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Three-dimensional rendering of otolith growth using phase contrast synchrotron tomography

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Corresponding Author:	Mark Henry Fisher, B.Sc., M.Sc., Ph.D. University of East Anglia Norwich, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of East Anglia
Corresponding Author's Secondary Institution:	
First Author:	James Mapp, M.Sc. Ph.D.
First Author Secondary Information:	
Order of Authors:	James Mapp, M.Sc. Ph.D.
	Mark Henry Fisher, B.Sc., M.Sc., Ph.D.
	Robert Atwood, Ph.D.
	Duncan Bell, M.D., M.Sc.
	Mark Greco, Ph.D.
	Sally Songer, B.Sc.
	Ewan Hunter, Ph.D.
Order of Authors Secondary Information:	
Abstract:	We present a three-dimensional computer reconstruction of a plaice (Pleuronectes platessa) otolith from data acquired by the Diamond Light synchrotron, beamline I12, X-ray source, a high energy (53 - 150 keV) source particularly well suited to the study of dense objects. Our data allowed non-destructive rendering of otolith structure, and for the first time allows examination of otolith annuli (internal ring structures) to be analysed in X-ray tomographic images.

Ethics questionnaire for JFB

This questionnaire relates to the Editorial published in JFB **68**, 1-2, which you have been asked to read. Please note that submitted manuscripts will only be considered if the experimental methods employed are ethically justified. PLEASE SUBMIT THE COMPLETED QUESTIONNAIRE WITH YOUR MANUSCRIPT ONLINE THROUGH EDITORIAL MANAGER.

Corresponding author's name: _ Mark Henry Fisher**___________________________________**

Question 1: If the fishes have been collected as part of faunal surveys, have the fishes, where feasible, been killed rapidly or returned to the wild after being held in aquaria?

Yes X

No ☐

Question 2: What method was used if they were killed?

The study used a single specimen (plaice) caught by a CEFAS survey vessel.

Question 3: If you have undertaken experimental work, has the care and use of experimental animals complied with local and or national animal welfare laws, guidelines and policies?

Yes ☐

No ☐

If 'Yes', state these and provide suitable evidence (*e.g*. for the U.K. a Home Office PPL number is sufficient) that protocols have undergone an ethical review process by an institutional animal care and use (or similar) committee, a local ethics committee, or by appropriately qualified scientific and lay colleagues.

N/A

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If 'No', because these laws do not exist in your country, please state this.

Please read the exceptions below (Questions 4 to 7). If any of these exceptions apply to your study, complete the appropriate section. Otherwise leave blank.

Question 4: Did you use experimental conditions that severely distressed the animals?

Yes ☐

No ☐

If 'Yes', state the conditions and how they can be justified.

N/A

Question 5: Did you use humane endpoints that minimized adverse effects?

Yes ☐
No ☐

Question 6: Have you performed surgical procedures?

Yes ☐
No ☐

If 'Yes', have you suitably described these in your manuscript?

Question 7: If the procedures caused more than slight pain or distress, did you use appropriate sedation, analgesia and anaesthesia, with appropriate post-operative care?

Yes ☐
No ☐

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Yes ☐
No ☐

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Yes ☐
No ☐

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Three-dimensional rendering of otolith growth using phase contrast synchrotron tomography

J. J. I. Mapp, M. H. Fisher*, School of Computing Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK.

R. C. Atwood, Diamond Light Source Ltd., Diamond House, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE, UK.

G. D. Bell, Faculty of Health and Science, University Campus Suffolk, Waterfront Building, Neptune Quay, Ipswich, IP4 1QJ, UK.

M. K. Greco, Faculty of Science, School of Dentistry and Health Sciences, Charles Sturt University, Panorama Avenue, Bathurst, New South Wales, 2795, Australia.

S. Songer, E. Hunter, Centre for Environment Fisheries and Aquaculture Science, Pakefield Rd., Lowestoft, Suffolk, NR33 0HT, UK.

Abstract

We present a three-dimensional computer reconstruction of a plaice (*Pleuronectes platessa*) otolith from data acquired by the Diamond Light synchrotron, beamline I12, X-ray source, a high energy (53 – 150 keV) source particularly well suited to the study of dense objects. Our data allowed non-destructive rendering of otolith structure, and for the first time allows examination of otolith annuli (internal ring structures) to be analysed in X-ray tomographic images.

Keywords

3-D Reconstruction; Fish Ageing; Otolith Growth; Phase Contrast; Synchrotron Tomography.

Fish otoliths have long played an important role in sustainable fisheries management (Campana 2005, Popper et al. 2005). The stock assessment models currently used rely on species specific age profiles obtained from the seasonal patterns of growth marks that their otoliths exhibit (Brophy 2014). Since the shape of otoliths is known to vary both within and between species due to the combined influences of genetic and environmental factors otolith shape is becoming increasingly important in determining the structure of mixed assemblages of fish stocks (Cadrin et al. 2014). Evidence of otolith microstructure, recovered by electron microscopy (Campana & Neilson 1985) and more recent chemical analysis of material at specific points in the accretion time-series and investigations using Raman microspectrometry have highlighted the physico-chemical characteristics of fish otoliths (Jolivet et al. 2008, Sturrock et al. 2012). But although these methods provide increasingly detailed information, all are invasive, ultimately resulting in degradation or even destruction of the tissue once the sampling is performed.

With X-ray tomography comes the possibility of recovering a full three-dimensional (3-D) otolith model of shape, density and composition, hitherto only approximated by stacking ablated serial sections (Bailey et al. 1995). Preliminary attempts to achieve this using conventional absorption X-ray micro-CT were reported by Hamrin et al. (Hamrin et al. 1999), but only the outer shape of the otolith was recovered (

Arneri et al.). Although the ambition of the implementation of phase-contrast micro-CT was to reveal the internal otolith structure, this ultimately proved unsuccessful. Working with SkyScan (http://bruker-microct.com) we recently repeated this experiment using a SkyScan 1172 desktop microCT with 5 micron spot size source operating at 60 kV. The detector used a 12-bit CCD camera fitted with Gallium Oxide scintillator, coupled by fibre optic connection. Our results show that a polychromatic X-ray source was unable to resolve sufficient X-ray absorption contrast to differentiate seasonal growth marks from the bulk composition within the otolith. These results are consistent with those reported previously by Hamrin et al. in 1999.

To investigate if higher energy X-rays could be used to recover internal growth features we initiated an experiment at Diamond Light (http://www.diamond.ac.uk/) to examine an otolith of the flatfish, plaice (*Pleuronectes platessa*). For this experiment we obtained X-ray images from a section of the otolith near the rostrum (highlighted in microscope image Fig. 1) with the aim of demonstrating proof of principle. The field of view is limited in this preliminary study due as the specimen was presented using general purpose mount. The images were processed using the method presented by Paganin et.al. (Paganin et al. 2002) that allows simultaneous phase and amplitude to be extracted from a single defocused image, then three-dimensional images were reconstructed using filtered back-projection (Kak & Slaney 1988). A reconstruction of the otolith is shown in Fig. 2.

Internal growth marks (rings or annuli) are visible in the individual slices (Fig. 3). The slice exhibits a number of primary rings, suggesting a fish age of 5 years. Software rendering programs such as DISECT (http://www.disectsystems.com/) and TOMOMASK (http://www.tomomask.com/) offer many possibilities for analyses of the reconstructed otolith volume. For example, while physical sections are limited to flat planes the virtual 3-D reconstructed volume can be cut along any curved surface or manifold. We illustrate this in Fig. 4 by reconstructing a surface orthogonal to the x-y plane along a spline curve fitted to the locus of maximum seasonal growth in a selection of slices (an example curve is plotted in Fig. 3). The reconstruction in Fig. 4 illustrates that otolith growth is complex and non-linear. This in turn highlights possible errors resulting from assessing age and growth of individuals from plane sections (current operational practice). An animation of the 3-D reconstruction and a virtual fly-through otolith can be found at https://www.youtube.com/channel/UCbvdIX_NY1EhJeJcYODG6tg. Although some imaging artefacts are evident in the reconstruction (e.g. the corona around the air/otolith interface), this proof of principle study provides a glimpse of the possibilities for otolith imaging using phase contrast synchrotron radiation and also demonstrates the potential for further 3-D rendered tomographic reconstructions using the Diamond Light I12 beamline.

Previous synchrotron studies of otoliths have used Synchrotron Rapid Scanning X-ray Fluorescence (SRS-XRF) to investigate trace elements (e.g. (Doubleday et al. 2014, Limburg et al. 2007)). This technique is inherently limited to an examination of the otolith surface and so may require thin sections of material to be prepared, resulting in damage to the specimen. The preliminary data from this study are encouraging because they illustrate the potential for more accurate measurement of total seasonal accreted volume (as opposed to growth estimates from 2-D sections). 3-D analysis is also potentially more robust to anomalous secondary growth signatures that do not correspond to seasonal deposits. Such artefacts continue to challenge even the most experienced readers of 2-D otolith sections thereby contributing to uncertainties in age estimates, and consequently stock assessments (de Pontual et al. 2006). Currently, synchrotron studies are very expensive but as the technology becomes more accessible virtual otolith studies using computer graphics could provide a historical perspective for each individual within their environmental context.

We hope to build on this preliminary study through funded access to the beamline to allow us to render whole otolith specimens, investigate species specific growth traits, and factors that affect the *direction* of maximal accretion, which seems to change with age (Fig. 3). This may help in understanding why certain species are particularly problematic for human otolith readers and provide a valuable insight into how and why the accreted biomineralisation is related to anatomy, physiology and lifecycle. Preliminary observations of the specimen suggest the study was totally non-invasive and that the otolith in this study was undamaged by the experimental process. This feature of tomographic analysis could be particularly valuable, for example where the otoliths of individual fish have been recovered accompanied by archival data storage tags which may have recorded ambient experience of the same fish for periods over seasons or sometimes years (Sturrock et al. 2012). Such otoliths with accompanying "ground-truth" data represent a rich resource of information concerning individual lifetime movements.

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Fig. 1: Microscope image of plaice otolith captured after exposure to synchrotron radiation. (Note: Area highlighted indicates the approximate location of the volume reconstructed by X-ray tomography).

Fig. 2: 3-D tomographic rendering of a section of plaice otolith highlighted in Fig. 1 recovered by the high energy Joint Engineering, Environmental and Processing (JEEP) I12 synchrotron beamline at the UK's national synchrotron science facility, Diamond Light (http://www.diamond.ac.uk/). The otolith was imaged using monochromatic X-rays of wavelength λ=.0234 nm (53 keV) and propagation phase contrast (Davis et al. 1995, Snigirev et al. 1995) inherent in such monochromatic X-ray images, was exploited to observe small variations in the sample. The camera was placed 1000mm beyond the sample to obtain this effect. This technique has been previously used with success for fossils and other specimens exhibiting weak variation in contrast (Tafforeau et al. 2006). The detector used was a CdWO4 (Cadmium Tungstate) scintillator viewed through bespoke radiation-hard microscope optics (SILL, Germany) by a PCO.EDGE (PCO, Germany) camera having 2560x2150 pixels, the resulting image pixel size being 5um square.

Fig. 3: Spline curve fitted to a set of maximal annular growth features identified in one of the image slices. Note: The direction of maximal growth varies throughout the life of the fish.

Fig. 4: Virtual section through the otolith taken along the curved surface orthogonal to the spline curve shown in Fig. 3.

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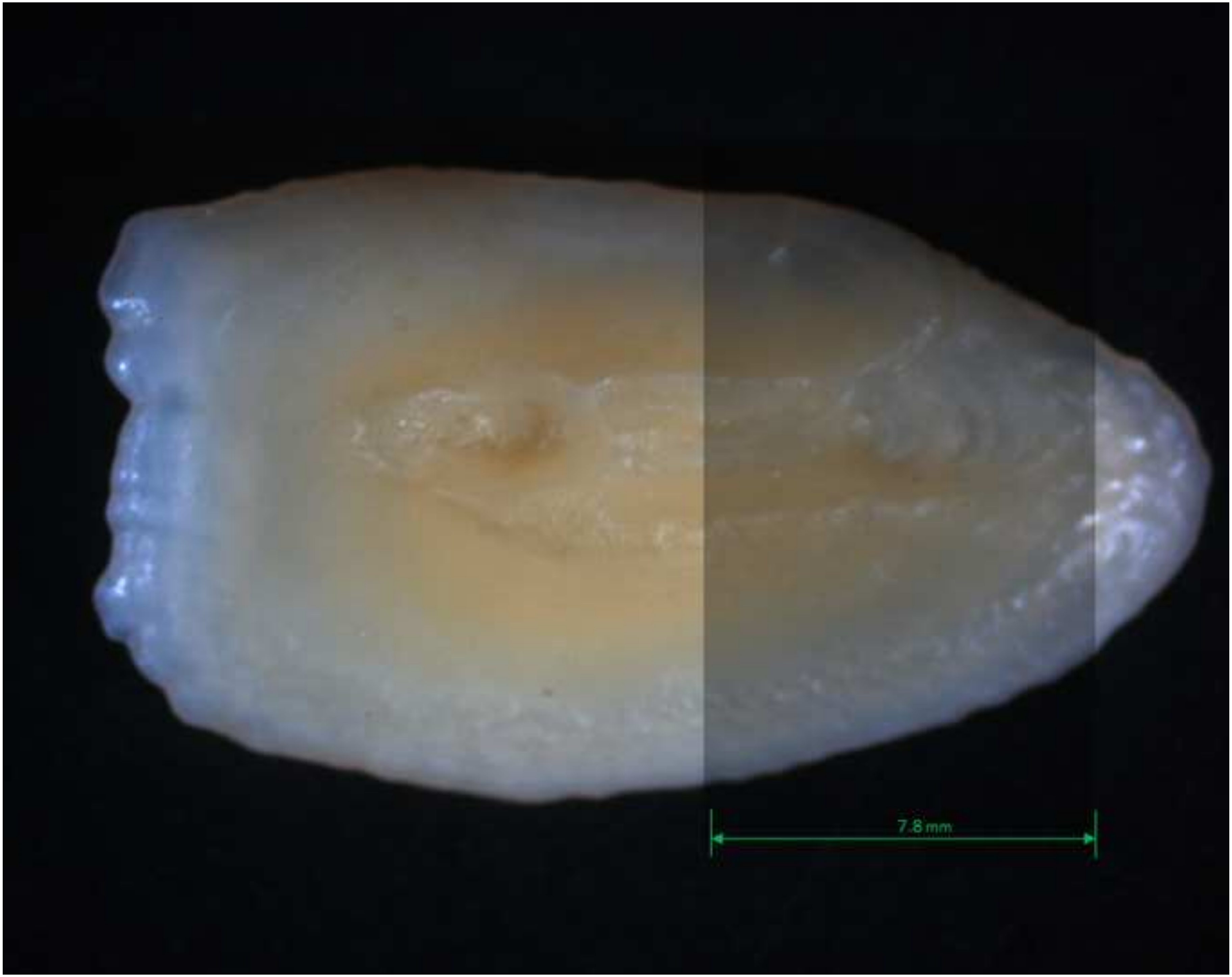

Figure

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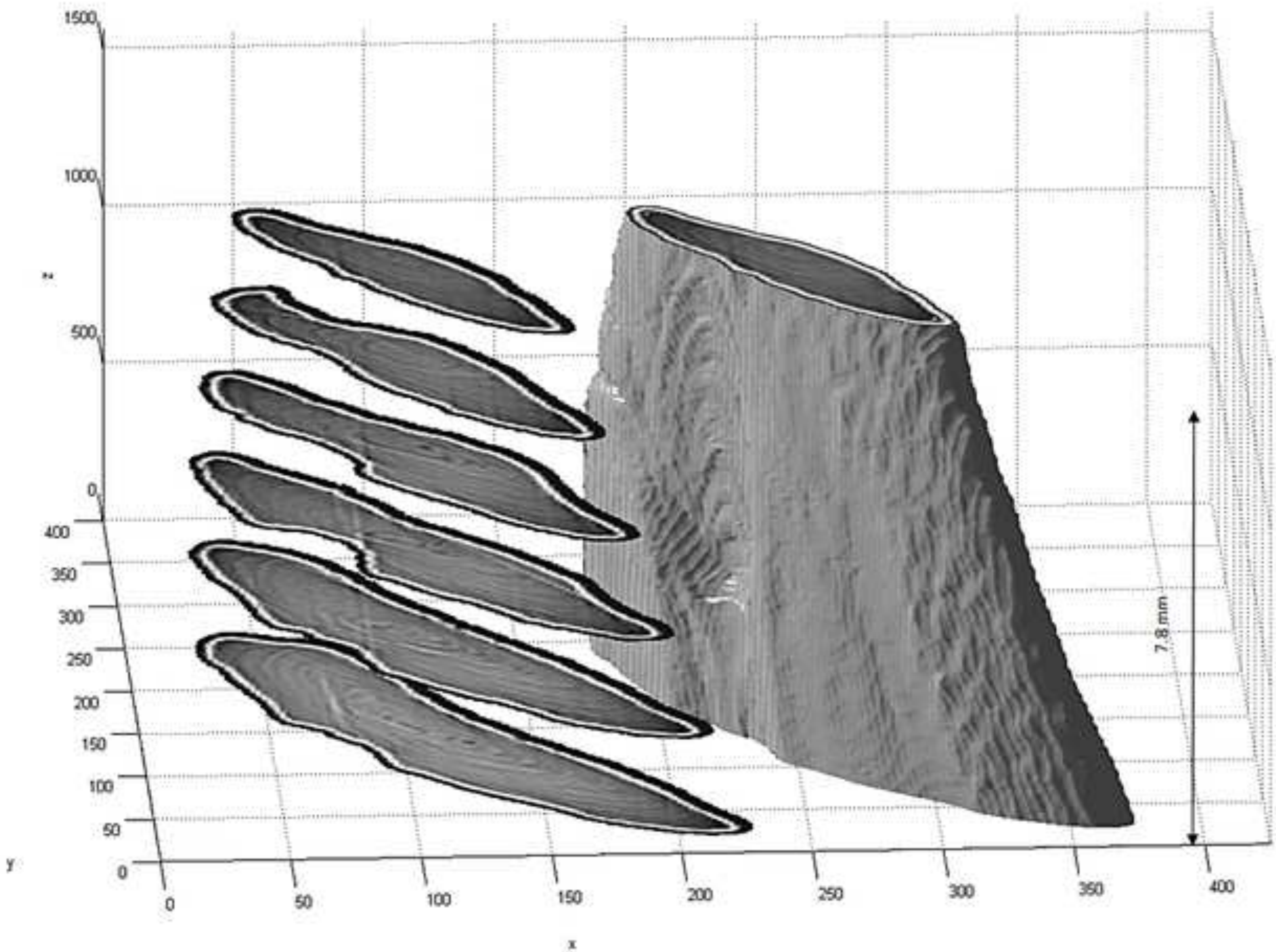

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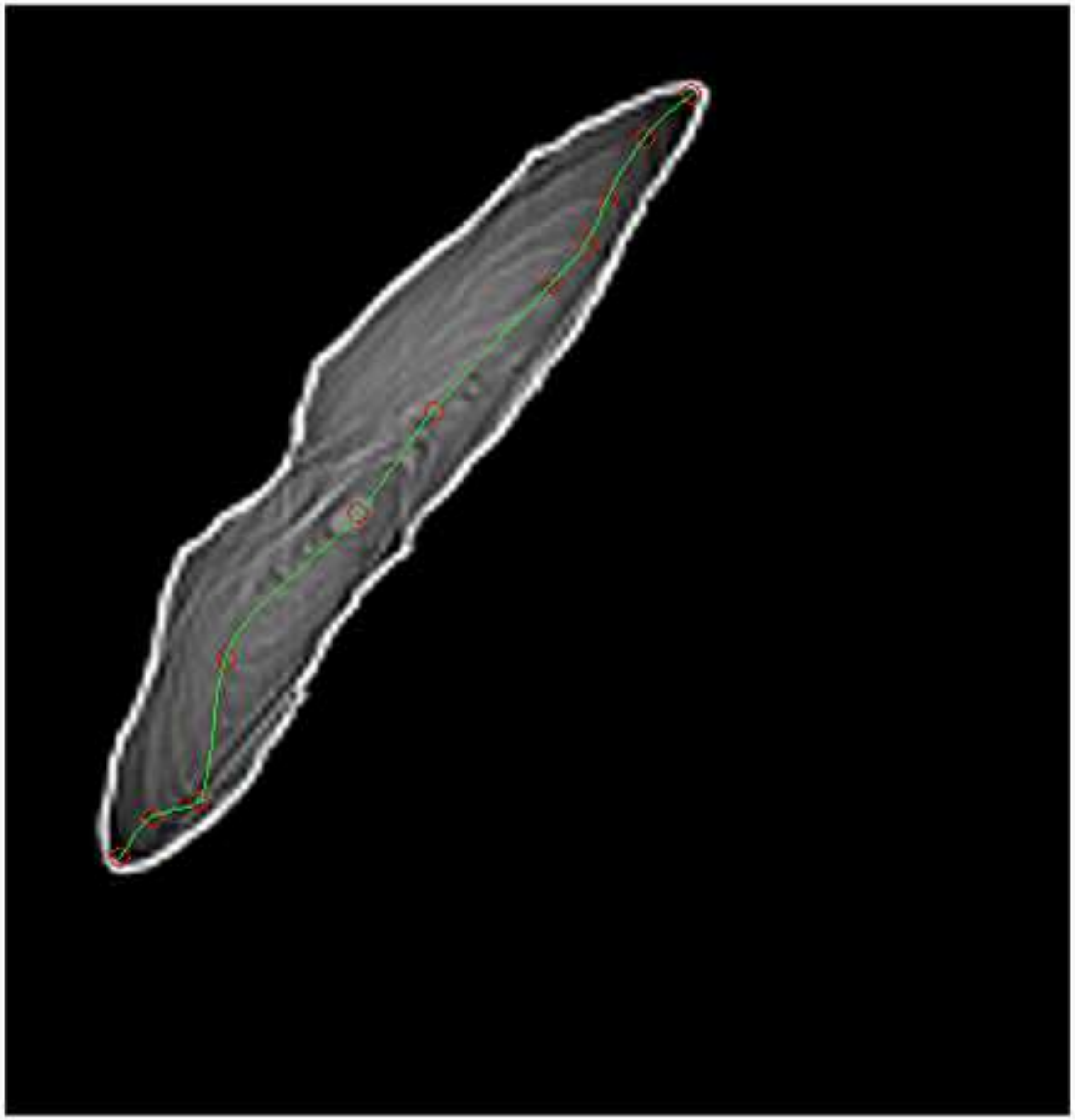

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