A new method for the analysis of soft tissues with data acquired under field conditions

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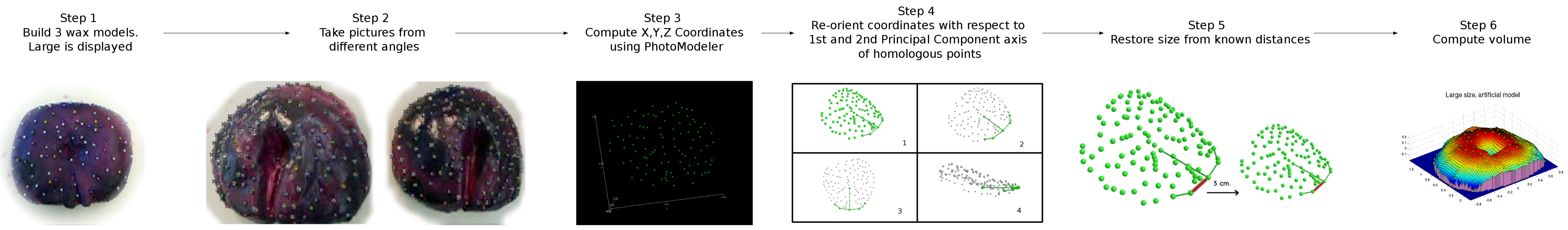
Introduction

Morphometric analysis of soft-tissue is particularly challenging due to the lack of homologous landmarks that can be reliably identified across time and specimens. This is particularly true when data are to be collected under field conditions. Our group has developed a method that combines photogrammetric techniques and geometric morphometrics methods (GMM) to quantify soft tissues for their subsequent volumetric analysis.

The method was developed and validated using wax models of the perineal region of female Barbary macaques. These regions, shown on figure 1, develop distinct anogenital swellings during sexually active phases and are of considerable interest not only to male macaques, but also to primatologists interested in their function. Their dynamic and landmark-poor structure, however, is a major impediment to their quantitative study.

Fig. 1. Barbary macaques sexual perineal swellings

Fig. 2. Steps of the proposed method using wax models.



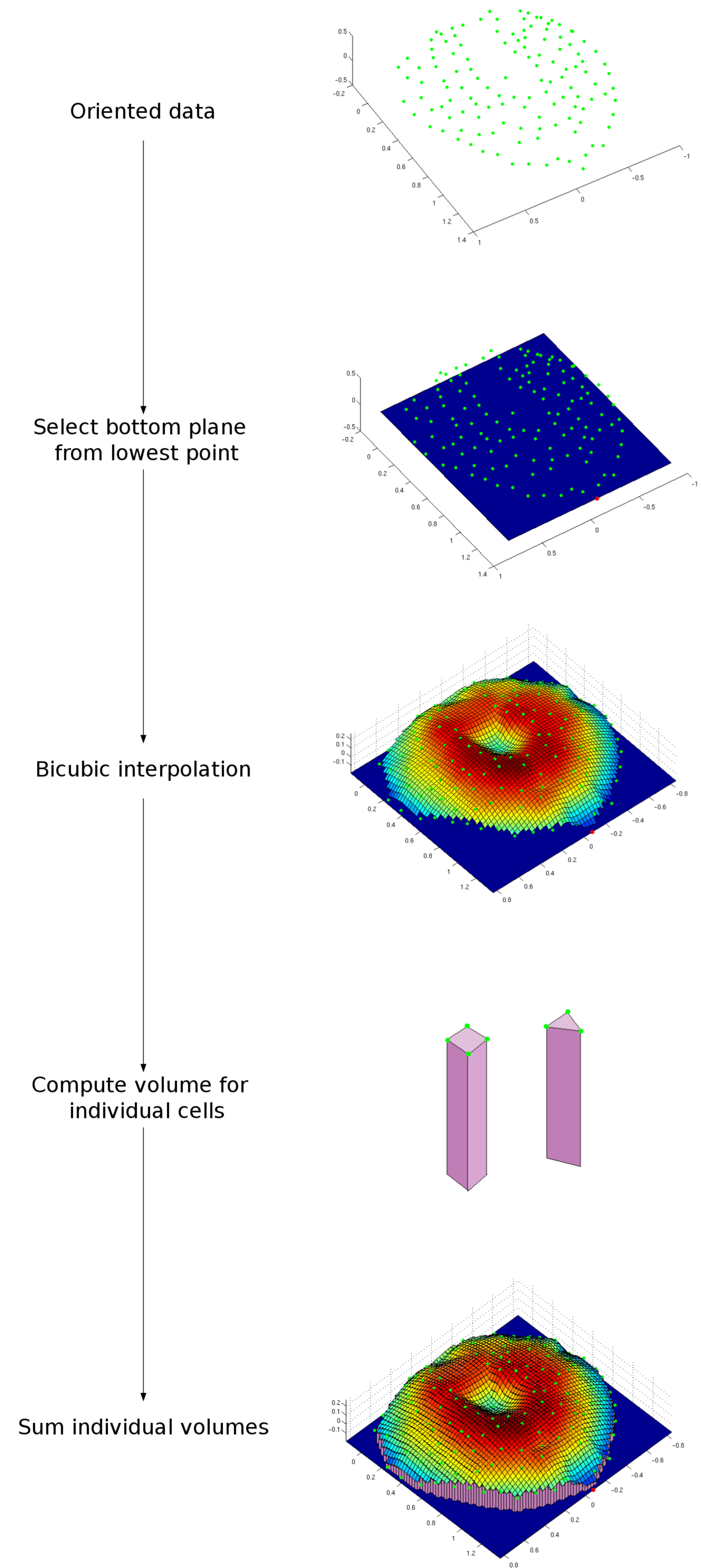
Methods

In geometric morphometric analyses, a set of points called landmarks describes specimens. But one core issue with the analysis of soft tissues is the lack of easily identifiable landmarks that can be found on all specimens at any given time in the same location. To overcome this problem, we use a limited number of points ("primary landmarks") that can be identified on all specimens at any time for orientation, and additional landmarks ("secondary points") for volume computations.

The proposed method is summarized by the folowing steps:

1. Three artificial swellings mimicking perineal sexual swellings at different stages were produced: one representing a maximally tumescent swelling (large), one moderately swollen (medium), and one in a non-engorged state (small).
2. From these models three single images (a frontal and two lateral with respect to the model surface) were taken.
3. The images were processed in PhotoModeler©5, where x, y, and z coordinates for each landmark in the model were obtained.
4. Processing of 3D coordinate data included the definition of primary and secondary landmarks. Primary points were six homologous points: (1) the middle of the anus, (2) the center of the upper end of the vaginal entrance, (3+4) the right and the left point where the medial side of the callosities, the labia and the perineal swelling meet and (5+6) the two points where the distal part of the callosities and the perineal swelling meet (left and right). All remaining landmarks were treated as secondary points (displayed in grey in Figure 2 at step 4). After demoting the secondary landmarks, a General Procrustes a General Procrustes Analysis (GPA) with a final orientation defined to be the spatial principal component axes of the primary points resulted in an orientation of the reconstruction along the plane through the six homologous points.
5. Actual size information was restored by measuring the distance between the third and the fourth homologous point on the artificial swellings and on the reconstructed point configuration. Afterwards, all the points were rescaled by the ratio of these measurements.
6. Obtain volume from rescaled model as shown in Figure 3.

Fig. 3. Steps for volume computation



Volume computation

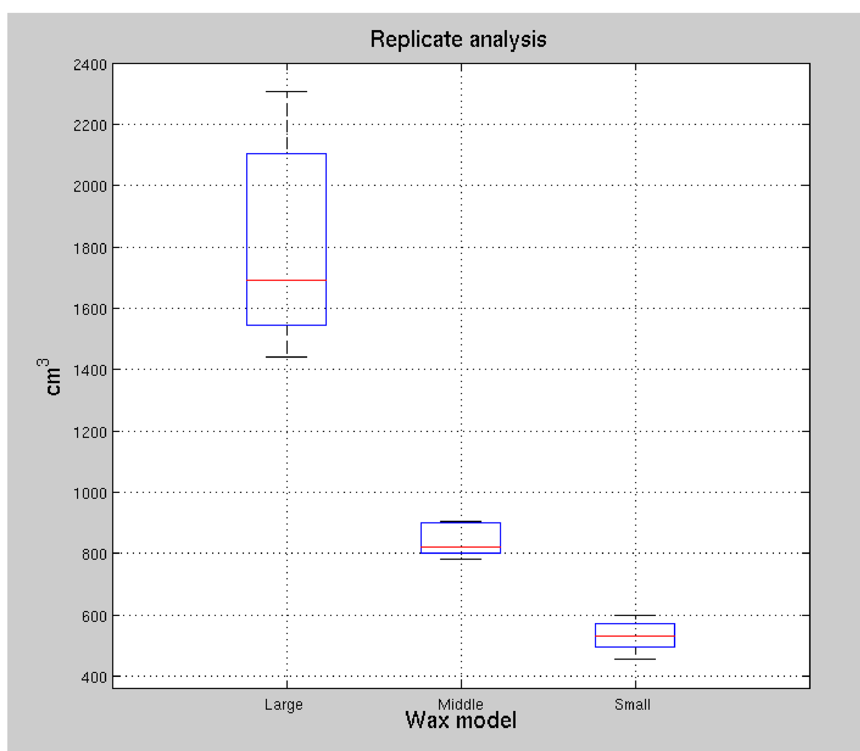
The volume computation of each model was obtained in the following manner:

- The bottom plane for each artificial model was computed as the plane parallel to the x-axis that passes through the lowest z-value of the set of all landmarks.
- The height of each reconstructed model of the artificial swellings was interpolated into a 60x60 grid, using bi-cubic interpolation of all of the reconstructed landmark coordinates, to obtain a smoothed surface of the reconstruction.
- A rectangular prism for each cell of the grid was obtained by taking the average heights of the artificial swelling model as the height of the prism and using the previously computed plane as the base of the prism.
- Volumes were computed for these individual rectangular prisms and then added together to obtain the overall volume of the artificial swelling.

Results

For validation of the proposed method, we performed a replicate analysis of the artificial swellings; made a bootstrap analysis of the model-volume estimates with respect to landmark density and observer; and computed volumes from high-density surface-scans of the artificial models and compared their volume results with our method.

To understand how stable the volume results of the artificial swellings were, the whole process of morphometric analysis from picture taking to volume computation was replicated. Figure on the right presents the volume results in cm^3 for the large, middle and small model obtained from five analyses procedures.



We bootstrapped our results by randomly adding 25, 50, 75 or 90 percent of the landmarks to the six homologous points. Both the type of the artificial swelling and the percentage of landmarks added affected the resulting volume values.

The table below shows the pair-wise comparisions of the Generalized Estimating Equations (GEE) for volume variances and percentage of landmarks. The lower diagonal shows the absolute values of the mean differences over the standard deviation (in parentheses) and the upper diagonal shows the lower and upper confidence limits (95%).

% of landmarks	25	50	75	90
25		(6321.86, 15253.5)	(9547.87, 23414.29)	(10664.42, 26665.80)
50	10787.68 (1787.97		(2661.64, 8725.18)	(3737.55, 12017.31)
75	16481.08 (2691.64)	5693.41 (1266.41)		(791.59, 3576.46)
90	18665.11 (3032.57)	7877.43 (1847.00)	2184.02 (710.44)	