1	Automated detection of microfossil fish teeth from slide images
2	using combined deep learning models
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25 Authorship contribution statement

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- 32

33 ABSTRACT

34 Microfossil fish teeth, known as ichthyoliths, provide a key constraint on the depositional age and 35 environment of deep-sea sediments, especially pelagic clays where siliceous and calcareous microfossils are 36 rarely observed. However, traditional methods for the observation of ichthyoliths require considerable time 37 and manual labor, which can hinder their wider application. In this study, we constructed a system to 38 automatically detect ichthyoliths in microscopic images by combining two open source deep learning models. 39 First, the regions for ichthyoliths within the microscopic images are predicted by the instance segmentation 40 model Mask R-CNN. All the detected regions are then re-classified using the image classification model 41 EfficientNet-V2 to determine the classes more accurately. Compared with only using the Mask R-CNN 42 model, the combined system offers significantly higher performance (89.0% precision, 78.6% recall, and an 43 F1 score of 83.5%), demonstrating the utility of the system. Our system can also predict the lengths of the 44 teeth that have been detected, with more than 90% of the predicted lengths being within $\pm 20\%$ of measured 45 length. This system provides a novel, automated, and reliable approach for the detection and length 46 measurement of ichthyoliths from microscope images that can be applied in a range of paleoceanographic 47 and paleoecological contexts.

- 49 Keywords:
 50 Deep learning;
 51 Object detection;
 52 Image classification;
 53 Microfossils;
 54 Ichthyolith
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- 57 1. Introduction

58 Pelagic clay is a type of deep-sea sediment that covers more than one-third of the global ocean floor (Dutkiewicz 59 et al., 2015) and has long been regarded as an important medium for recording changes in atmospheric and 60 oceanic circulation, surface ocean productivity, and the influx of extraterrestrial material in the pelagic realm 61 (Kyte et al., 1993; Kyte, 1998; Kyte and Bostwick, 1995; Nozaki et al., 2019; Tanaka et al., 2022; Zhou and 62 Kyte, 1992). The chemical composition of pelagic clay varies considerably despite its homogenous appearance, 63 reflecting the fractions of its components, such as terrigenous dust, volcanic materials, hydrogenous and 64 hydrothermal Fe-Mn oxides, and biogenic components (Dunlea et al., 2015a, 2015b; Leinen, 1987; Ren et al., 65 2021; Tanaka et al., 2022; Yasukawa et al., 2016, 2019; Ziegler et al., 2007).

Pelagic clay has recently received attention as a novel type of mineral resource for rare-earth elements and yttrium (REY), which are industrially critical metals, especially for technologies and products aiming toward carbon-neutrality. Pelagic clays enriched in REY, termed as "REY-rich mud" (Kato et al., 2011), were originally discovered in the deep-sea basin of the central North and eastern South Pacific Ocean. To date, they have been reported from the global ocean, including the western North Pacific (Bi et al., 2021; Fujinaga et al., 2016; Tanaka et al., 2020a, 2020b), central and South Pacific (Ohta et al., 2021; Sa et al., 2018; Zhou et al., 2020, 2021), Indian (Yasukawa et al., 2014, 2015; Yu et al., 2021; Zhang et al., 2017) and Atlantic Oceans (Menendez 73 et al., 2017; Nakamura et al., 2015). Notably, the existence of "extremely REY-rich mud" in the western North 74 Pacific Ocean (Iijima et al., 2016; Mimura et al., 2019; Takaya et al., 2018), together with the investigations of 75 physical beneficiation techniques (Takaya et al., 2018) and by-product metal extraction (Yasukawa et al., 2018, 76 2020), further highlights the significance of pelagic clay as a promising mineral resource. Interestingly, the 77 accumulation of REY in pelagic clay was caused by changes in bioproductivity and ocean circulation, which 78 reflects changes in the Earth's climate system (Ohta et al., 2020). This indicates that examining environmental 79 changes recorded in pelagic clay is essential for understanding the genesis and distribution of industrially critical 80 metal resources, emphasizing the increasing importance of analyzing pelagic clay.

Depositional age is key information for understanding depositional environments of the seafloor sediment because the environment has been affected by a secular change in global climate (Westerhold et al., 2020; Zachos et al., 2008) and plate motion over geologic timescales (Müller et al., 2018). However, calcareous or siliceous microfossils, which have commonly been used for constraining depositional ages of the seafloor sediment, are not found in pelagic clay, owing to the dissolution of the fossils by undersaturation of carbonates and silica in the deep-sea environment. This has hampered examination of the depositional environment and exploration of the origin that controls distribution of deep-sea resources.

88 In contrast, fish teeth and denticles, known as ichthyoliths, are well preserved in almost all kinds of seafloor 89 sediments because they are composed of calcium phosphate, which is not easily dissolved (Sibert et al., 2014). 90 Therefore, ichthyoliths have been used as a key for constraining the depositional age of pelagic clay (Doyle et al., 91 1974; Doyle and Riedel, 1979, 1985; Ohta et al., 2020). In addition, ichthyoliths are regarded as indicators of 92 depositional environments recently. The productivity of pelagic fish has been measured based on the 93 accumulation rate of ichthyoliths (Sibert et al., 2014, 2016, 2020; Sibert and Rubin, 2021), the evolution of 94 pelagic ecosystems has been explored based on variations in morphotypes (Sibert et al., 2018; Sibert and Rubin, 95 2021), and the distribution of pelagic fish has been studied based on variation in the length of fish teeth (Britten 96 and Sibert, 2020). Hence, establishing an effective method for ichthyolith observation will enable understanding

97 of the records on the evolution of pelagic realms which has long been a black box in Earth science.

98 Traditionally, ichthyolith analysis first involves extracting coarse-grained particles from the target sediment. By 99 observing these grains under a stereomicroscope, ichthyoliths are manually picked up and moved on to a slide 100 using a fine-pointed brush. This process, called 'handpicking', remains a common technique for both 101 stratigraphic and environmental research (Ohta et al., 2020; Sibert et al., 2017) and is one of the most 102 time-consuming processes in ichthyolith analysis. Slides with the ichthyoliths are then observed under a 103 microscope for detailed description and identification. Observers describe a range of features including their 104 outer shape, inner structures, and size (Britten and Sibert, 2020; Doyle and Riedel, 1979; Sibert et al., 2018), 105 which also requires considerable time and effort by experienced experts.

106 In comparison to these manual techniques, recent developments in computer vision have achieved promising 107 results in various fields including medicine, neuroscience, and robotics (Jo et al., 2017; Kim et al., 2018; Sakai et 108 al., 2018; Shoji et al., 2018; Suleymanova et al., 2018). Techniques in computer vision have also been applied in 109 the field of microfossil research for the tasks of classification and detection. The classification of microfossils 110 was first attempted by obtaining key morphological parameters from microfossil images (Marmo et al., 2006; Yu 111 et al., 1996), with support vector machines (SVMs) contributing to their classification according to the acquired 112 values (Apostol et al., 2016; Bi et al., 2015; Hu and Davis, 2005; Solano et al., 2018; Xu et al., 2020). Owing to 113 the development of convolutional neural networks (CNNs), deep learning based classification models have 114 successfully been used to determine the taxa of various microfossils including foraminifera and radiolarians 115 (Carvalho et al., 2020; Hsiang et al., 2019; Itaki et al., 2020; Keçeli et al., 2017; Marchant et al., 2020; Mitra et 116 al., 2019; Pires de Lima et al., 2020; Xu et al., 2020). Although some of these classification models achieve an 117 accuracy of > 85% (Hsiang et al., 2019; Itaki et al., 2020; Marchant et al., 2020), large training datasets are often 118 required, which creates the challenge of generating a large number of images for each microfossil species. To 119 address this problem, previous studies (Hsiang et al., 2018; Itaki et al., 2020; Tetard et al., 2020) have proposed a 120 method that captures the entire area of a slide. In these studies, individual particles were extracted from the 121 image based on thresholding, which may reduce the efficiency of ichthyoliths observations for the following two 122 reasons. First, particles have to be positioned on the imaged slides without overlap, which can be practically 123 difficult when using glass slides. Second, ichthyoliths are translucent when observed under a polarized light 124 microscope, which makes determining an appropriate threshold challenging.

Here, as a first step toward using deep learning for ichthyolith observation, we describe a deep learning based system that can detect microfossil fish teeth from glass slide images and predict their lengths. The system is composed of open-source libraries, so that it can be readily applied to a range of detection problems within the geosciences.

129 2. Materials and Methods

130 2.1. System overview

Our system is divided into two parts: (1) the detection of fossil fish teeth from slide images and (2) the preciseclassification of the detected particles (Fig. 1), each described in the following sections.

133 2.1.1 Detection using Mask R-CNN

The slide images are processed using the object detection model "Mask R-CNN." Mask R-CNN is an open-source model that is capable of semantic segmentation and has a deep-learning-based algorithm that

136 predicts the label in every pixel of an image (He et al., 2017). The input image size was set to 640×640 pixels.

137 2.1.2 Re-classification using EfficientNet-V2

Although the fully trained Mask R-CNN model can predict the classes of the objects detected, we found that the model was unable to learn the features of fish teeth with our dataset. Therefore, we combined it with another open source deep learning model, 'EfficientNet-V2' (Tan and Le, 2021), which discriminates the classes of the particles detected by the Mask R-CNN model. Images of particles detected by Mask R-CNN were resized to 224 × 224 pixels without changing the aspect. These images are then classified into 'tooth' or 'noise' classes by the trained EfficientNet-V2 model. The class determined by the image-classification model was taken as the final class predicted by the system. In other words, even if a particle was predicted as a "tooth" by the Mask R-CNN 145 model, it was considered "noise" if it was classified as such by the EfficientNet-V2 model.

146

147 2.2 Experiments

148 2.2.1 Preparation of slide images

149 Glass slides were prepared from the pelagic clay samples collected at Ocean Drilling Program (ODP) Site 1179 150 and piston core site MR15-E01 PC11 in the western North Pacific Ocean, and Integrated Ocean Drilling 151 Program (IODP) Sites U1366 and U1370 in the South Pacific Ocean. The locations and water depths of these 152 sites are summarized in Table S1. The method for preparing the slides followed previous studies on the 153 determination of depositional ages (Doyle and Riedel, 1985; Ohta et al., 2020) with some modifications as 154 described by Sibert et al. (2017). Approximately 5 g of the wet sediment sample was first well mixed with 155 deionized water in a plastic bottle, and then sieved through a 62 µm mesh to collect the larger particles. Heavy 156 liquid separation was then used to concentrate biogenic calcium phosphate grains. The particles were well mixed 157 with a solution of sodium polytungstate (SPT; specific gravity = 2.80-2.85 g/cm³) and centrifuged at 1000-1500 158 rpm. The collected particles were washed with deionized water, placed on glass slides using a pipette, dried at 159 40 °C, and then sealed with a cover glass using a light-curing adhesive. Microscopic images of the entire area of 160 the prepared slides were automatically captured using an RX-100 digital microscope (Hirox Co., Ltd.). This 161 microscope has a motorized stage that moves gradually to divide the observation area into small squares, which 162 can be continuously imaged. The magnification of the microscope was $200 \times$ (each pixel = $0.96 \times 0.96 \mu$ m), and 163 approximately 1000 images of 1200 × 1200 pixels were generated from a single slide.

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165 2.2.2. Training of the object detection model

A total of 958 slide images with at least one ichthyolith were prepared to train the Mask R-CNN model. For these images, ichthyolith contour and class information was annotated using the VGG Image Annotator (Dutta and Zisserman, 2019). The dataset was randomly split into a training dataset, which was composed of 958 169 images with annotation data for 1625 teeth, and a validation dataset composed of 92 images and annotation data170 for 165 teeth.

171 The mask R-CNN model training was conducted using the online cloud service Paperspace 172 (https://www.paperspace.com/). To augment the dataset, the images were randomly flipped upside down and/or 173 left-to-right during the training. The initial learning rate was set at 0.001, and the model was trained for 80 174 epochs. The progress of learning was monitored by calculating the losses implemented in the Mask R-CNN 175 library for both the training and validation datasets.

176

177 2.2.3. Training the image classification model

Particles within the slide images were trimmed from the classification model dataset. These particles were manually labeled into the 'tooth' and 'noise' classes. Examples of 'noisy' particles are fish bones, opaque grains that are possibly micro ferromanganese (Fe-Mn) oxides (Yasukawa et al., 2020), and the edges of light-curing adhesives (see Fig. 1b). The EfficientNet-V2 model was trained using the Google Colaboratory Cloud service (Carneiro et al., 2018). During the training, the images were randomly flipped upside down and/or left-to-right to prevent overfitting. The learning rate was set at 0.005, and the model was trained for 20 epochs. The progress of the learning was monitored by calculating the losses and accuracies for both the training and validation datasets.

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186 2.2.4. Tests for the practical use of the system

In addition to the validation of each model, we conducted a practical test to verify the performance of the entire system. A total of 5177 slide images from six glass slides were generated from a single sample (ODP Site 1179, section 24, Core 5, 75–77 cm interval). This sample was not used for any of the training or validation datasets. Annotation data for the locations of the 431 teeth within the images were prepared. The images were first subjected to detection using the trained Mask R-CNN model. By comparing the annotated data and the model predictions, the number of true positives (TPs), false positives (FPs), and false negatives (FNs) were determined. 193 TPs represent the numbers of teeth that were correctly predicted as teeth by the model. FPs represent the 194 numbers of non-teeth particles that were incorrectly predicted as teeth. FNs represent the numbers of teeth that 195 were not detected by the model. Using these values, several evaluation parameters were calculated as follows:

196
$$\operatorname{Precision} = \frac{TP}{TP + FP} \dots (1)$$

197
$$\operatorname{Recall} = \frac{TP}{TP + FN} \dots (2)$$

198 F1 score =
$$\frac{2(Precision \times Recall)}{Precision + Recall}$$
 ... (3)

Precision represents the extent to which the model misclassified particles as teeth. Recall represents the extent to which the model failed to detect teeth. The F1 score is the harmonic mean of precision and recall, indicating the overall balance of the model. After evaluation of the Mask R-CNN model detection results, all of the detected particles were re-classified using the EfficientNet-V2 model, and the precision, recall, and F1 scores were recalculated.

204

205 2.3 Measurement of ichthyolith length

206 The dimensions of ichthyoliths are key for their accurate classification. Here, we defined the length of a tooth as 207 the perpendicular length from the apex of the outline to the lowest level (Fig. S1a) based on the traditional 208 ichthyolith description system (Doyle and Riedel, 1979). Given that variation in tooth length can be used as an 209 indicator of variation in the body sizes of pelagic fish (Britten and Sibert, 2020), we attempted to predict the 210 lengths of teeth automatically, by approximating the detected contours of each tooth within a rectangle and 211 measuring the length of the longest side (Fig. S1b). This approach was based on the assumption that most teeth 212 have an elongated shape (Britten and Sibert, 2020). To evaluate the accuracy of the acquired lengths, tooth 213 lengths were manually measured in the same images following the traditional methods for ichthyolith 214 biostratigraphy.

- 216 3. Results and Discussion
- 217 3.1 Detection of fish teeth
- 218 3.1.1 Mask R-CNN

219 Figure 2 shows the trend of the loss function for each training epoch. Although the loss values for the training 220 dataset gradually decreased, the loss for the validation dataset oscillated within the range of 0.5-1.2 and did not 221 show any significant decrease. This indicates that the model could not sufficiently learn the general features of 222 the teeth. A practical test was performed using the trained model up to epoch 80. Although the model showed 223 99.3% recall, the precision was 5.5%. Thus, while almost all of the ichthyoliths were correctly detected, many 224 non-tooth particles were incorrectly classified as teeth by the trained Mask R-CNN model (see Fig. 1b). 225 Therefore, detection by the Mask R-CNN model alone does not represent a time-saving approach because 226 manual intervention is still needed to correctly identify ichthyoliths from a large number of detected particles.

227

228 3.1.2 EfficientNet-V2

The trends in the loss functions and accuracies for the training and validation datasets during each epoch of the EfficientNet-V2 model training are shown in Fig. 3. For the training data, there was a decrease in loss and an increase in accuracy up to epoch 20. The validation data also showed a decrease in loss and an increase in accuracy up to epoch 10, and maintained low losses and high accuracy without oscillation during epochs 10–20. This suggests that the model successfully learned the general features of the teeth without overfitting the training dataset. The model trained up to epoch 19 was selected as the best model, when the lowest validation loss was recorded, and was subjected to the practical test.

By combining the Mask R-CNN model trained up to epoch 80 and the EfficientNet-V2 model trained until epoch 19, the practical test was performed as described in Section 2.2.4. By testing several thresholding confidence scores, we found that the highest F1 value was achieved when the particles predicted by the EfficientNet-V2 model to be teeth, with a confidence of more than 0.45, were treated as teeth (Table S2). Compared to the Mask R-CNN model alone, the combined system showed significantly higher precision and slightly lower recall
(89.0% and 78.6%, respectively, Fig. 4). This indicates that the EfficientNet-V2 model is effective at identifying
fish teeth from the large numbers of particles detected by the Mask R-CNN model. The F1 score was 83.5%,
which is eight times higher than that of the Mask R-CNN model when used alone.

For application of this system in stratigraphic research, it is important to detect clear and distinct ichthyoliths with a small number of false positives, even if small and obscure ichthyoliths are not detected. In this case, a threshold score of 0.45 should be used to obtain the highest F1 score. In environmental research, the total number of ichthyoliths within a sample is an important proxy. A threshold score of 0.1 and manually checking the detection results can minimize the occurrence of false negatives. Although this approach requires some manual labor, it is much more time-efficient than the previous handpicking process.

250

251 3.2 Measurement of ichthyolith length

The scatter diagram for the lengths of the teeth predicted by the contours of the detection results and manually measured lengths is shown in Fig. 5. In three cases (out of 341), the predicted lengths were significantly shorter than the measured length, which occurred when the Mask R-CNN model was unable to determine the contours of the model. However, overall, the predicted lengths of 90.6% of the detected teeth were within \pm 20% of their measured lengths. This indicates that as well as their detection and classification, our system provides an efficient means of determining the length distribution of fossil fish teeth.

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259 3.3 Implications for the wider application of object detection in the geosciences

There are many fields within the geosciences in which images are used to detect and/or count target objects (Ohta et al., 2016, 2020; Takahashi et al., 2009; Usui et al., 2017). Automation of these tasks using object detection techniques has the potential to acquire a greater number of results and enable more comprehensive investigation than has been previously possible. However, object detection has not yet been widely applied in the 264 geosciences, with the exception of remote sensing (Zhang et al., 2020). This can be attributed to the difficulty in 265 generating the large learning datasets required for precise detection. This is hindered by the requirement for 266 special equipment, such as microscopes (polarizing microscopy, stereoscopic microscopy, and scanning electron 267 microscopy) and computed tomography (CT) scanners. This has cost, time, and manual labor implications that 268 can make the acquisition of a large number of images impractical. Second, the annotation process of object 269 detection often requires skilled expertise, compared with more applied fields of research such as robotics, 270 medicine, and materials science, and devoting sufficient resources (both budgetary and personnel) to the 271 annotation process may be less prioritized in this field.

272 Our study shows that a relatively small dataset (< 1000 microscopic images containing approximately 1800 273 teeth) is sufficient to train the Mask R-CNN model to detect the contours of possible teeth, although when used 274 alone, it was not sufficient to distinguish the teeth precisely. Therefore, the best overall performance was 275 achieved by fully training a model focused on the classification of the predicted regions, which requires much 276 less time and manual labor than preparing a large dataset for the Mask R-CNN model. This indicates that 277 challenging object detection problems can be efficiently addressed by dividing the task into two subtasks i.e., 278 extracting the contours of candidate objects and then precisely classifying the objects based on the extracted 279 contours. This implies that object detection may be applied in various fields in the geosciences, especially where 280 the acquisition of large training datasets for object detection has proven to be challenging.

281

282 4. Conclusions

We developed and tested a system to detect fossil fish teeth from slide images by combining two open source deep learning models—the object detection model 'Mask R-CNN' and the image classification model 'EfficientNet-V2'. The system provided results with 89.0% precision, 78.6% recall, and an F1 score of 83.5% in a test that assumed realistic conditions, indicating its potential for practical application. In addition, the system successfully derived the lengths of 90% of the detected teeth with an accuracy of \pm 20%. As such, the system has

288	potential for constraining both the depositional ages and environments of deep-sea sediments and, more broadly,
289	contributing to research on the evolution of the marine ecosystem. Additional work is now being undertaken to
290	update the EfficientNet-V2 model so that ichthyoliths can be further classified into morphological taxa. This
291	requires a larger dataset of ichthyolith images, which could be compiled with the support of the system
292	constructed in this study.
293	Competing interests
294	The authors declare that they have no competing interests.
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305	Data Availability
306	Datasets related to this article can be found at http://dx.doi.org/10.17632/zdpz6m9gzf.1, an open-source online
307	data repository hosted at Mendeley Data (Mimura, 2022).
308	
309	Code Availability
310	
010	The sample codes for application of Mask R-CNN and EfficientNet-V2 for microfossils detection proble

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529	Li	st of Figures
530	1.	Figure 1: An overview of the ichthyolith detection system constructed in this study. (a) First, possible
531		regions of fish teeth in slide images were proposed using the object detection model 'Mask R-CNN'. Both
532		teeth and other particles were predicted as class 'tooth' by Mask R-CNN, and the precise classes were
533		re-predicted by an image classification model 'EfficientNet-V2'.
534		
535	2.	Figure 2: Losses for training and validation datasets during the training of the Mask R-CNN model.
536		
537	3.	Figure 3: (a) Losses and (b) accuracies for training and validation datasets during the training of the
538		EfficientNet-V2 model.
539		
540	4.	Figure 4: Precisions, recalls, and F1 scores from the practical test. The bars on the left side indicate the
541		results when the regions of fish teeth were predicted by the Mask R-CNN alone, while the bars on the right
542		represent the results predicted by the combined system of Mask R-CNN and EfficientNet-V2.
543		
544	5.	Figure 5: Comparison of mechanically predicted and manually measured ichthyolith lengths.
545		
546	6.	Figure S1: Additional information on acquiring the (a) manually measured and (b) mechanically predicted
547		length of ichthyoliths.
548		
549	Li	st of Tables
550		
551	1.7	Table S1. Locations and water depths of the analyzed sites.

553	2.	Table S2. Results of the practical test with varying threshold confidence scores of the EfficientNet-V2
554		model.
555		

(a) detection

object detection model "Mask R-CNN"



inputs: slide images



outputs: locations of possible teeth



(b) classification

inputs: particles detected by Mask R-CNN

outputs: classes of the images

class 'tooth'



Figure 1



Figure 2



Figure 3







Figure 5

(a) Manually measured length

(b) Prediction of length by contour

contour predicted by Mask R-CNN model

100 µm

The smallest rectangle that encloses a contour

Table S1. Locations and water depths of the analyzed sites.

ODP/DSDP	Leg/Cruise	Site	Hole	Latitude	Longitude	Water depth [m]
ODP	191	1179	С	41°04.7871' N	159°57.7856' E	5,563.9
IODB	Exp. 329	U1366	С	26°03.0845' S	156°53.6700' W	5,129.5
IODP		U1370	D	41°51.1156' S	153°06.3812' W	5,073.0
-	MR15-E01	PC11	-	21°58.2732' N	153°47.7461' W	5,770

threshold score	Recall	Precision	F1 value
0.1	90.8	61.9	73.6
0.15	86.4	74.1	79.8
0.2	84.8	79.0	81.8
0.25	83.2	81.1	82.1
0.3	80.9	82.8	81.8
0.35	80.2	85.1	82.6
0.4	79.0	87.3	83.0
0.45	78.6	89.0	83.5
0.5	77.6	89.9	83.3
0.55	77.4	90.3	83.4
0.6	76.3	91.4	83.2

 Table S2. Results of the practical test with varying threshold confidence scores of the EfficientNet-V2 model.

 threshold score
 Recall
 Precision
 F1 value