

# Technical Note: CT Thresholding Protocols for Taking Measurements on Three-Dimensional Models

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Computed tomography (CT) is a powerful analytical tool that is becoming increasingly popular for investigating small and difficult to visualize (internal) structures. However, along with this capability come numerous potential difficulties. One vexing problem is how to interpret the images that result from CT scanning. A typical CT slice displays a spectrum of gray-scale values that represent the densities of the structures in the image. To measure a particular structure it is necessary to establish a threshold value (i.e., gray-scale value), which clearly distinguishes the structure of interest from surrounding structures. However, determining the proper threshold is not a trivial task. One problem arises from the fact that the boundaries between adjacent structures (e.g., bone and air) are never clearly defined, but are instead represented by a continuum of gray-scale values. Another problem is related to the viewer control settings (window and leveling values), which can greatly affect the visual appearance of images, particularly along the edges of structures. Simply basing of the threshold value on the apparent (visual) boundaries has been shown to yield erroneous results (Koehler et al., 1979; Baxter and Sorenson 1981; Hara et al., 2002).

A handful of studies have sought to investigate this problem, and these have led to several proposals regarding the appropriate method for establishing the threshold value. One approach that has enjoyed increasing attention is the half-maximum height protocol (HMH), which calculates the threshold value as the mean of the maximum and minimum gray scale values along a row of pixels that spans the boundary transition (Ulrich et al., 1980). This method has been found to produce accurate measurements of modern human vertebrae (using regular CT images—Ulrich et al., 1980; Baxter and Sorenson 1981; Seibert et al., 1981; Eubanks et al., 1985) and of extant and fossilized long-bones and teeth (Spoor et al., 1993). More recently, Fajardo et al. (2002) used a variation of the HMH method (applied to high resolution X-ray computed tomography) to accurately reconstruct and measure trabecular architecture of long bones. These researchers also found that it was important to sample the appropriate region of interest because bone types of different density (e.g., cortical versus trabecular) yield different HMH values (Fajardo et al., 2002).

One of the limitations of these studies is that the HMH protocol and validation measurements were applied to individual slices. However, when a researcher wishes to take measurements on three-dimensional models created from CT, a single threshold value must be applied to the slices of interest. One potential concern is

that applying a single threshold value to a dataset of dozens or hundreds of slices may not accurately represent the real morphology of various structures. Thus, this study sought to investigate the effects of taking linear measurements on three-dimensional models using a modified version of the HMH protocol.

To examine the accuracy of the thresholding protocol, measurements were taken from high resolution X-ray computed tomography data and compared with measurements taken on dried specimens. The oval window was selected as the variable of interest because it represents a relatively small structure and also because it is possible to obtain these measurements on the dried skulls of certain taxa with a reasonable amount of accuracy. A Zeiss Discovery V.12 stereo digital microscope was used to take the measurements on the dried specimens (hereafter referred to as Zeiss-based measurements).

All scanning was done at the University of Texas High-Resolution X-ray CT facility. These scans were taken with a 68- $\mu\text{m}$  slice thickness and 68- $\mu\text{m}$  interslice spacing. The images were reconstructed from 1,000 views and the field of view was 64 mm yielding a pixel size of 62.5  $\mu\text{m}$  (1024  $\times$  1024 pixel matrix). The final images were 16-bit TIFF files. The image stacks were imported into ImageJ 1.35f (NIH), cropped, converted to 16-bit signed files (to be compatible with the measurement software), and saved as raw stacks. These stacks were then used to construct three-dimensional models using 3D Slicer 2.6 and the measurements were taken using the fiducials module. This module allows the researcher to place markers (fiducials) anywhere on the model and take linear measurements between the markers. It is useful since it permits structures to be measured that are not in the same plane. Each measurement was taken three times and the mean of the three measurements was taken as the final value.

Grant sponsor: National Science Foundation; Grant numbers: NSF BCS-0408035, NSF BCS-0109130; NSF BCS-9904925, and NSF EAR-0345710.

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Received 13 October 2006; accepted 8 January 2007

DOI 10.1002/ajpa.20583

Published online 26 February 2007 in Wiley InterScience (www.interscience.wiley.com).

TABLE 1. Oval window measurements taken using three different procedures as described in the text

Genus	Specimen	Zeiss length	HMH length	Visual length	Zeiss width	HMH width	Visual width	Zeiss area	HMH area	Visual area
Alouatta	Nal 13	1.90	1.88	1.78	0.95	0.92	0.80	1.42	1.36	1.12
Aotus	Nao 1	1.30	1.28	1.11	0.63	0.60	0.43	0.64	0.60	0.37
Aotus	77299	1.31	1.30	1.15	0.64	0.67	0.56	0.66	0.68	0.50
Callithrix	15915	1.22	1.19	0.89	0.59	0.60	0.38	0.57	0.56	0.27
Callithrix	133688	1.16	1.18	1.14	0.64	0.63	0.56	0.58	0.58	0.50
Cebus	Ncb 9	1.67	1.65	1.56	0.85	0.86	0.78	1.11	1.11	0.96
Erythrocebus	19003	1.96	1.97	1.73	0.80	0.80	0.67	1.23	1.24	0.91
Erythrocebus	34852	2.09	2.09	1.94	0.92	0.93	0.88	1.50	1.53	1.34
Galago	81458	1.16	1.14	0.90	0.57	0.60	0.43	0.52	0.54	0.30
Macaca	103730	1.87	1.90	1.79	0.72	0.77	0.67	1.06	1.15	0.94
Nycticebus	183827	1.16	1.16	1.12	0.67	0.70	0.65	0.61	0.64	0.57
Perodicticus	52704	1.15	1.15	1.08	0.66	0.66	0.57	0.60	0.60	0.48
Perodicticus	52700	1.34	1.30	1.24	0.71	0.73	0.64	0.75	0.75	0.62
Saimiri	NSm 7	1.29	1.29	1.15	0.62	0.58	0.45	0.63	0.59	0.41
Percent diff			1	11		3	21		3	37
Mean diff			0.02	0.14		0.02	0.11		0.03	0.19
Max diff			0.04	0.33		0.05	0.21		0.09	0.32

Length and width measurements are in millimeters (mm) and areal measurements are in mm<sup>2</sup>. The percent diff, mean diff, and max diff represent the mean of the absolute values for differences between Zeiss-based measurements and the HMH and visually-based measurements.

Two different threshold values were used to construct the models, one following a modified HMH protocol and the other achieved by visually adjusting the threshold value until the threshold boundaries appeared to correspond to the image boundaries. The HMH protocol used here is a modified version of the protocol outlined by Fajardo et al. (2002). The threshold value was determined by calculating the HMH for a row of pixels that crossed the bone-to-air transition around the circumference of the oval window for 10 randomly selected slices. The mean value of the 10 HMH values was then taken as the threshold for the entire stack.

Table 1 presents the measurements (length, width, and calculated area) taken on 14 primate specimens representing a wide range of body sizes. In all cases, the measurements made using the HMH protocol were in close agreement with those made on the dried specimens (Zeiss-based). A paired-samples *t*-test found there to be a nonsignificant difference between the two sets of data (length  $P = 0.217$ ; width  $P = 0.414$ ; area  $P = 0.745$ ). The average difference for oval window length was about 1% and the mean difference in absolute terms was 0.02 mm with a maximum difference of 0.04 mm. For oval window width, the average difference was 3% with a mean difference of 0.02 mm and a maximum difference of 0.04 mm. Oval window area showed a similar pattern with an average difference of 3%, a mean difference of 0.02 mm<sup>2</sup>, and a maximum difference of 0.09 mm<sup>2</sup>.

In contrast, the measurements made using the visually determined threshold values were consistently smaller than the other two measurement sets, and were found to be significantly different from the Zeiss-based measurements for all three variables ( $P < 0.001$ ). The average difference for oval window length was 11%, the mean absolute difference was 0.14 mm, and the maximum difference was 0.33 mm. Oval window width showed an even larger difference of 21%, with a mean absolute difference of 0.11 mm and maximum difference of 0.21 mm. Oval window area illustrated the largest discrepancies averaging 37% smaller than the Zeiss-based calculations, with a mean absolute difference of 0.19 mm<sup>2</sup> and a maximum difference of 0.32 mm<sup>2</sup>. The larger disparities displayed by the area calculations are most likely the prod-

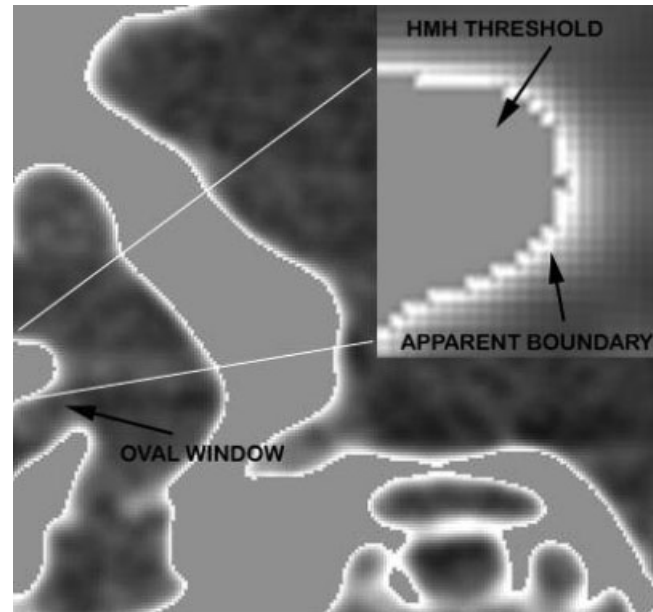


Fig. 1. A representative CT slice of the oval window illustrating the difference between the HMH-based threshold and the apparent (visually-based threshold) bone-to-air boundary. Using the visually-based threshold protocol often results in underestimating the measurement values.

uct of compounding the errors associated with the length and width measurements. When comparing the HMH-based boundaries with the apparent boundaries (Fig. 1), the HMH boundaries were nearly always within the apparent bone-to-air transition. Therefore, an oval window measurement from one bone boundary to an adjacent bone boundary using the HMH protocol will be larger and this explains why the visually-based threshold measurements were generally smaller. A similar directional trend between HMH and visually determined protocol measurements was also found for other auditory structures such as the volume of the middle-ear cavity and the length of the cochlea (Table 2). Each of these

TABLE 2. Tympanic cavity volumes (CV) and cochlear length (CL) measurements taken using the visually-based and HMH-based thresholding protocols

Genus	Specimen	Visual CV	HMH CV	Visual CL	HMH CL
Aotus	NAo1	0.31	0.40	20.14	23.74
Callithrix	NCx 1	0.13	0.15	19.73	20.62
Lemur	22912	0.55	0.58	19.74	21.73
Lemur	100596	0.77	0.78	19.74	21.30
Lemur	100821	0.65	0.66	18.33	20.30
Nycticebus	112991	0.83	0.84	22.35	23.27
Nycticebus	101508	0.28	0.29	16.26	17.41
Nycticebus	183827	0.46	0.46	22.12	23.16
Perodicticus	52704	0.26	0.28	20.93	21.97
Perodicticus	52700	0.36	0.43	18.31	20.12
Perodicticus	52699	0.38	0.40	17.56	21.01
Percent diff			8		9
Mean diff			0.03		1.76
Max diff			0.09		3.60

The CV values are in ml<sup>3</sup> and the CL values are in mm.

measurements used a HMH threshold value derived by sampling the specific region of interest (i.e., the middle-ear bullar walls and the cochlear wall, respectively).

In general, the difference between Zeiss-based measurements and the HMH-based measurements were on the same order as the size of the voxel dimensions, whereas the dissimilarity with the visually-based measurements was an order of magnitude larger. These results agree with previous studies that found problems with visually adjusted thresholding protocols, but add confidence to the HMH procedure for estimating a regional threshold value for a particular structure of interest.

One final point to consider is that in this study the small size of the oval window was advantageous because it helped test the limits of measurement resolution. However, the small size also meant that the structure spanned a limited number of slices (several dozen) and therefore represented a limited range of gray-scale values. In addition, the oval window is a fairly simple structure and has only one bone-to-air boundary. Therefore, when measuring structures that span many slices (>100) it would seem advisable to sample a larger number of slices to determine the mean HMH value. Furthermore, if a structure has more than one bone-to-air boundary (e.g., cortical bone) the best estimate for the mean HMH would probably be determined by sampling all the rele-

vant boundaries (in the case of cortical bone, both the periosteal and endosteal surfaces). Of course, if the differences between the gray-scale values for the various boundaries are too great, this approach may not be practical. Future studies should investigate this possible shortcoming concerning multiple boundary structures. With these considerations in mind, the modified HMH protocol described here is recommended for most studies taking measurements on three-dimensional models obtained from CT data.

## ACKNOWLEDGMENTS

The authors thank Andrew Farke, Nathan Kley, and Doug Boyer for helpful comments on the manuscript. They also acknowledge the help of Richard Ketcham, Andrew Farke, Anthony Olejniczak, Robert Fajardo, Callum Ross, Fred Spoor, Eileen Westwig, and Justin Georgi with CT collection and analysis.

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