshold is applied, you do not need to set a constant (the constant value can be left at zero).

A certain amount of trial-and-error is needed to get a correct result. In this specific case, the adaptive threshold (mean of min and max values) was run in 3D with a pre-thresholding of 50-255 and a radius of 3. The result is shown in table 2. Comparing this result to the global thresholding (table 1) shows that fine structures are accurately segmented without overestimation of the thick structures.

Table 2. Adaptive thresholding of a bone cross-section.

<table>
<thead>
<tr>
<th>Raw image</th>
<th>Adaptive threshold</th>
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</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Raw Image" /></td>
<td><img src="image2.png" alt="Adaptive Threshold" /></td>
</tr>
</tbody>
</table>

When running an adaptive threshold, be careful to avoid the appearance of false voids inside solid objects. This segmentation artefact can readily occur in this type of adaptive thresholding if parameter values are not correct – e.g. if radius is too small. They are indicated in table 2 by the blue arrows. In case the appearance of false voids can’t be avoided, one can switch to a third type of thresholding: double or combined thresholding. (In the future, the availability of an upper pre-threshold level will also solve this issue. See below.)
**Combined thresholding**

Combined or double thresholding is a combination of adaptive thresholding and global thresholding. First, the adaptive thresholding is run as described above. In a second step, this result is copied to the region of interest by running a bitwise operation ‘region of interest = COPY image’.

Next, the image is reloaded and a global threshold is run to adequately select the high dense structures in the images (in this case 90-255 according to table 1).
Finally, both the result of the adaptive threshold (in the region of interest view) and the global threshold (in the image view) are combined by running the bitwise operation 'Image = Image OR Region of interest'. The result of this double thresholding can be found in table 3. Comparing the images with the global thresholding (table 1) and adaptive thresholding (table 2) shows that the combined thresholding results in adequate segmentation of both the thick and thin structures in the image without creating false voids in solid objects. Whether you first run an adaptive threshold followed by a global threshold, or the other way around doesn’t matter.

![Bitwise operations](image)

Table 3. Combined thresholding of a bone cross-section.

<table>
<thead>
<tr>
<th>Raw image</th>
<th>Combined threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Raw image" /></td>
<td><img src="image" alt="Combined threshold" /></td>
</tr>
</tbody>
</table>
NOTE:
From (future at time of writing) CTAn version 1.13.0.0 onwards, the adaptive thresholding plug-in also allows specifying an upper pre-thresholding level and avoids running a double thresholding. One will thus specify a threshold range (between the pre-threshold low and pre-threshold high greyscale values) in which the adaptive thresholding is performed. All greyscale values lower than the pre-threshold low value are set by default as background, while greyscale values higher than the pre-threshold high value get selected by default. In this specific case, one would run an adaptive threshold with pre-threshold values of 50 (low) and 90 (high).