

## MicroCT scanning of flowers: a critical step in geometric morphometric analyses of floral shape variation

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### Customer need

Flowers come in all shapes and sizes. Although this is easily appreciated by the human eye, it has proven much more difficult to develop a quantitative method to compare and describe this variation. Analyses of biological shape variation have undergone a revolution with the launch of geometric morphometrics [1]. This powerful and intuitive method is different from traditional morphometrics (which is based on simple length measurements) in that it uses 'landmarks'. These are defined as homologous points on biological structures, which can be compared across species. Given enough landmarks, any biological structure can be represented in multiple dimensions and variation in shape can be quantified, visualized, and analysed statistically.

Placing the landmarks at these homologous points is a critical step of a geometric morphometric analysis. Methods to set landmarks can be divided into two strategies. The first approach is to use a contact

digitizer directly onto an object. Although this works for large, hard structures, such as bones, most flowers are too small and fragile for this.



Figure 1. 3D reconstruction of a flower of the rare orchid *Satyrium rhodanthum*, using microCT scanning (see insert for an image of real flowers).

### Customer need

Representing the structure of flowers in multiple dimensions for the application of landmarks, to obtain a visual and quantified description of shape variation.

### Materials and methods

SCANCO Medical  $\mu$ CT 80 scanner was used to produce the virtual 3D reconstruction of the flowers.

### Results

The virtual reconstructions of flower surfaces, obtained using microCT scanning, strongly resembled real flowers and were therefore highly suitable for landmarking.

One way to get around this problem, is to use a digitizer in conjunction with a photograph of a flower, instead of a real flower [2]. This, however, would limit analyses to only two dimensions. For flowers, which are typically complex three-dimensional (3D) objects, this is clearly highly unsatisfactory. The second approach is to obtain a virtual 3D reconstruction of an object, which can subsequently be used to place landmarks using

software specifically designed for this purpose. Such reconstructions can be obtained by using laser surface scanners. Most flowers are, however, too small and complex in shape to use this method. More recently, researchers have started using microCT scanning to obtain 3D reconstructions of objects [e.g. 3]. The great advantage of this method is that it is non-invasive (i.e. does not pose any damage to the object), and that it provides extremely fine resolution. Although this technique has been applied to flowers before [4], it has never been associated with geometric morphometrics in plants. This has seriously hampered progress in analyses of floral shape.

Here we show that 3D representations of flowers for landmarking can be obtained by using microCT scanning, following a relatively simple protocol. This method is a critical step of any geometric morphometric study of flowers, and facilitates powerful analyses that are visually appealing and easy to interpret.

## Materials and methods

### *Flower collection and storage*

A typical morphometric analysis will be conducted in a comparative context (i.e. including several plant species). Furthermore, these species may not flower at the same time or occur in close proximity of where the microCT scanner is housed. Fresh flowers therefore need to be collected and stored in such a way that they retain their shape until they can be scanned. It was found that by depositing flowers in 70% ethanol (ethanol : H<sub>2</sub>O = 7 : 3), flowers can be stored for periods of up to several years without changing shape. Our application was based on flowers of c. 1 cm in diameter of the orchid family, which are typically sturdy. Flowers of species with thinner flower parts, which are not fused to a large degree, may require additional treatment before storage.

### *Treatment prior to microCT scanning*

To determine the optimal conditions for maximizing contrast between flowers and the surrounding area (a critical condition for obtaining an accurate representation of the flower surface), we experimented with two treatments prior to scanning. 1) We used critical-point drying to remove any liquid traces to prepare dry flowers with their shapes intact for microCT scanning. This method is also frequently used as a step to prepare samples for Scanning Electron Microscopy

(SEM). 2) We used Osmium Tetroxide to stain the cell membranes of flowers with a heavy metal. This method is also used for SEM, as an alternative to sputter-coating, to increase contrast to images and it was therefore deemed potentially useful for our application. As a control, we scanned flowers that received no treatment. These flowers were taken out of the 70% ethanol used for storage, and were dried briefly on filter paper prior to scanning.



**Figure 2** Flowers of the South African orchid species *Satyrium erectum*. Note the similarity in shape between the real flower (left) and a 3D reconstruction of a flower that was scanned using microCT scanning (right).



**Figure 3** Flowers of the European orchid species *Ophrys insectifera* from the Ofenpass. Note the similarity in shape between the real flower (left) and a 3D reconstruction of a flower that was scanned using microCT scanning (right).

We were also concerned about movement of flowers during the scanning process. We therefore embedded flowers in a matrix of agarose gel to prevent movement. As an alternative method, we cut shapes resembling the flower shape as much as possible in styrofoam discs that fit the microCT scanner tubes.

## MicroCT scanning

We optimized a microCT scanning protocol that took into consideration length of scanning time, as this affected potential changes of shape for flowers during scanning (i.e. the longer the scanning, the higher the likelihood flowers would change shape), as well as the resolution required for subsequent landmarking.

Flowers were positioned in such a way to capture the entire flower during scanning but at the same time to minimize the vertical depth of a particular individual (i.e. to reduce scanning time).

Specimens were scanned on a Scanco  $\mu$ CT80 device, using the following parameters: X-ray tube energy/intensity: 70kV/114 $\mu$ A; integration time: 500ms. Depending on flower size, serial cross-sectional images were reconstructed at a spatial resolution of 36, 50, or 60 $\mu$ m (1024x1024 pixel matrix), and at equivalent interslice distances yielding isotropic voxel sizes [5]. These image volume data were then transferred to a high-performance computer graphics workstation. Three-dimensional (3D) surface models can then be constructed with the Scanco built-in 3D viewer. The data is also available to be exported to third-party software such as Amira (Visage Imaging, Inc.).

## Results of the analysis

Optimal contrast for reconstructing flower surfaces for landmarking was obtained with flowers that did not receive any treatment. Embedding flowers in agarose was not necessary, as flower movement was found to be minimal during the scanning process. In addition, the agarose gel matrix melted during the scanning process.

Virtual reconstructions of flower surfaces, obtained using microCT scanning, strongly resembled real flowers (Figs. 1-5) and were therefore highly suitable for landmarking. This applied to flowers that varied in size between several mm up to several cm.

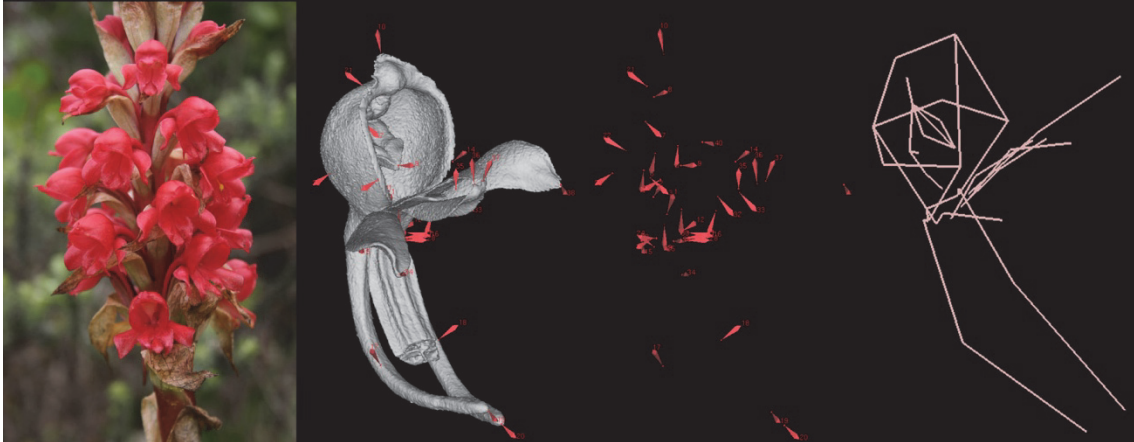
## Conclusion

- MicroCT scanning is a successful way of obtaining a virtual 3D reconstruction of flowers that can be used for landmarking and, thus, geometric morphometric analyses [e.g. 5].
- Because pre-treatment of flowers is unnecessary, this method can be applied to any flowers that are stored in 70% ethanol. This includes flowers that are collected at remote sites, far away from a microCT scanner, and flowers of rare or now extinct species that are stored in herbarium collections. These samples can even be deposited back into 70% ethanol after scanning and the method is therefore non-destructive.



**Figure 4.** Flowers of the South African orchid species *Satyrium macrophyllum*, which is pollinated by long-tongued flies with tongues of up to 8 cm long. 3D reconstructions of flowers scanned using microCT scanning, strongly resemble real flowers in various positions (see insert for an image of real flowers).

## From real flowers to a schematic representation using microCT scanning and landmarks



**Figure 5.** Overview of the process to get from real flowers to a schematic representation of floral shape using landmarks. On the far left is an image of a real flower of the orchid species *Satyrium princeps*, to the right a 3D reconstruction of a scanned flower using microCT scanning (in silver grey) with red landmarks placed at homologous points (see text for explanation). These landmarks are shown without the 3D reconstruction as red cones on a black background, representing the basic shape of the orchid flower. If the landmarks are connected with lines, a schematic reconstruction of the basic floral shape emerges. If shapes of several species are compared in a geometric morphometric analysis, this representation can be used to visualize interspecific shape variation.

## References

[1] Rholf FJ, Marcus LF. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* 8:129-132.

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[3] Zollikofer CPE, De Leon MSP, Chaimanee Y, Lebrun R, Tafforeau P, Khansubhaand S, Jaeger JJ. 2009. The Face of Siamopithecus: New Geometric-Morphometric Evidence for Its Anthropoid Status. *Anatomical Record – Advances in Integrative Anatomy and Evolutionary Biology* 292: 1734-1744.

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Numerous references to similar studies can be found on the SCANCO Medical webpage: [www.scanco.ch](http://www.scanco.ch)

### SCANCO equipment

SCANCO Medical  $\mu$ CT 80 scanner

### SCANCO software

Measurement program incl. Scout View

Evaluation program

✓ Segmentation

Visualization program