TOWARDS UNDERSTANDING SPECIATION BY AUTOMATED EXTRACTION AND DESCRIPTION OF 3D FORAMINIFERA STACKS

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ABSTRACT

The sheer volume of 3D data restricts understanding of genetic speciation when analyzing specimens of planktonic foraminifera and so we develop an end-to-end computer vision system to solve and extend this. The observed fossils are planktonic foraminifera, which are single-celled organisms that live in vast numbers in the world's oceans. Each foram retains a complete record of its size and shape at each stage along its journey through life. In this study, a variety of individual foraminifera are analyzed to study the differences among them and compared with manually labelled ground truth. This is an approach which (i) automatically reconstructs individual chambers for each specimen from image sequences, (ii) uses a shape signature to describe different types of species. The automated analysis by computer vision gives insight that was hitherto unavailable in biological analysis: analyzing shape implies understanding spatial arrangement and this is new to the biological analysis of these specimens. By processing datasets of 3D samples containing 9GB of points, we show that speciation can indeed now be analyzed and that automated analysis from morphological features leads to new insight into the origins of life.

Index Terms— automated shape measurement, machine learning, 3D Krawtchouk moments, understanding speciation, foraminifera

1. INTRODUCTION

Foraminifera are single-celled biomineralizing organisms that live in the ocean. They are very small, typically up to a millimetre in diameter. As a rich marine resource, their Calcium Carbonate shells are ubiquitously retained in sediments in the form of fossils. The internal structure of these fossils is an increasingly important material for systematically studying evolution, because it enables the reconstruction of the growth rate of dead individuals and thus better biological insight into the selection pressures experienced as these organisms lived [1]. The high-resolution micro-CT scan technology enables the non-destructive observation of internal structures.

To investigate how variations among individuals affect evolution, biologists need to collect large numbers of specimens to measure the internal dimensions of these fossils in order to analyze the factors that lead to evolution over a long timeline. Traditional methods require an experienced taxonomist to manually label each chamber of foram for processing. Although accurate data can be obtained in this way, this approach is obviously time-consuming and labour intensive for research projects that demand analysis of thousands of samples. In our work we are using computer vision to enable understanding of variation by using automated measurement of shape of geological specimens, thereby giving new insight concerning spatial layout and arrangement.

In this work, we propose an end-to-end framework to avoid manual effort and remove subjectivity as much as possible. The framework takes a computed tomographic (CT) scanned image sequence as input. The output is the detected chambers with corresponding measurements (see Fig. 1). We use manually labelled images as ground-truth to train a neural network for foram chamber prediction. We reconstruct the space inside each chamber as a surface mesh based on the detected chamber contours, which helps measuring the each chamber's size, volume and elongation. This framework will help biologists to prepare the required measurements with reduced manual effort. The results are reasonable and reproducible. We also propose a new shape signature for foraminifera to perform species recognition.

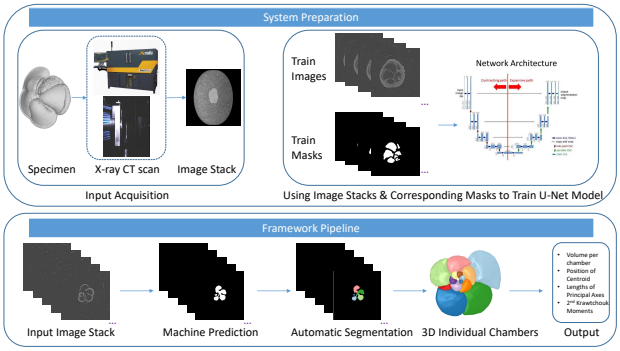


Fig. 1: Framework of the end-to-end automatic foraminifera measurement.

2. RELATED WORK

In this section, we will introduce the case studies of evolution using foraminifera. Followed by that, leveraging computer vision techniques to benefit inter-disciplinary research works will be discussed.

2.1. Speciation

Speciation encapsulates the processes by which new and distinct biological species form. Studying speciation requires data that reveals how differences among individuals become differences among species. The need to study variation mandates large datasets gathered using repeatable protocols that minimise subjective treatments of successive data points.

Dr. Zhang did this work when she was a Research Fellow at University of Southampton. She is currently a Lecturer at Cardiff Metropolitan University.

Under favourable geological conditions, huge numbers of planktonic foraminifera accumulate in continuous sediments and enable study of millions of years of evolutionary history from a single location. The maturity of the group's fossil taxonomy and the abundance of fossil material allows ancestor-descendant relationships to be hypothesized with a high degree of confidence [1]. Multivariate statistical procedures including Gaussian Additive Mixture Models can then be applied to large data sets spanning speciation events to provide quantitative, non-subjective evidence for when the naming of new species corresponds to diagnostic features supporting the emergence of distinct biological forms [2].

2.2. Computer Vision for Foraminifera Study

Image based research A team of researchers at North Caroline State University has conducted a series of works on foraminifera identification. [3] introduced a machine learning method to segment foraminifera images initially targeted at identification. They captured 16 images for each (individual) sample under different illuminations to use the variation for coarse edge map generation. They also trained a convolutional neural network (CNN) and random forest to extract features and refine edge maps to obtain the final chamber segmentation. [4] trained and tested different machine learning algorithms by using the dataset mentioned above. They compared automatic foraminifera identification performance with that of human expertise thus validating results that show the foraminifera can indeed be identified. They have listed more detailed comparison results in [5].

Volume Based Research Although the machine performed competitive results in automated foram identification, it cannot provide a quantitative analysis for each specimen. High resolution X-ray scanning enables a new way of investigating quantification of foraminiferal interiors. [6] explained how X-ray CT scanning with a submicron resolution helps observe inner structure of foram. In [7, 8], the three dimensional reconstructions of the large benthic foraminifera have been introduced. This helps biologists to profile their biometrics and reveal complex shape informations. This type of foram has an obvious chamber shape in most cases. Therefore the reconstructions can be easily done by the popular software like Avizo or ImageJ, with user annotations.

2.3. Moment Based Shape Features

There is an abundance of methods of object recognition which have been well studied. Among them, moment-based techniques are widely used to generate scale, translation and rotation independent features [9]. Krawtchouk moments were first introduced for image analysis by [10], and are based on a set of discrete orthogonal polynomials. This avoids any numerical approximation compared with other traditional moments like Zernike and Legendre moments. According to performance, 3D Krawtchouk moments have been proposed for content-based search and retrieval in [11]. Others have carefully examined their accuracy and efficiency on 3D object analysis and recognition [12]. Recent research [13] on protein local surface shape comparison first employed 3D Krawtchouk moments on irregular shapes instead of well-known 3D shape database. The experimental results in [13] showed that a shape descriptor consists of lower 3D Krawtchouk moments could present a promising protein recognition performance.

2.4. Image Segmentation

As mentioned before, currently foram processing requires much human effort. An automatic individual inner chamber reconstruction is much desired. Normally, segmenting and numbering chambers in each slice is the most intensive work. Fortunately, there has been the rapid development of machine learning techniques, especially on applications of biomedical image segmentation [14, 15]. These examples successfully pioneered computer aided cross-disciplinary research. U-Net is one of the most popular convolutional networks for biomedical image segmentation . The network has excellent performance on segmentation tasks even when trained using few images. It inspires our study as only fewer than 1000 manually labelled images are available for training a network.

3. METHODOLOGY

We propose an end-to-end framework of automatic measuring foraminiferal chambers in this paper. The framework takes X-ray CT scanned image sequences as input and returns individual chamber shape measurements. Importantly, we seek to isolate distinct subcomponents (growth chambers) within each sample (individual) to provide a more coherent representation of the functional constraints of the image and organism under investigation. The detailed structure is demonstrated in Fig. 1.

3.1. Preparation

In our study, we have a high resolution image stack for each specimen shape reconstruction and measurement. To avoid manual labelling, we train a neural network to automatically predict possible chambers. The trained data is randomly chosen from the prepared image stacks. The corresponding masks used for training are manually labelled. The manual processing for one specimen can take up to one day. Therefore we only have several image stacks processed for training. U-Net [16] is then selected as a segmentation network in this paper since it requires only a few images. The trained model is employed to generate image masks to initially segment possible chamber regions from the original images.

3.2. Chamber Tracing

As a single-cell organism, all the chambers in foraminifera share the same corridor to pass food, which will form openings between adjacent chambers when observing from a 2D image. In this situation, the trained network cannot provide a precise prediction. The connected region will need an additional partition to get individual chamber contour.

Assuming all the chambers have been segmented before the current image slice, we can place the centroids of each chamber from last one as seed points to initialize segmentation. For each pixel inside the connected area, we calculate a geodesic distance to every seed point. Those pixels with the shortest distance to the same seed point will be clustered together. The results are shown in Fig. 2.

3.3. Shape Measurement

To investigate evolutionary rates and patterns on multi-million-year time scales, a large number of statistics on quantifying foraminiferal growth is necessary. The fundamental information includes growth rates as represented by volume and equivalent radius (principal axis length). In this paper, we use lower order of Geometric Moments

(a) (b) (c) (d) (e)

Fig. 2: Further segmentation on predicted mask. Use the centroids of previously segmented chambers (a) as the initial seed points on current mask (b) to run partition (c). Use the centroids of current segmented chambers (d) to divide next mask (e).

to capture basic geometric information per chamber for individual specimen.

We use the image masks obtained from section 3.1 to define a binary volume function $f(x, y, z)$ on each specimen (Voxelization). After that, we compute Krawtchouk polynomials and apply them on the aforementioned volume function to calculate 3D weighted Krawtchouk moments $\bar{Q}_{nml}$ of $f(x, y, z)$ up to order $(n + m + l)$ (see Eq. 1 introduced by [11]). This translation, rotation and scaling independent moments will form a shape signature and be discussed in next section.

$$\bar{Q}_{nml} = \sum_{x=0}^{N}\sum_{y=0}^{M}\sum_{z=0}^{L} f(x, y, z) \cdot \bar{K}_n(x; p_x, N) \cdot \bar{K}_m(y; p_y, M) \cdot \bar{K}_l(z; p_z, L). \quad (1)$$

4. EXPERIMENT AND DISCUSSION

4.1. Compare Manual Labelling & Machine Learning Prediction

Normally, there is no standard rule for segmenting individual chambers inside the foram, as long as the connected space has been isolated. We compared the chamber contours from manual labelling with machine prediction, with examples of both in Fig. 3a and 3b.

The main limitation of this work is loss of volume due to imperfect chamber mask prediction. There is an average loss of 15.5% in our experiment, which compares with a previous difference analysis [6] which observed a similar error rate of 15%. It is of course not known from which source the differences derive: the manual or the automated labelling. However, the performance of deep learning prediction relies on the quality of scanned specimens. There are two situations in which the volume loss increases dramatically. The first one is when the samples have a broken surface, there is no chance of generating a correct prediction (see Fig. 4a and 4b). Second is when there are too many sediment particles inside the chambers, the prediction generates a shrunk chamber mask (see Fig. 4c and 4d). It is very tricky to study the correct growth rates from these imcomplete samples. When holes occured on the largest chamber, we truncated the last one as some research believes that the last two chambers will not affect growth rate analysis and can confuse taxonomic identification [17]. And for samples with large sediment data inside chambers, future work will enable the framework with image shadow removal to improve the quality of input.

4.2. Growth Pattern

To understand the biological question of how do differences among individuals make differences among species, we will not only analyze the growth pattern of a single specimen but also compare the patterns as groups to study speciation. Fig. 3c shows the chamber by chamber growth pattern of individuals from three different species, *M.pertenius*, *M.exilis* and *M.limbata* respectively.

According to the observed data, the largest size of the three species is not detectably different (Wilcox Rank Sum Tests on chamber Q: *M.pertenius* vs *M.exilis* W = 144, p = 0.594; *M..limbata* vs *M.exilis* W = 103, p = 0.287; *M.limbata* vs *M.pertenius* W = 94, p = 0.336), but this masks clear differences in their growth rates: the smallest chambers of *M.exilis* are smaller than those of *M.pertenius* (coef = 0.0042, s.e. = 0.0002, p<0.05) and *M.limbata* (coef = 0.0054, s.e. = 0.0002, p<0.01). There are no detectable differences in linear growth rates (coef = $1e^{-5}$, s.e. = $3e^{-5}$, p>0.05 for *M.pertenius* & *M.exolis*) but *M.limbata* has a faster quadratic growth rate (coef = 0.042, s.e. = 0.016, p<0.01) than *M.exilis*. The differences among growth rates and in the earliest lifestages emphasizes the need to study developmental sequences to reveal the structure of variation among individuals.

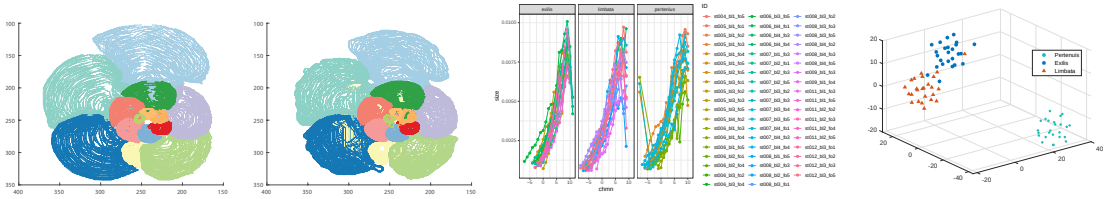
4.3. Shape Signature

The chamber's growth rate is a fundamental property for distinguishing different species because it represents changes in the organism's pace of life. However, we try to investigate the intrinsic shape information of forams during the evolution. We proposed a shape signature for foraminifera to describe the appearance of shape. The 3D Krawtchouk moments up to second order are collected as the shape signature. The binary volume function $f(x, y, z)$ is defined on a grid of $512 \times 512 \times 512$ for every specimen in order to normalize them under the same scale. Because we introduced translation, rotation and scale independent 3D Krawtchouk moments, the specimens have the identical zero order and first order of 3D Krawtchouk moments. Thus we omit them to only compare the second order of 3D Krawtchouk moments in the experiment.

There are 75 specimens randomly chosen from our dataset to validate the hypothesis and 25 of each species *M.pertenius*, *M.exilis* and *M.limbata*. In Fig. 3d, the t-SNE algorithm [18] is used to reduce the signature dimension and visualize the clusters. According to this, the *M.pertenius* has a clear difference on shape appearance compared to its ancestor *M.exilis* and *M.limbata*. Meanwhile, the boundary between clusters *M.exilis* and *M.limbata* is not well defined. There are only few samples falling into incorrect clusters. Because the species label is manually assigned to each specimen, human bias will be one of the reasons of generating this problem.

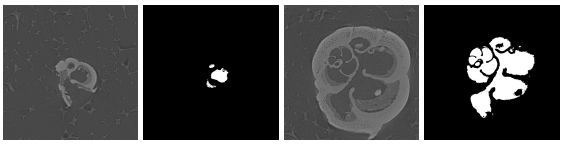
5. CONCLUSION

This paper is an introductory work in understanding the important factors affecting speciation by leveraging computer vision algorithms to reconstruct the internal features of computed tomographic scans. We proposed an end-to-end framework of automatic measurement of foraminiferal shape, by bringing the vast armoury of computer vision to bear on an evolutionary question, does developmental plasticity inhibit speciation? The experimental results reveal the individual life cycle as well as the shape patterns differ between species. In addition, the reproducible shape measurements and signatures reduced human bias. Our protocol provides an efficient access to generate and analyze biological trait based on a large number of samples. Our automated processes provide for analysis of spatial arrangement which has already given new insight into growth processes. As this information is new to biologists, we await with interest their development and analysis of this new information. Future work will focus on improve the performance of



(a) (b) (c) (d)

Fig. 3: Experiment & Discussion. (a) Chamber contours generated from manual labelling. (b) Chamber contours generated from machine learning prediction. (c) Growth patterns of *M.exilis*, *M.limbata* and *M.pertenius*. (d) Dimension reduction and visualization of foram shape signature.



(a) (b) (c) (d)

Fig. 4: Example of imperfect samples. (a) Broken sample. (b) Incorrect prediction. (c) With sediment. (d) Incorrect prediction.

machine learning and expand the number of specimens and diversity of morphological forms as broadly as possible.

6. ACKNOWLEDGEMENT

This work was funded by the Natural Environment Research Council award NE/P019269/1. The authors thank μ-VIS X-ray Imaging Centre at University of Southampton for supporting micro-CT scanning of foreminifera.

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