

Interactive comment on “A new automated radiolarian image acquisition, stacking, processing, segmentation, and identification workflow” by Martin Tetard et al.

Martin Tetard et al.

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Interactive comment on “A new automated radiolarian image acquisition, stacking, processing, segmentation, and identification workflow” by Martin Tetard et al.

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Comment: The development of an automated system for the collection of radiolarian census-data using a neuronal network is a consequent step to give over time-

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consuming workflows to machines. Posting the codes as well as the organisation of a discretionary image-based radiolarian (training) dataset are a good practice, but it also means that maintaining the dataset will be one of the most important tasks for the future. The manuscript is well done and requires only minor revisions. The following annotations and questions should be considered and/or answered in the final publication.

Response: We thank the reviewer for its comments about the MS, a detailed answer to each of its raised point is given below.

Comment: I miss a short introduction about neuronal networks and the “k-nearest neighbors” algorithm for readers who are not familiar with these terms.

Response: A short sentence was added about the neural network that was used, however, we refer to the very detailed paper of Marchant et al. (Accepted) for more information about accurate description of the algorithm.

Comment: As mentioned in the script’s introduction, one and the same specimen may be referred to different species/classes depending on the experience, subjective interpretation and/or taxonomic “education” of the researcher (e.g. Fenton et al. (2018) for planktonic foraminifera). Thus, to reduce the number of possibly mistakenly identified specimens in the training dataset, having at least one more taxonomic expert checking the correctness of the species determination of specimens within the dataset could increase the reliability of the dataset.

Response: Overall, three expert reviewed the database: Martin Tetard, based on numerous and consistent publications, Giuseppe Cortese a radiolarian taxonomist expert, and the first reviewer, David Lazarus, that points out numerous correction on the database.

Comment: Does the transparency of the radiolarian shells produce any problems for the image stacking, especially in case of smaller and more delicate specimens? Figure

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3j) shows a specimen of the species *Collosphaera tuberosa*. Its contours are diffuse. Is this a common “problem” for this species? Does this affect the identification accuracy for this species and may be one reason for the relatively high value of confusion with *Solenosphaera zanguebarica*?

Response: As the shells are outlined by the different refractive indices between the shell and the mounting medium, we don't experience any issue with the stacking step, even with small and / or delicate shells. Lots of time was spent for finding the best parameters with regard to the stacking method on Helicon Focus. The diffuse contours of the specimen of *Collosphaera tuberosa* is due to its position on the slide. This specimen was slightly out of focus from the 150 μm range imaged using the automated stacking technique. However, this specimen is the best preserved we have for now, explaining why it was chosen for a plate. This problem is not common but on some slides, it may happen that some specimens are out of focus from the stacking range. This does not usually affect the identification. Most of the confusion between *C. tuberosa* and *S. zanguebarica* was due to the few number of specimens that was imaged in both classes. Now, with more images, these two classes are no longer confused with each other in the new version of the CNN.

Comment: The collection of census data for planktonic foraminifera avoid juvenile specimens (e.g. Davis et al. (2019) only investigated the $>125\mu\text{m}$ fraction), because their identification is often very difficult (Fenton et al., 2018). Is there a lower size limitation for radiolarian specimens to be detected and identified by the new system? Is the system able to distinguish between early ontogenetic stages and broken specimens? Does the size of specimens affect the accuracy of the automated species determination?

Response: We only work on the fraction $> 50 \mu\text{m}$ to avoid lots of juvenile specimens that are often difficult to identify as early features are shared between numerous species, and lots of broken shells. However, the ontogenetic stages are visible in numerous classes and, when they are sufficiently imaged, can be distinguished. The size

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of specimens do not seem to affect the accuracy of the system as most of the images are above the image size (256 px) used to train the CNN.

Comment: What is the procedure for (intact) specimens which extend over the borders of the 324 FOV and are parted/bisected? Is the program able to identify these specimens as being intact? In this case, are these specimens prevented from being “double-counted” by the system?

Response: For specimens that are “cut” between two FOVs, if a part of the shell contains the first chambers (usually for nassellaria) it should be identified as the correct class, and the second part should be identified as “broken”, to prevent a double identification in the correct class.

Comment: Closely related species tend to show a similar morphology and are often only distinguishable by details. Since the sample preparation bases on random settling, the orientation of a single specimen may not be ideal to enable the program to recognise these morphological details. What is the procedure for specimens which do not show an ideal orientation for determination?

Response: The intra-specific morphological variability generated by the orientation of specimens in images is mostly covered by the number of images present in each class. However, most of the specimens settled the same way for each specimen of a class. Also, if a specimen is oriented in a way that its identification is not possible (e.g. the under view of a nassellaria, which is very rare), it is identified as “broken”.

Comment: I give the authors credit for implementing morphometric measurements. In combination with census data they may provide additional and valuable information for palaeoenvironmental reconstructions and evolutionary studies. Although this paper clearly focuses on the collection of census data, the accuracy of the morphometric measurements should be given as well. To what extent do differences in specimen orientation affect the accuracy and intraspecific comparability?

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Response: The morphometric measurements of every specimens, averaged for each class, will be presented for two cores in a next study that is in preparation. As the morphometric measurements are performed on the outline of each specimens, we are confident regarding their accuracy. Regarding the bias generated by the specimens' orientation, as specimens usually fall on the same way for every specimen of each class, we are confident that most of the observed variability is due to actual change in shape, and not to change in their orientation between samples.

Comment: -l. 62-63: The sentence contains two times the phrase "promising results". -l. 86: A comma is missing after "6.3ka". "[. . .] 3-4cm, (6.3ka[,] de Garidel-Thoron et al., 2005) [. . .]" -l. 273: there is a closing bracket at the end of the sentence, but I could not figure out the corresponding, opening counterpart. "[. . .]and that 150 images represent the C3 original dataset for this class; Fig. 5 green square). [. . .]" -l. 359: The semicolon may be replaced by a closing bracket. "[. . .] palaeoenvironmental proxies such as SSTs (e.g., radiolarian-based palaeotemperatures for [. . .], Kamikuri, 2017;[]] and paleoproductivity [. . .] -Fig. 2: A space is missing in the text for step 7. "7.[]Identification of every single particle using a trained CNN." -Fig. 4: The printed version is difficult to read, because the font size of the species names is relatively small. The digital figure requires a lot of scrolling. -Fig. 5: Several names of species overlap and make it impossible to read them. -Fig. 6 e,f: The percentage numbers are difficult to read, because they overlap with black bars within the figure.

Response: -l. 62-63: The was corrected and the double word was removed. -l. 86: The comma was added. -l. 273: The closing bracket was removed. -l. 359: The semicolon was replaced by a closing bracket. -Fig. 2: A space was added. -Fig. 4: This figure was revised and the species is now slightly bigger. To help with the reading of this figure, an excel spreadsheet was added as a supplementary material, with a fixed first column (classes name) that help with the scrolling. -Fig. 5: This figure was removed as discussed in the reviewer 1's response to comment. -Fig. 6 e,f: This was corrected.

Thank you for all your comments.

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