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¹ A machine learning based approach to

- ² the segmentation of micro CT data in
- ³ archaeological and evolutionary sciences.
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2

1 Abstract

Segmentation of high-resolution tomographic data is often an extremely time-consuming task and
until recently, has usually relied upon researchers manually selecting materials of interest slice by
slice. With the exponential rise in datasets being acquired, this is clearly not a sustainable workflow.
In this paper, we apply the Trainable Weka Segmentation (a freely available plugin for the
multiplatform program ImageJ) to typical datasets found in archaeological and evolutionary
sciences. We demonstrate that Trainable Weka Segmentation can provide a fast and robust method
for segmentation and is as effective as other leading-edge machine learning segmentation

9 techniques.

10 Introduction

11 Three-dimensional imaging using micro CT scanning has rapidly become mainstream in the

12 archaeological and evolutionary sciences. It enables the high-resolution and non-destructive analysis

13 of internal structures of scientific interest. In archaeological sciences it has been used for a variety of

14 purposes, from imaging pottery (Barron et al., 2017; Tuniz and Zanini, 2018) understanding soil

15 compaction (McBride and Mercer, 2012) imaging early bone tools (Bello et al., 2013) and mummies,

both human and animal (Charlier et al., 2014; Du Plessis et al., 2015; Romell et al., 2018). In

17 evolutionary sciences, it is employed even more widely, from scanning hominin remains for

morphological reconstruction (Gunz et al., 2012; Hershkovitz et al., 2018, 2015; Hublin et al., 2017)

19 to diagnosing ancient pathologies (Anné et al., 2015; Odes et al., 2016; Randolph-Quinney et al.,

20 2016). It is used extensively in vertebrate palaeontology (Abel et al., 2012; Chapelle et al., 2019;

Hechenleitner et al., 2016; Laloy et al., 2013) and invertebrate palaeontology (Garwood and Dunlop,

22 2014; Wacey et al., 2017) and increasingly, palaeopalynology (Collinson et al., 2016).

23 In comparative anatomy, it is now part of the standard non-destructive analytical toolkit, alongside

24 geometric morphometrics (GMM) and finite element analysis (FEA) (Borgard et al., 2019; Brassey et

25 al., 2018, 2013; Brocklehurst et al., 2019; Cuff et al., 2015; Marshall et al., 2019; Polly et al., 2016).

26 Unfortunately for researchers, if one wishes to quantify biological structures, the data does not

27 simply appear from scanners ready to use. It requires processing through the segmentation of the

structures of interest, followed (usually) by the generation of 3-dimensional models.

29 There are 5 main approaches to image segmentation.

• Global thresholding based upon greyscale values in scans.

• Watershed based segmentation

• Locally adaptive segmentation

- 1 Manual segmentation of structures
- 2 Label based segmentation, in conjunction with machine learning.
- 3 One can broadly classify greyscale segmentation and edge-based segmentation as passive
- 4 approaches, as very little input is required from the user, and Region or label based segmentation as
- 5 active, in that they require more explicit input from the user.
- 6 Greyscale thresholding
- 7 Greyscale thresholding is the oldest approach to the processing of tomographic data (Spoor et al.,
- 8 1993) and has been refined to the use of the half width, full maximum height approach based upon
- 9 stack histograms (Spoor et al., 1993). This however is only useful for materials which have a single
- 10 range of X-ray absorption and several passes are therefore required for the segmentation of multiple
- 11 tissue types.

12 Watershed based segmentation

13 Watershed based segmentation has enjoyed a lot of popularity for segmenting complex structures 14 such as brain folds but is also of some utility when segmenting fossil structures. A recent innovation 15 has been the application of Ray-casting and similar techniques to the processing of data (Dunmore 16 et al., 2018; Scherf and Tilgner, 2009) which helps to ameliorate problems with fuzzy data and 17 automates the processing of this. A problem is that it is only feasible to process a single material (although the others are also detected) and an aspect of 're-looping' the procedure is then required, 18 19 which can create a bottleneck for scans where multiple materials are of equal interest (for example, 20 mummies, where the skeleton, desiccated flesh and wrappings are all of equal scientific interest.

21 Locally adaptive segmentation

22 Locally adaptive segmentation is increasingly carried out using deep learning in an automated 23 fashion. Algorithms use combinations of edge detection, texture similarity and image contrast to 24 create rules for the classification of different materials. (Prasoon et al., 2013; Radford et al., 2015; 25 Suzani et al., 2015). It has become increasingly popular with the availability of massive datasets from 26 healthcare providers and several recent reviews cover this suite of techniques in-depth (Greenspan 27 et al., 2016; Litjens et al., 2017; Shen et al., 2017; Suzuki, 2017). A criticism of unsupervised methods 28 such as convoluted neural networks is that they can demand huge computing resources while still 29 often yielding false positives including highlighting artefacts in data (e.g. ring artefacts in CT 30 scans(Nguyen et al., 2015; Szegedy et al., 2013; Wang, 2016). Another set of related techniques 31 include kmeans and c-means clustering algorithms. K-means is known as a 'hard' clustering 32 algorithm, introduced independently by Forgy and MacQueen (Forgy, 1965; MacQueen, 1967). This 33 method, and extensions of it, have been used widely in MRI processing (e.g. (Dimitriadou et al.,

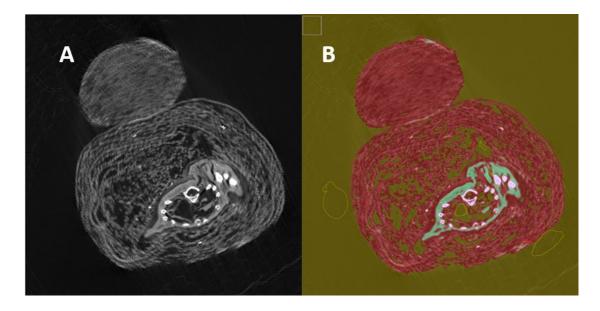
- 1 2004; Juang and Wu, 2010; Singh et al., 1996). An interesting note is that the original publication
- 2 cautioned against using k-means clustering as a definitive algorithm, but as an aid to the user in
- 3 interpreting clusters of data. Another popular clustering algorithm is that of fuzzy c-means (Bezdek,
- 4 1980, 1980, 1975; Pham and Prince, 1999) which is an example of 'soft' clustering methods, where
- 5 probabilities of group allocation are given. Again, this is popular for the automated segmentation of
- 6 MRI data (e.g. Dimitriadou et al., 2004) and computational speed can be further improved by an
- 7 initial clustering using k-means partitioning (Dunmore et al., 2018).

8 Manual segmentation

- 9 Manual segmentation is usually carried out using a graphics tablet and the contours of each material
- 10 of interest are manually traced by a researcher who is familiar with the characteristics of the
- 11 material of interest. This technique is intrinsically reliant on the skill of the researcher carrying out
- 12 the segmentation and is also extremely time consuming for large datasets.

13 Label based segmentation

14 Label based segmentation is commonly used to 'seed' areas of interest and a contour is propagated 15 until a significant difference in the material absorption is observed (within user set parameters such 16 as kernel size and diffuseness of boundaries). In more recent applications, these approaches have 17 been combined with machine learning, such as in (Arganda-Carreras et al., 2017; Glocker et al., 2013). Most user guided approaches utilise a variant of the Random Forest Algorithm for training 18 19 (Breiman, 2001; Tin Kam Ho, 1998). The Weka segmentation method is an example of supervised 20 label based segmentation, augmented by machine learning and as such, is our preferred technique 21 for the segmentation of complex anatomical and archaeological/palaeontological data which may 22 suffer from artefacts in scanning and material inhomogeneity (defined here as differences in 23 material x-ray absorption). It has previously been tested on ground truth images applicable to 24 geological samples and found to perform at least as well as other leading algorithms (Berg et al., 25 2018). It provides an easy to interpret overlay on training datasets (see figure 1) and can be used to rapidly process multiple complex materials simultaneously. 26



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Figure 1 An example of Weka segmentation. A: Original slice B: Weka classification of rodent mummy showing previews of
 masking

4 In summary, a significant roadblock to more rapid and precise advances in micro CT imaging in

5 archaeological and evolutionary sciences is that the structures of interest are often non-

6 homogenous in nature and until recently, have required extensive manual processing of slices .

7 Recent advances in machine learning, combined with user-friendly interfaces mean that an

8 acceleration of data processing is seriously possible, especially when combined with the potential to

9 process data through either clusters or multiple GPUs. In this article, we demonstrate for the first

10 time the implementation of the Trainable Weka Segmentation (Arganda-Carreras et al., 2017), to

11 typical micro CT data encountered in archaeological and evolutionary sciences. The Trainable Weka

12 Segmentation is available through the FIJI fork of ImageJ (Schindelin et al., 2012). We demonstrate

13 the efficacy of these algorithms as applied to six distinct examples: an entirely synthetic dataset;

14 micro CT scans of a machine wire phantom; a defleshed mouse tibia; a lemur vocal tract; a juvenile

15 Neanderthal humerus (Kostenki 2) and a small rodent animal mummy. These represent a range of

16 the type of samples commonly encountered by researchers working in imaging in evolutionary

17 sciences and each presents different segmentation challenges. To further demonstrate its efficacy,

18 we compare this algorithm with other methods that have typically been used.

1 Materials and methods

2 Synthetic Dataset

- 3 A synthetic dataset of 12 images of white triangle outlines on a black background was made. The
- 4 original was kept as the ground truth. To simulate partial volume averaging and scanner noise, the
- 5 following filters were applied in ImageJ: Noise: Salt and Pepper; Gaussian Blur of radius 2.0 pixels;
- 6 Shadow from south (base) of the image. The original data, the data with noise added, and, all
- 7 segmentations are included in the supplementary material. A render is in figure 2 with an arbitrary
- 8 voxel depth of 10.

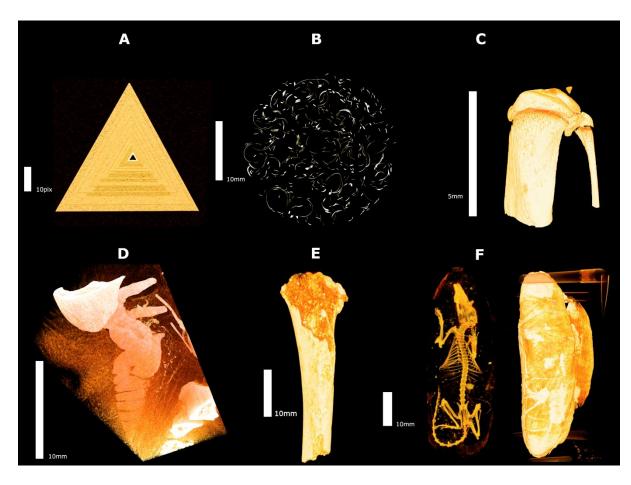
9 MicroCT scans

- 10 A wire phantom object from (Dunmore et al., 2018). This is a coil of randomly crunched stainless
- 11 steel wire of thickness 4mm.
- 12 A wild type mouse proximal tibia and from (Ranzoni et al., 2018).
- 13 Rodent mummy Manchester Museum number 6033. This is thought to be a shrew, based upon size
- 14 of the wrappings and earlier medical X-Rays (Adams, 2015).
- 15 Primate vocal tract-this is a scan of a wet preserved *Nycticebus pygmaeus* individual from the Duke
- 16 Lemur Center, catalogue DLC_2901 and is more fully described in (Yapuncich et al., 2019)..
- 17 A partial proximal humerus from a juvenile Neanderthal from the site of Kiik-Koba. It has been
- 18 described in detail by (Trinkaus et al., 2016) and has matrix and consolidant adhering to it which
- 19 obscure some more detailed aspects of its morphology.
- 20 Full scan parameters are shown in Table 1 and volume renders of the tested datasets are shown in
- 21 Figure 2.

7

Object	Reference	Scanned with	KeV	μA	Voxel	Filter	Medium
	number				size/µm		
Rodent mummy in	Manchester	Nikon XTH225				Air	
wrappings	museum 6033						
Nycticebus pygmaeus	Duke Lemur	Nikon XTH225	155	110	35.47	None	Air
vocal tract	Center 2901						
Kiik-Koba 2 juvenile	KunstKamera	Custom MicroCT	135	35	30	None	Air
neanderthal humerus	Museum	(microCT MPKT-					
		01)					
Wild type mouse tibia	Number 6	Skyscan1172	49	200	5.06	Aluminium	70% Ethanol
10mm thickness	n/a	SkyScan 1172	100	100	12.87	None	Air
machine wire artefact							

2



3 4

5

Figure 2. Volume renders of items analysed. A: Synthetic dataset; B: Machine Wire; C: Wild mouse type tibia section; D: Nycticebus pygmaeus vocal tract; E: Kiik Koba 2 partial humerus; E: Mummified rodent

Table 1. Scan parameters

8

Sample processing

1 2 All samples were subjected to segmentation using the Interactive Weka Segmentation editor plugin in ImageJ (Arganda-Carreras et al., 2017) with the following settings (adjusted after Somasundaram 3 4 et al., 2018 who have applied this to medical images): Gaussian blur, Sobel Filter, Hessian Filter, Membrane projections (Thickness 1, patch size 10, difference of Gaussian filters, median filter 5 6 (minimum sigma=1.0, maximum sigma=4.0); Kuwahara filter (Kuwahara et al., 1976). These filters 7 help to counteract potential artefacts in the original scan slices and were found by (Somasundaram 8 et al., 2018) to give the closest values to their 'gold standard' which was manual segmentation by a 9 specialist. 10 Individual slices which contained all the materials of interest were trained using the Weka 11 segmentation plugin. Briefly, areas containing each material of interest were selected using a 12 graphics tablet and areas at the interface between materials were also selected (e.g. where a bone 13 came into contact with air, near the edge of the bone was selected and added to the 'bone' label 14 and a part near the edge of the air was selected and added to the 'air' label). This helped the 15 algorithm to effectively select the correct labels at interfaces between materials. To propagate this 16 label selection across the whole image, the Random Forest Algorithm was used, with 200 hundred 17 trees. Although the use of fewer trees is more computationally efficient, the tradeoff between 18 efficiency and efficacy starts to plateau after ~250 trees (Probst and Boulesteix, 2018). All images 19 were then segmented using the appropriate training dataset. All stacks were processed on of two machines with 32GB RAM, PCIeM2 SSD and either a 6 core i7 at 20 21 3.6GHz (4.2GHz at boost) or an 8 core AMD 2700 at 3.2 GHz (4Ghz at boost). Due to the way the Java

23 All datasets were also in ImageJ segmented with the following competing algorithms:

virtual machine is configured, graphic card parameters are not currently relevant for this workflow.

- 24 Greyscale thresholding (using the half-maximum-height algorithm) •
- K-means segmentation 25 •

22

26 C-means segmentation •

27 • Localised fuzzy c-means segmentation, with pre-selection through k-means clustering

28 All variants of k-means and c-means segmentation used the ImageJ plugin available from

29 https://github.com/arranger1044/SFCM. Spatial fuzzy c-means used the settings recommended by

30 Dunmore et al. (2018).

- 9
- 1 For visual purposes, 3D renderings of each of the complete models were created using Avizo with no
- 2 smoothing applied and the distances between the Weka segmentation and meshes generated with
- 3 competing algorithms was visualised using the package Rvcg in R (Schlager, 2017).

4 Statistical comparisons

- 5 The effects of the varying segmentation algorithms on real world results is the most important
- 6 consideration as it is sensible to anticipate that improvements will be made to the accuracy of these.
- 7 In the case of the wire phantom and the tibia ROI it was also possible to compare average
- 8 wire/trabecular thickness and thickness distribution of the samples (with the wire also having a
- 9 ground truth thickness of 4mm). One further real-world test was a comparison of the ellipsoidness
- 10 (after Salmon et al., 2015) and degree of anisotropy in the trabecular ROI, to demonstrate what
- 11 effect the segmentation would have on biomechanical analyses. All thickness and anisotropy
- 12 calculations were calculated with BoneJ 2 (Doube et al., 2010). We also assessed the degree of bone
- 13 volume in the trabecular ROI, as many publications use this as a proxy for levels of bone formation in
- response to weight bearing or mechanical stimulation (e.g. Acquaah et al., 2015; Farooq et al., 2017;
- 15 Li et al., 2016; Milovanovic et al., 2017; Turner, 2002).

10

1 Results

2 Segmentation times

3 Segmentation times for Weka segmentation are listed for representative individual slices from each

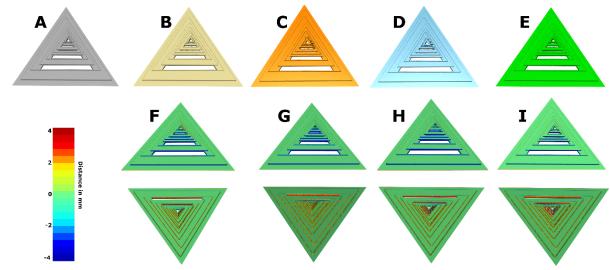
- 4 stack in table 2.
- 5

Table 2. Segmentation times for each dataset

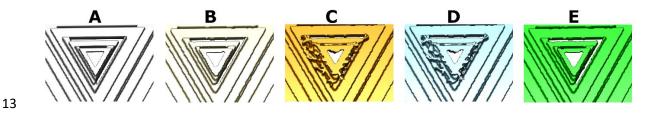
Scan	Training	Classification	Bit	Image x	Image y	No.
	time/ms	time/ms	depth	length	length	materials
Synthetic dataset	15	0.5	8	814	814	2
Machine wire	18	6.8	32	3240	3240	2
phantom						
Mouse tibia	10	0.59	8	960	960	2
Lemur larynx	50	4	16	1329	1271	3
Kiik-Koba	49	0.3	8	480	576	3
Rodent mummy	50	1.5	8	1117	1141	3

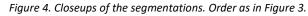
6

7 Synthesised dataset



- 9 Figure 3. Synthesised data results. A: Original non-modified data; B: Weka Segmentation; C: local c-means; D: k-means; E:
 10 Watershed. F-I comparisons of above meshes with original data.
- 11 The majority of the data segmented relatively easily, but both k-means and local c-means struggled
- 12 with the smaller triangles, where noise was closer to the dimensions of the object of interest.



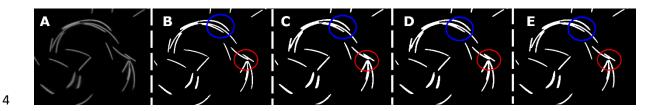


14

11

1 Wire phantom

- 2 The Weka segmentation performed as well as the local c-means segmentation and improved some
- 3 aspects of fine detail retrieval (figure 5).



- Figure 5. A: Original wire phantom scan; B: Weka segmentation. C: Local c-means segmentation; D: K-means segmentation;
 E: Watershed segmentation. Modified after Dunmore et al., (2018) Red circles indicate the areas highlighted by Dunmore et
 - al., (2018) and blue are areas where Weka segmentation retrieves more fine detail.
- 8

7

9 *Wild type mouse tibia*

- 10 The Weka segmentation performed better than the other types of segmentation, with improved
- 11 quality on fine features.

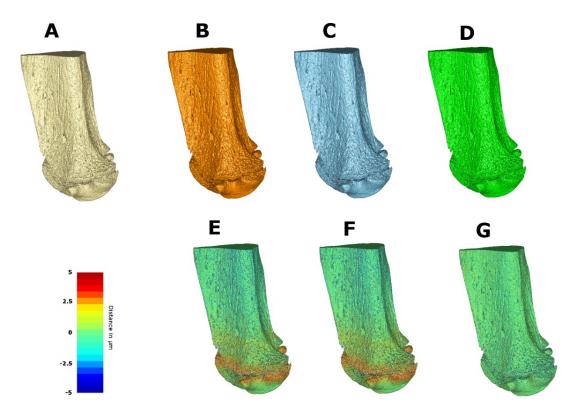


Figure 6. Comparisons of segmentations of the tibia. A: Weka; B: Local C-means; C: K-means; D: Watershed. E-G: heatmap
 comparisons of the above specimens with Weka segmentation. Blue to red scale, Blue indicates values which are concave
 compared with Weka; Red indicates areas that are concave compared with Weka.

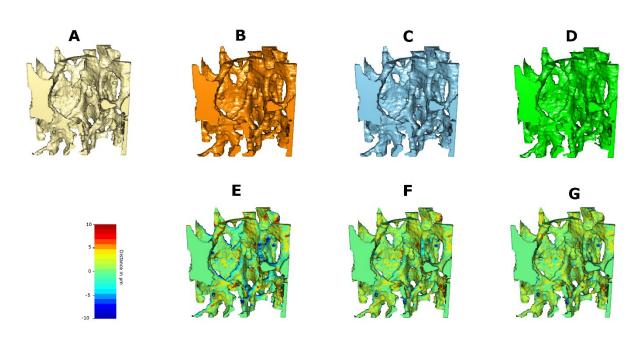
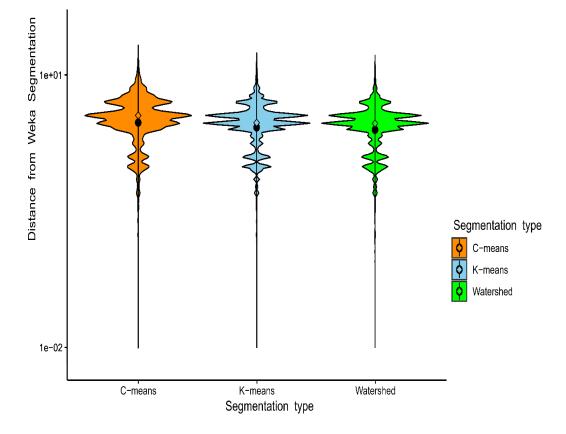


Figure 7. Comparisons of segmentations of ROI. A: Weka; B: Local C-means; C: K-means; D: Watershed. E-G: heatmap
 comparisons of the above specimens with Weka segmentation. Blue to red scale, Blue indicates values which are concave
 compared with Weka; Red indicates areas that are concave compared with Weka.

- 5 Violin plots indicate that alternative segmentation methods have subtly different distributions in
- 6 terms of distance from the Weka segmentation. All suffer from arbitrary spiking in the data.



7 8

Figure 8. Violin plots of mesh distances. Cut off at 1e⁻² to illustrate the main trends in the data

- 13
- 1 It is also apparent that differing segmentation techniques have a marked effect on the degree of
- 2 anisotropy detected in trabecular bone, with Weka tending towards more anisotropic structures.
- 3 This may be because of the lack of spiking in the resulting segmentation when compared with the
- 4 other methods here. The Ellipsoid Factor (a replacement for the Structure model index (Doube,
- 5 2015; Salmon et al., 2015) also varies considerably, with a difference of almost 4% between Weka
- 6 and watershed segmentation. It is noticeable also that Weka segmentation classifies a relatively low
- 7 percentage of bone and also trabecular thickness.
- 8

Table 3 Comparison of the morphometric measures of the tibia ROI

Segmentation technique	Degree of anisotropy	% of foreground volume filled with ellipsoids	% of ROI classified as bone	Tb thickness/μm	Tb. Th S.D. /μm
Weka	0.44	14.32	12.7	35.1	12.7
Local c-means binarized	0.38	14.39	15.1	39.2	12.6
K-means	0.39	16.52	15.2	40.9	13.2
Conventional watershed	0.41	18.28	15.3	41.0	13.2

9

10 Lemur scan

- 11 The Weka segmentation was able to account for the ring artefacts in the scan and successfully
- 12 segmented the materials of interest. It was also more successful at segmenting the finer structures
- 13 in the larynx (see Figure 8). It also generated much cleaner data than all other segmentations.

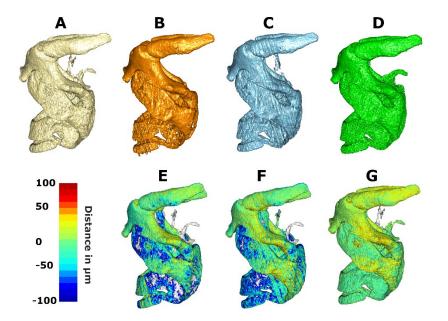
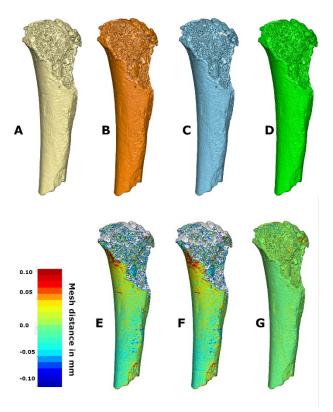


Figure 9. Comparison of Nycticebus pygmaeus scan segmentations. A: Weka; B: Local C-means; C: K-means; D: Watershed.
 E-G: heatmap comparisons of the above specimens with Weka segmentation. Blue to red scale, Blue indicates values which
 are concave compared with Weka; Red indicates areas that are concave compared with Weka.

14

1 Kiik-Koba Neanderthal humerus

- 2 The Weka segmentation was able to track trabecular structure successfully, without eroding the
- 3 material. It also was able to take into account the slight 'halo' effect on the bone/air interface, which
- 4 conventional segmentation used to create an external border of the matrix material. The c-means
- 5 and k-means segmentation both created this 'halo' like border (Figure 11).

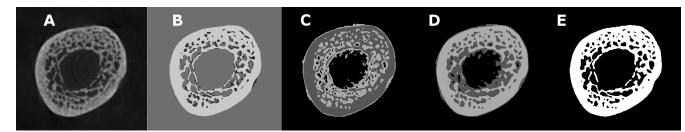


6

7

Figure 10. Kiik-Koba 2 reconstructions.

- 8 A: Weka; B: Local C-means; C: K-means; D: Watershed. E-G: heatmap comparisons of the above specimens with Weka
 9 segmentation. Blue to red scale, Blue indicates values which are concave compared with Weka; Red indicates areas that are
 10 concave compared with Weka.
- 11 12





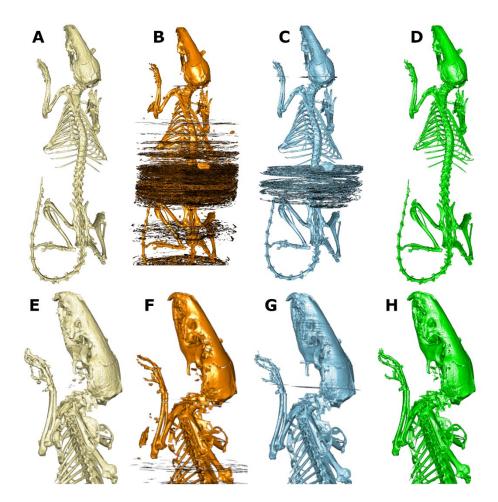
15

Figure 11. Orthoslice from Kiik-Koba microCT scan. A: Original scan; B: Weka; C: Local C-means; D: K-means; W: Watershed

15

1 Rodent Mummy

- 2 The Weka segmentation was able to detect the majority of the skeletal features and also
- 3 discriminate the mummified tissues from the outer wrappings. There were a few artefacts around
- 4 the front paws and mandible which would require some manual correction to fully delineate the
- 5 structures. The smoothing steps introduced in the segmentation were able to remove many of the
- 6 scanning artefacts which made borders of materials harder to resolve with conventional methods. K-
- 7 means segmentation was able to discriminate materials relatively well also, although it did
- 8 misclassify several slices. Localised C-means was unsuccessful in several slices, missing bone material
- 9 entirely, where other segmentation techniques succeeded.



- 11
- 12 Figure 12. Rodent mummy 3D model reconstructions. Order as before. Second row are closeups showing extra detail
- 13
- 14

16

1 Discussion

2 The Weka segmentation algorithm generated improved results when compared with standard tools 3 available in Avizo and was especially good at tracking fine structures throughout the samples. It is 4 interesting that all algorithms apart from Weka seem to struggle to differentiate between materials as contrast is low and details approach the resolution of the scans (e.g. they struggle with details 5 6 that are around 10 microns in size, when the scan is 5 microns in resolution). Although it did not 7 perfectly segment out the bone in the rodent mummy sample, it is a noted improvement on current 8 methods, and is extremely easy to implement. It compared favourably with the MCIA data and has 9 the added advantage that ImageJ is widely available, easy to learn and platform independent. It also 10 has the advantage over the MCIA algorithm in that it is able to compensate for the material interface 11 artefacts in scans (Dunmore et al., 2018). A suggestion for further processing of data from especially noisy scan data is that the user applies the 'despeckle' filter in imageJ, either on the original data, or 12 13 the resulting segmentation. 14 Our results with the trabecular ROI add to those of Verdelis et al., (2011), who caution that inter 15 system microCT results at high resolutions may not necessarily be entirely comparable. We would

16 add an extra caution to this and would suggest that segmentation choice should be explicitly

17 referred to as this can also have a large effect on results. It also appears to contradict the results of

18 Christiansen, (2016) who found that different watershed methods did not really affect results at high

19 resolutions. This is definitely an area which warrants more investigation.

20 The Weka algorithms can be applied to a wide range of image types, as it was originally developed 21 for microscopy (Arganda-Carreras et al., 2017). We have tested this on image datasets from 8 to 32-22 bit depth. Given workflow constraints, most images used in everyday analysis will be either 8 or 16-23 bit. DICOM data requires the conversion to TIFF or other standard image formats to processing with 24 Weka. ImageJ/Weka segmentation is multi-platform and has a user-friendly GUI. This make it an 25 ideal toolbox to teach researchers (who may be unfamiliar with the subtleties of image processing) a fast and free way to process their CT data. Key parameters t observe are to use a fast CPU with 26 27 multiple cores, which will enable users to fully leverage multi-threading; as well as the use of fast 28 hard drives (preferably Solid State Drives) if working on a desktop. Training the segmentation using 29 fine structures will also improve delineation of edge features. Finally, the use of a graphics tablet is 30 also recommended.

A major disadvantage currently is that the Weka algorithms are extremely CPU intensive but in
 ImageJ, do not utilise the GPU. K-means and fuzzy c-means algorithms are also extremely CPU
 intensive, regardless of if they are written in ImageJ or Matlab. Interestingly, the MCIA algorithms

are very RAM intensive (Dunmore, pers. comm.). Implementation of the Weka algorithm, either 1 2 through virtualised clusters (e.g. FIJI archipelago,) or through GPU optimisation (either through CLIJ 3 (Haase et al., 2019) or Matlab bridging) may work to ameliorate bottlenecks in processing speed 4 somewhat. The yield in minimising user time in segmentation (as once trained, segmentation can 5 process independently of the user) does however make the current implementation an ideal 6 approach for the first pass segmentation of structures in archaeological and evolutionary studies. 7 Localised C-means segmentation in both ImageJ and Matlab also tend to flip the order of labels in 8 some images, which then necessitates further steps of interleaving different stacks to obtain one

- 9 segmentation. This is probably fairly straightforward to address by a forcing of order of labels in the
- 10 algorithm but is beyond the scope of this paper.

11 Conclusions

12 For the first time, we have presented the implementation of the Weka Machine learning library to 13 archaeological and palaeontological material. It yields results that are the equal of leading edgebased methods and superior to conventional segmentations produced by commercial packages. The 14 15 implementation of Weka segmentation is fast, with no software cost to the end user and it enables 16 an easy introduction to both image segmentation and machine learning for the inexperienced user. 17 Future work will seek to apply this algorithm to larger and more varied samples, as well as exploring the possibility of increasing the speed of computation, either through GPU based acceleration or use 18 19 of virtual clusters.

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18

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