Automated Segmentation of Insect Anatomy from Micro-CT Images Using Deep Learning

Evropi Toulkeridou[1], Carlos Gutierrez[1], Daniel Baum[2], Kenji Doya[1], and Evan Economo[1]

[1]Okinawa Institute of Science and Technology Graduate University
[2]Zuse-Institut Berlin

May 2, 2023

Abstract

Three-dimensional (3D) imaging, such as micro-computed tomography (micro-CT), is increasingly being used by organismal biologists for precise and comprehensive anatomical characterization. However, the segmentation of anatomical structures remains a bottleneck in research, often requiring tedious manual work. Here, we propose a pipeline for the fully-automated segmentation of anatomical structures in micro-CT images utilizing state-of-the-art deep learning methods, selecting the ant brain as a test case. We implemented the U-Net architecture for 2D image segmentation for our convolutional neural network (CNN), combined with pixel-island detection. For training and validation of the network, we assembled a dataset of semi-manually segmented brain images of 76 ant species. The trained network predicted the brain area in ant images fast and accurately; its performance tested on validation sets showed good agreement between the prediction and the target, scoring 80% Intersection over Union (IoU) and 90% Dice Coefficient (F1) accuracy. While manual segmentation usually takes many hours for each brain, the trained network takes only a few minutes. Furthermore, our network is generalizable for segmenting the whole neural system in full-body scans, and works in tests on distantly related and morphologically divergent insects (e.g., fruit flies). The latter suggests that methods like the one presented here generally apply across diverse taxa. Our method makes the construction of segmented maps and the morphological quantification of different species more efficient and scalable to large datasets, a step toward a big data approach to organismal anatomy.

Automated segmentation of insect anatomy from micro-CT images using deep learning

Evropi Toulkeridou[1*], Carlos Enrique Gutierrez[2], Daniel Baum[3], Kenji Doya[2*], Evan P. Economo[1*]

1 Biodiversity and Biocomplexity Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna, Okinawa, 904-0495, Japan
2 Neural Computation Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna, Okinawa, 904-0495, Japan
3 Zuse Institute Berlin, Takustraße 7, 14195, Berlin, Germany

*evropi.toulkeridou@oist.jp
*doya@oist.jp
*economo@oist.jp

Abstract

Three-dimensional (3D) imaging, such as micro-computed tomography (micro-CT), is increasingly being used by organismal biologists for precise and comprehensive anatomical characterization. However, the segmentation of anatomical structures remains a bottleneck in research, often requiring tedious manual work. Here, we propose a pipeline for the fully-automated segmentation of anatomical structures in micro-CT images utilizing state-of-the-art deep learning methods, selecting the ant brain as a test case. We implemented the U-Net architecture for 2D image segmentation for our convolutional neural network (CNN), combined with pixel-island detection. For training and validation of the network, we assembled a dataset of semi-manually segmented brain images of 76 ant species. The trained network predicted the brain area in ant images fast and accurately; its performance tested on validation sets showed good agreement between the prediction and the target, scoring 80% Intersection over Union (IoU) and 90% Dice Coefficient (F1) accuracy. While manual segmentation usually takes many hours for each brain, the trained network takes only a few minutes. Furthermore, our network is generalizable for segmenting the whole neural system in full-body scans, and works in tests on distantly related and morphologically divergent insects (e.g., fruit flies). The latter suggests that methods like the one presented here generally apply across diverse taxa. Our method makes the construction of segmented maps and the morphological quantification of different species more efficient and scalable to large datasets, a step toward a big data approach to organismal anatomy.

Key Points

- Development of a deep learning based pipeline for the fully-automated segmentation of micro-CT images of insects, using ant brains as a starting point.
- Creation of an open access dataset of micro-CT images of ant heads for training and testing.
- Generalizable computer vision methodology, extendable across diverse taxa and anatomical features.

Keywords

Automated Segmentation, Deep Learning, Computer Vision, Ants, Comparative Biology, Insect Anatomy

INTRODUCTION

Three-dimensional (3D) imaging of animals by X-ray micro-computed tomography (micro-CT) has become popular in morphological biology as a non-destructive method to acquire high-precision data on organismal anatomy [1, 2, 3, 4, 5]. The high-resolution 3D data enables the users to visualize and quantify internal and external structures, forming the basis for a wide range of biological applications.

A key challenge for the use of micro-CT lies in the analysis of large amounts of acquired data. In particular, while the 3D images are usually reconstructed after scanning, the reconstructed 3D images do not yet provide measures for morphological studies. What is needed for this is the segmentation of the 3D images. Only then can they be visualized and quantified. Thus, segmentation for the processing of the data is essential.

The most common segmentation method is manual processing, which is extremely time-consuming and compromises reproducibility [6]. This limits the number of samples that can be included in a given study, and thus the scientific applications of 3D scanning. For example, developmental biologists may want to analyze large numbers of experimental treatments and replicates. Or, in comparative biology, we may seek to analyze the evolution of a body part across hundreds or thousands of species. The recent emergence of large databases and coordinated projects to scan many species in specific taxonomic groups (e.g., oVert) [7] offers rich opportunities for new research directions if limitations on segmentation can be overcome.

In the medical literature, image segmentation methods have recently become more powerful and efficient due to significant developments in machine learning algorithms. To date, the main focus of automated segmentation methods has been on cells and human organs (e.g., human CT or MRI image segmentation for cancer detection [8, 9] or bone structure [10]. However, there is a great potential for automated segmentation to accelerate biological research on organisms across the tree of life [11, 12, 13, 14].

New software for biomedical image analysis has steadily progressed during recent years, with the capability for analysis and segmentation of 2D or 3D biological images and to build own data processing pipelines [15]. However, despite the unconstrained accessibility to free general-purpose software tools, the development of specific segmentation algorithms is essential to achieve high accuracy, objectivity, and reproducibility. Recently, deep learning and convolutional neural networks (CNNs) have been successfully applied in numerous image classification and semantic segmentation problems [16, 17]. CNNs have recently become widely used in image processing due to their high performance, the efficiency of GPUs, and the availablity of free software platforms and pre-trained networks [18].

Toolsets and pipelines that use classical statistical methods [19, 20, 21] such as ANTs [22], Biomedisa [23], and Freesurfer [24] are accessible and accurate for the segmentation of high-resolution images. However, these are either not fully automated and still require an expert user and considerable amounts of time and effort [25] (requiring training examples within the same scan), or are not adaptable to diversity and complexity in the target set. On the other hand, accurate and general toolkits and application frameworks that use machine learning techniques such as SlideCam have been successfully used for medical image segmentation as well as computer-aided diagnosis and analysis of images spanning from human brain segmentation to cancer

detection [26]. However, to date no toolkit has been designed to recognize homologous parts across a wide diversity of animal species, which would require an appropriate choice of network architecture, fine-tuning of hyperparameters, and the production and curation of substantial, high-quality datasets. When it comes to analyzing such images, segmentation remains a most challenging task, and often manual or semi-automated segmentation is still the only way to analyse the data.

U-Net is a CNN architecture that has shown high accuracy and robustness for biomedical image segmentation [27]. It uses relatively small amounts of training images to achieve precision even for segmentation of areas with unclear borders. The simple architecture of U-Net makes it easy to develop and very fast to train. Once a U-Net is trained, the acceleration of the segmentation is extreme: for example, the segmentation time for one ant brain, which may be up to a whole day's work if performed manually, is reduced to merely 1-2 minutes by automatic segmentation.

In this paper, we present an automated pipeline for segmentation of different parts of insects in volumetric data, using micro-CT scans, and specifically ant brains across a diverse set of different ant species, as a test case. A basic question for such studies is how general algorithms can be applied across the tree of life. Can an algorithm trained to recognize a part in one type of organism be used on more distant relatives, or do they break down once applied outside the group for which they were developed? Ants are a well-defined clade following a similar overall body plan but reflect >100 million years of diversification and a large range in ecological, sensory, and behavioral modes [28, 29]. We expect ant brains to have an intermediate level of diversity and thus be a reasonable test case: they will change in size and shape across species, while the general organization and tissue composition should be conserved [30]. As a secondary experiment, we assess whether the ant brain segmenting algorithm we developed can be applied with minimal modification to recognize brains in distantly related insects.

RESULTS

Overview of segmentation pipeline

Our strategy for the development of an automated micro-CT image segmentation pipeline can be broken down into eight steps, as listed below and schematically illustrated in Figure 1. More methodological details can be found in the first section of the Supplementary Information document. A proposed segmentation workflow for potential users should contain modules 1, 2, 4, 6, and 7.

1. **Sample preparation:** Before scanning, all specimens were kept in 97% ethanol until they were transferred to iodine staining solution (2%) for an average of two weeks. Subsequently they were washed with ethanol before they were transferred to a specimen holder. As a specimen holder, we chose a plastic pipette tip filled with 99% ethanol.

2. **Image acquisition and reconstruction:** An X-ray micro-CT image dataset was acquired from 76 species of ants. The acquired images were reconstructed along all three perpendicular dimensions that comprise a Cartesian system forming a detailed cross-section dataset.

3. **Volume rendering:** The reconstructed raw images were used for creating a 3D model for volume rendering, to be used for visual inspection and future morphological studies.

4. **Semi-automated segmentation:** Raw images of heads were segmented semi-automatically using the seed-based watershed tool of the Amira software first and manually cleaning its result subsequently. Labels were assigned to areas of interest, starting with the brain. The databases of both raw and labeled images were pre-processed to enhance their homogeneity and used as training and validation data.

5. **CNN development:** An implementation of the U-Net architecture was built for automated segmentation.

6. **Training:** 60% of the acquired segmented brain images (46 species) were used for network training.

7. **Testing:** The remaining 40% (30 species) were reserved for testing.

8. **Pixel island detection and post-processing:** After segmentation by U-Net, pixel island detection was used to identify the largest continuous areas to remove isolated segments.

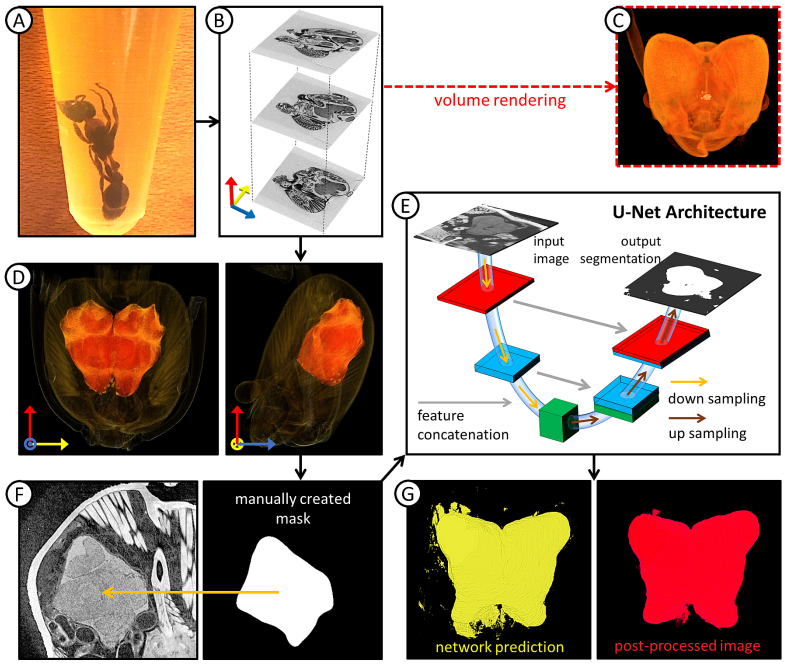

FIGURE 1. Segmentation pipeline overview. (A) Specimens are placed in iodine for staining for two weeks and then placed in small vials containing 99% ethanol to prevent them from moving during scanning. (B) The CT scanner acquires successive X-ray images of the stepwise rotating specimen, and, using a user-defined reference image, automatically reconstructs them to produce orthogonal cross-section stacks that are used for the volume reconstruction of the specimen. (C) Volume rendering for future morphological studies is performed using Amira software. (D) Semi-automated segmentation of the brain volume of each scan (in orange) using the watershed method in Amira. (E) Schematic representation of the U-Net architecture used as the core of the pipeline for the development of a fully automated brain segmentation method. (F) The acquired brain images are used for training after pre-processing augmentation and manual creation of masks. (G) The network's prediction (in yellow) is post-processed for smoothing out over-predicted areas (in red).

Segmentation of ant brains

First, we applied our method to our primary taxonomy group of choice, i.e., ants, and trained our network to segment the brain areas in micro-CT scans from different ant species. Our processed data of 38,000 520×520 pixel images from 46 species were used for training and validation (randomly split into 80% for training and 20% for validation) and the remaining 20,000 520×520 pixel images from 30 species were used for testing.

As shown in Table 1, both IoU and F1 scores steadily increased as we added more 2D images from planes along the same x-y directions of different species, and even more so after we included reconstructed 2D images from planes along all three directions of our 3D brain scans. To estimate the generalized performance of our network, we calculated the true positive rate (TPR) values and false positive rate (FPR) values of our images by changing the discrimination threshold of our network [31], shown in Figure 2. The deviation from the no-discrimination diagonal (which would be the result of random guessing) toward the top-left corner, produced by the relatively high TPR and low FPR for both graphs (testing and training), indicates that our network predicted the brain region and its border accurately but without over-predicting. Results for test and training images are similar, suggesting good generalization capabilities for optimized hyperparameters of our network.

Accuracy scores		
Number of images of training set	IoU	F1
3,500 - xy plane	50%	62%
10,000 - xy plane	63%	71%
38,000 - along all three directions - no post-processing	72%	80%
38,000 - along all three directions - after post-processing	80%	90%

Table 1. Performance evaluation of our proposed pipeline. Both performance descriptors studied (IoU and F1 scores) increase steadily with increasing number of images and post-processing.

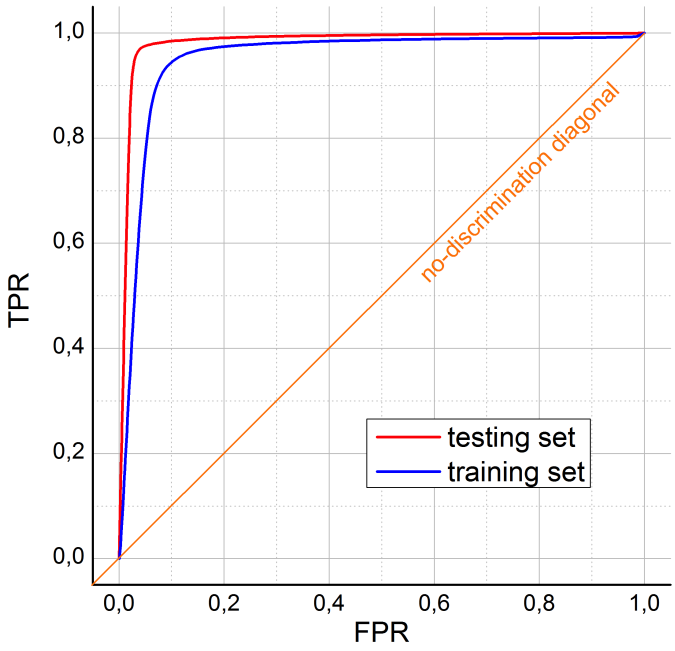

FIGURE 2. Network performance evaluation. High TPR and low FPR values for training (blue) and testing data (red) indicate the network's high generalizability.

Finally, a post-processing step also boosted the performance of our network further. Example results of our network's performance on validation and testing data are shown in Figure 3 demonstrating a predicted area in good agreement with the ground truth. The overpredicted areas of the brain are removed as we form the 3D prediction of the brain. As such, it is essential to see the prediction of all three directions and evaluate the results accordingly. Our automated segmentation pipeline achieves an approximate maximum of 80% IoU and 90% F1 score. Prediction times were in the order of only a few minutes, significantly lower than for the semi-automated segmentation commonly used.

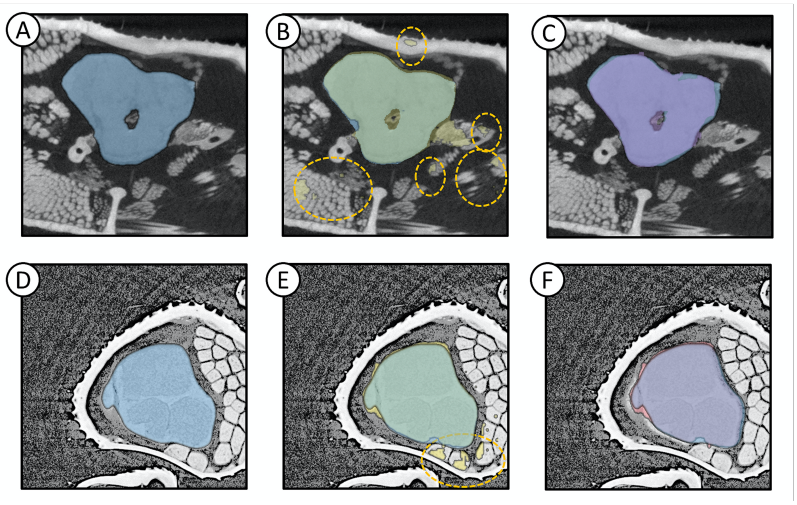

FIGURE 3. Pipeline performance demonstrated both for validation (top row) and testing (bottom row) sets. (A, D) Raw images of head of *Acromyrmex versicolor* and *Carebara atoma* ant specimens, cropped along the x-y axes. The manually segmented brain areas are indicated in blue. (B, E) Network predictions before post-processing (in yellow). Areas in yellow dotted circles are pixel islands not connected to the brain area that were over-predicted. (C, F) Predictions after post-processing (in red). The borders of the predicted areas show good agreement with the manual segmentation in both sets. Note that in overlapping manually and automatically segmented areas in B, C, E, and F, colors appear green or purple.

3D volume rendering

After segmenting the 2D slices, the 3D brain volume was readily computed by loading the stack of images in Amira or ITK-snap (version 3.4.0, PICSL-SCI). Thus, using a 2D network allowed us to maintain high accuracy, performing 3D segmentation in a faster and easier to train way. An exemplar predicted brain area is shown in Figure 4; 3D volume was reconstructed from the 2D predicted images with Amira software. The switch from 2D to 3D is straightforward, giving the user of the pipeline the ability to adapt it to their own dataset circumventing the complications of using an actual 3D CNN.

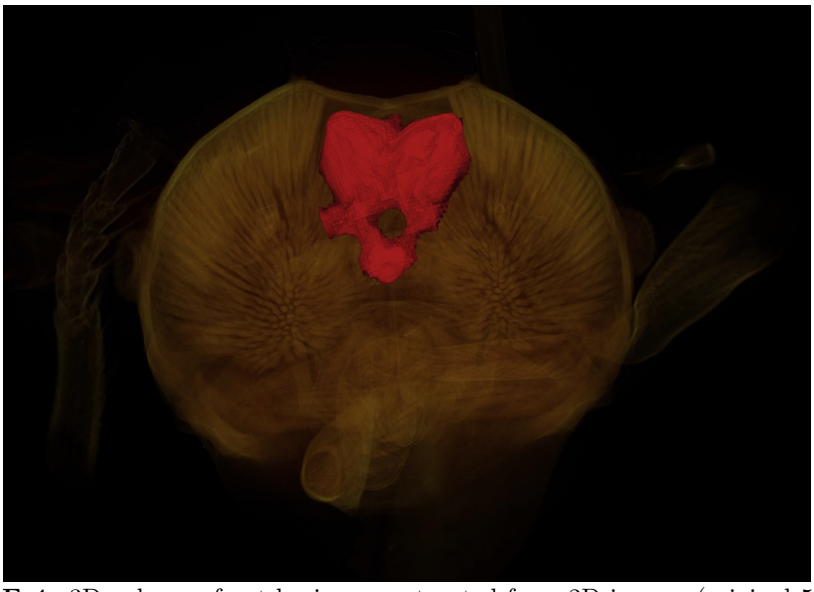

FIGURE 4. 3D volume of ant brain reconstructed from 2D images (original 520×520 px) predicted by the algorithm. 3D reconstructed brain prediction of an *Atta texana* worker.

Generalization to other neural systems and other insects

The U-Net step appears to be largely driven by textures, with the pixel island detection step used to isolate the brain. Even though our customized U-Net was designed for the segmentation of ant brains, it was also successfully applied for the segmentation of neural tissue in other parts of ants and works on distantly related species. Our network was able to predict the whole neural system in full-body scans of ants, as shown in Figure 5, being able to predict the same texture as the brain in different ganglia in the thorax (called mesosoma in ants).

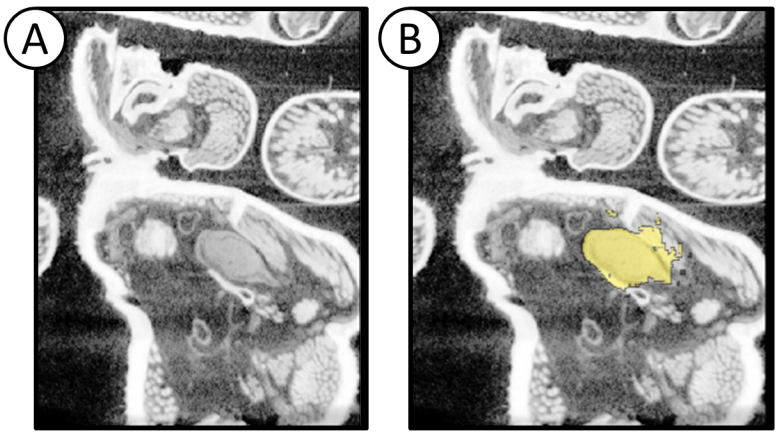

FIGURE 5. Prediction of ganglia in the thorax. As the tissue texture in the image is similar to that of the brain, the network accurately predicts other areas of nervous tissue in the organism. The pixel island detection step isolates the brain, but without this step neural tissue can be isolated.

Our network also gave good prediction for the brain area in scans of various different

distantly related insect species. We used our pre-trained (on ant-brains) network to segment the brain areas of micro-CT scans of model organisms such as flies (*Drosophila*) and wasps, as well as closely related insects such as praying mantises (*Leptomantella*) and termites. Both wasp and praying mantis scan samples were stained with iodine and kept in ethanol, using a similar protocol as with ants. For both scans, the same CT scanner was used as for ants. Naturally, the scanning parameters were different, as these were larger samples; thus, a vertical stitch method was used. Since its prediction capability relies mainly on identifying the texture of the brain area, which does not differ significantly among different insect species, our pre-trained network was able to perform satisfactorily without further adaptation on the data. Exemplar results are shown in Figure 6 for (A-B) wasp and (C-D) praying mantis brain prediction, respectively (also for termite and fly brain prediction in Supplementary Information Figure S7). Our network was successful in segmenting the brains of different insects without significant prediction accuracy losses (when compared to predictions for ants), indicating its flexibility and its lack of necessity for training on each specific distinct species. This is remarkable, considering, for example, that the eyes of the praying mantis are very large, directly connected to the brain, and show the same texture as the brain; none of these features help the segmentation task. It should be noted, however, that in these cases our stated good prediction is merely qualitative, based on the figures themselves, as no metrics were calculated.

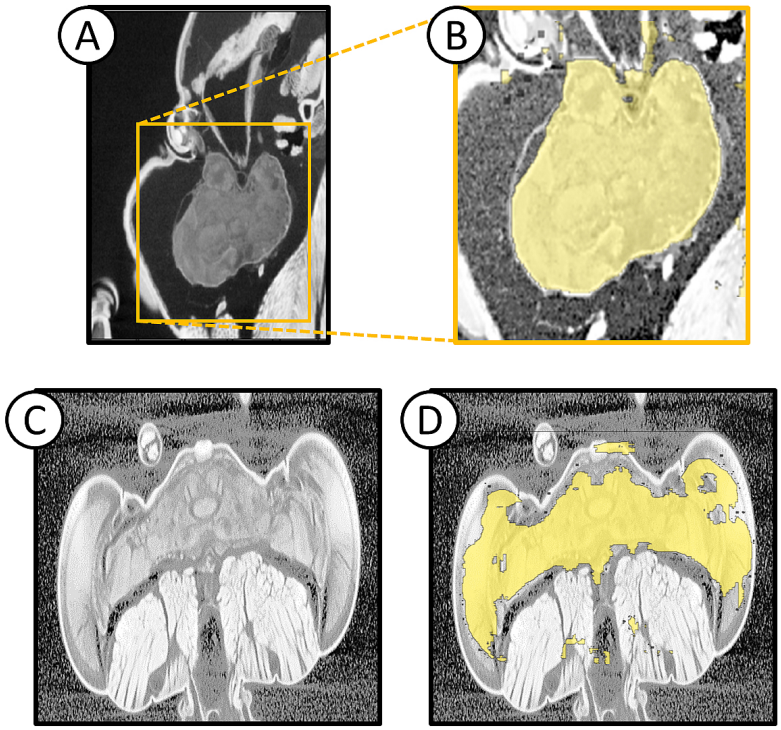

FIGURE 6. Application of pipeline for other insect species. The brain textures of various insect species can be very similar to those of ants, facilitating the prediction by the network even without pre-training on specific insect brain scans. (A) Raw image of wasp head (original 1000×1000 px) and (B) its prediction without post-processing (original 520×520 px), indicating satisfactory identification of the borders of the brain area. (C) 2D image of praying mantis head (520×520 px) and (D) the prediction of its brain area without post-processing. Even though the network over-predicts some small pixel islands, it excludes from its prediction areas of the muscles, fibers and cuticle.

DISCUSSION

To bring morphology fully into the big data era, we need automated methods to retrieve biological meaning from large volumes of images. The proposed automated pipeline is a step in that direction, presenting considerable advantages over other standard methodologies. First of all, automated segmentation is achievable within a few minutes for each specimen, producing user-independent and accurate results faster than manual or semi-automated segmentation. A noteworthy additional advantage is that once algorithms have been trained, advanced expertise in morphology is not required, while manual and semi-automated segmentation usually require advanced knowledge [32]. In fact, during testing our network often outperformed even experienced users and compensated for their oversights or misjudgments, predicting correctly brain areas that were accidentally missed out during manual segmentation.

The two approaches in our method, U-Net and pixel-island detection, represent two complementary steps which suggest a path forward for automated segmentation of structures in complex organisms. U-Net was efficient at retrieving tissue with similar properties in the image, but in our implementation did not make use of shape and position. Thus, we found it retrieved all the structures of neural tissue across the body,

even though it was trained on the brain alone. The brain was then delineated with the pixel-island detection by isolating the largest structure in the head. In general, we expect a combination of tissue-level identification followed by other methods that make use of size and spatial organization to be a powerful combination that should generalize to a wide range of anatomical tissues and parts. Additionally, our pre-trained network's weights can be transferred and further fine-tuned through transfer learning algorithms to be used with other desired datasets.

During testing with other insect species, we used both high and low resolution/quality images acquired from different laboratory and synchrotron-based micro-CT scanners. Our results showed that our segmentation pipeline can perform without significant loss of accuracy to predict the brain area across highly divergent insect species and across scanning methods. Our network's generalizability is high and it can be widely used not only for head but also for whole-body scans of ants and other insects. Finally, the prediction performance of low-resolution images indicates that there is a threshold in the image resolution below which our network does not perform well.

The fact that in this preliminary testing our network worked reasonably well on other groups of insects separated by 300 million years of evolution is noteworthy. It implies that, in principle, such algorithms can identify and shed light on highly conserved features across taxa and/or genera. By this we do not imply that the algorithm we trained for ants is ready out of the box to use for all insect brains in its current form. Instead, we are postulating that discovery of such highly conserved features raises hope for generalized algorithms that could work satisfactorily for different taxa (perhaps with the help of additional tuning).

Last, it should be noted that both automated classification and segmentation tasks typically require big datasets for training and validation, which can be a challenge for researchers to produce for any given application. Since no publicly available dataset of micro-CT images of ant brains existed for our case study, we created a new, extensive dataset across a wide variety of ant species. Since neural anatomy across insects share features that make them targets for segmentation, our dataset can act as a starting point for the development of an even bigger library of micro-CT images of insects, and work as a pre-training dataset for future CNNs [33]. To this end, we have provided all our data available online.

CONCLUSION

In this paper, we introduce a U-Net based CNN for the fully-automated segmentation of micro-CT images of insects. We also present an extensive dataset of manually segmented brain images that can be used to pre-train other networks of interest. Our trained network predicted the brain area in ant images quickly and with high accuracy. Further, our network was able to generalize and predict the whole neural system in full-body scans, as well as to predict ganglion areas that were missed by manual segmentation. After training, the network's performance was tested on training and validation data showing good agreement between prediction and mask scoring 80% IoU and 90% F1. Our pipeline allows successful segmentation in only a few minutes instead of hours which are typically required for manual segmentation.

One of the most important features of the framework described here is that it can be applicable to other anatomical features. Preliminary results on other organs have shown that it can be easily tuned and trained to predict muscles as well as the cuticle of the insect bodies. Specific attention was paid so that the application of the pre-trained network is straightforward and user-friendly, which we aspire will enable the community to adopt it as a valuable resource.

The development of large-scale 3D datasets across phylogenetically diverse taxa

opens up new vistas for comparative research [34]. Likewise, developmental biologists may want to use high-throughput scanning to image hundreds or thousands of specimens as part of an experiment. However, just as DNA sequence data needs bioinformatic algorithms to process massive datasets, large scale image collections require algorithms to digest and extract biologically meaningful data. Algorithms such as this one offer a way forward for powering a "big data" approach to organismal morphology.

ACKNOWLEDGEMENTS

This work was supported by funding from the Okinawa Institute of Science and Technology Graduate University (OIST).

The authors would like to gratefully acknowledge P. Puchenkov from OIST Scientific Computing and Data Analysis section for designing and providing the Table of Contains graphic and supplementary movie, H. Skibbe for useful discussion, G. Fischer, F. Hita Garcia, S. Gautam, J. Katzke, F. Azuma and A. Casadei-Ferreira for their help on micro-CT scanning of ants, and A. Buček and T.A. Schoborg for providing fly and termite scans used for the analysis of this project.

ETHICS STATEMENT

The authors confirm that they have followed the ethical policies of the journal.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Code to generate trained model, and code for analysis described in this study are available at the following GitHub repository: https://github.com/evropi/U-Net. All data used in training and testing, and raw images to test pre-prcocessing methods are available in DRYAD: https://doi.org/10.5061/dryad.qz612jmgv.

References

[1] Francisco Hita Garcia et al. “X-Ray microtomography for ant taxonomy: An exploration and case study with two new Terataner (Hymenoptera, Formicidae, Myrmicinae) species from Madagascar”. In: *PLOS ONE* 12.3 (2017), e0172641. DOI: 10.1371/journal.pone.0172641. URL: https://doi.org/10.1371/journal.pone.0172641.

[2] Ryuta Mizutani and Yoshio Suzuki. “X-ray microtomography in biology”. In: *Micron* 43.2 (2012), pp. 104–115. ISSN: 0968-4328. DOI: https://doi.org/10.1016/j.micron.2011.10.002. URL: http://www.sciencedirect.com/science/article/pii/S0968432811001788.

[3] Thomas van de Kamp et al. “A Biological Screw in a Beetle’s Leg”. In: *Science* 333.6038 (2011), pp. 52–52. DOI: 10.1126/science.1204245. URL: http://science.sciencemag.org/content/sci/333/6038/52.full.pdf.

[4] Willi Ribi et al. “Imaging honey bee brain anatomy with micro-X-ray-computed tomography”. In: *Journal of Neuroscience Methods* 171.1 (2008), pp. 93–97. ISSN: 0165-0270. DOI: https://doi.org/10.1016/j.jneumeth.2008.02.010. URL: http://www.sciencedirect.com/science/article/pii/S0165027008001179.

[5] Kleoniki Keklikoglou et al. “Micro-CT(vlab): A web based virtual gallery of biological specimens using X-ray microtomography (micro-CT)”. In: *Biodiversity Data Journal* 4 (2016), e8740. ISSN: 1314-2828. DOI: 10.3897/BDJ.4.e8740. URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5139143/.

[6] Sebastian Schmelzle et al. “The NOVA project: maximizing beam time efficiency through synergistic analyses of SRμCT data”. In: *SPIE Optical Engineering + Applications*. Vol. 10391. SPIE, 2017, p. 17.

[7] Gabriel S. Yapuncich et al. “A digital collection of rare and endangered lemurs and other primates from the Duke Lemur Center”. In: *PLOS ONE* 14.11 (Nov. 2019), pp. 1–47. DOI: 10.1371/journal.pone.0219411. URL: https://doi.org/10.1371/journal.pone.0219411.

[8] Hoo-Chang Shin et al. “Deep Convolutional Neural Networks for Computer-Aided Detection: CNN Architectures, Dataset Characteristics and Transfer Learning”. In: *IEEE Transactions on Medical Imaging* 35.5 (2016), pp. 1285–1298. ISSN: 0278-0062. DOI: 10.1109/TMI.2016.2528162.

[9] Dan C. Cireşan et al. “Mitosis Detection in Breast Cancer Histology Images with Deep Neural Networks”. In: *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2013*. Ed. by Kensaku Mori et al. Springer Berlin Heidelberg, pp. 411–418. ISBN: 978-3-642-40763-5.

[10] Jared Hamwood et al. “A deep learning method for automatic segmentation of the bony orbit in MRI and CT images”. In: *Scientific reports* 11.1 (July 2021), p. 13693. ISSN: 2045-2322. DOI: 10.1038/s41598-021-93227-3. URL: https://europepmc.org/articles/PMC8249400.

[11] Michael Staab et al. “Systematics of the ant genus Proceratium Roger (Hymenoptera, Formicidae, Proceratiinae) in China – with descriptions of three new species based on micro-CT enhanced next-generation-morphology”. In: *ZooKeys* 770 (2018), pp. 137–192. ISSN: 1313-2989. DOI: 10.3897/zookeys.770.24908.

[12] Daniel Baum et al. “High-Throughput Segmentation of Tiled Biological Structures using Random-Walk Distance Transforms”. In: *Integrative and comparative biology* 59.6 (Dec. 2019), pp. 1700–1712. DOI: 10.1093/icb/icz117. URL: https://pubmed.ncbi.nlm.nih.gov/31282926.

[13] Claire McQuin et al. “CellProfiler 3.0: Next-generation image processing for biology”. In: *PLOS Biology* 16.7 (July 2018), pp. 1–17. DOI: 10.1371/journal.pbio.2005970. URL: https://doi.org/10.1371/journal.pbio.2005970.

[14] Chensi Cao et al. “Deep Learning and Its Applications in Biomedicine”. In: *Genomics, Proteomics & Bioinformatics* 16.1 (2018), pp. 17–32. ISSN: 1672-0229. DOI: https://doi.org/10.1016/j.gpb.2017.07.003. URL: https://www.sciencedirect.com/science/article/pii/S1672022918300020.

[15] Hao Chen et al. “VoxResNet: Deep voxelwise residual networks for brain segmentation from 3D MR images”. In: *NeuroImage* 170 (2018), pp. 446–455. ISSN: 1053-8119. DOI: https://doi.org/10.1016/j.neuroimage.2017.04.041. URL: http://www.sciencedirect.com/science/article/pii/S1053811917303348.

[16] Yann LeCun et al. “Gradient-based learning applied to document recognition”. In: *Proceedings of the IEEE* 86.11 (1998), pp. 2278–2324. ISSN: 0018-9219. DOI: 10.1109/5.726791.

[17] Geoffrey E. Hinton et al. “Improving neural networks by preventing co-adaptation of feature detectors”. In: *CoRR* abs/1207.0580 (2012). arXiv: 1207.0580. URL: http://arxiv.org/abs/1207.0580.

[18] Régis Vaillant, Christophe Monrocq, and Yann LeCun. *Original approach for the localisation of objects in images*. Vol. 141. 1994, pp. 245–250. DOI: 10.1049/ip-vis:19941301.

[19] Mackenzie Weygandt Mathis and Alexander Mathis. “Deep learning tools for the measurement of animal behavior in neuroscience”. In: *Current opinion in neurobiology* 60 (Feb. 2020), pp. 1–11. ISSN: 0959-4388. DOI: 10.1016/j.conb.2019.10.008. URL: https://doi.org/10.1016/j.conb.2019.10.008.

[20] Xiongwei Wu, Doyen Sahoo, and Steven C. H. Hoi. “Recent Advances in Deep Learning for Object Detection”. In: *Neurocomputing* 396 (2020), pp. 39–64.

[21] Fuyong Xing et al. “Deep Learning in Microscopy Image Analysis: A Survey”. In: *IEEE transactions on neural networks and learning systems* 29.10 (Oct. 2018), pp. 4550–4568. ISSN: 2162-237X. DOI: 10.1109/tnnls.2017.2766168. URL: https://doi.org/10.1109/TNNLS.2017.2766168.

[22] Brian B. Avants et al. “A reproducible evaluation of ANTs similarity metric performance in brain image registration”. In: *NeuroImage* 54.3 (2011), pp. 2033–2044. ISSN: 1053-8119. DOI: https://doi.org/10.1016/j.neuroimage.2010.09.025. URL: https://www.sciencedirect.com/science/article/pii/S1053811910012061.

[23] Philipp D. Lösel et al. “Introducing Biomedisa as an open-source online platform for biomedical image segmentation”. In: *Nature Communications* 11.1 (2020), p. 5577. DOI: 10.1038/s41467-020-19303-w. URL: https://doi.org/10.1038/s41467-020-19303-w.

[24] Bruce Fischl. “FreeSurfer”. In: *NeuroImage* 62.2 (2012), pp. 774–781. ISSN: 1053-8119 1095-9572. DOI: 10.1016/j.neuroimage.2012.01.021. URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3685476/.

[25] Konstantinos Kamnitsas et al. “Efficient multi-scale 3D CNN with fully connected CRF for accurate brain lesion segmentation”. In: *Medical Image Analysis* 36 (2017), pp. 61–78. ISSN: 1361-8415. DOI: https://doi.org/10.1016/j.media.2016.10.004. URL: http://www.sciencedirect.com/science/article/pii/S1361841516301839.

[26] Daniël M. Pelt and James A. Sethian. “A mixed-scale dense convolutional neural network for image analysis”. In: *Proceedings of the National Academy of Sciences* (2017). DOI: 10.1073/pnas.1715832114. URL: http://www.pnas.org/content/pnas/early/2017/12/21/1715832114.full.pdf.

[27] Olaf Ronneberger, Philipp Fischer, and Thomas Brox. “U-net: Convolutional networks for biomedical image segmentation”. In: *International Conference on Medical Image Computing and Computer-Assisted Intervention. Springer* (2015), pp. 234–241.

[28] Edward O. Wilson. “Causes of Ecological Success: The Case of the Ants”. In: *Journal of Animal Ecology* 56.1 (1987), pp. 1–9. ISSN: 00218790, 13652656. DOI: 10.2307/4795. URL: http://www.jstor.org/stable/4795.

[29] Ignacio Arganda-Carreras et al. *Group-wise 3D registration based templates to study the evolution of ant worker neuroanatomy*. 2017, pp. 429–432. DOI: 10.1109/ISBI.2017.7950553.

[30] Sabrina Amador-Vargas et al. "Specialization and group size: brain and behavioural correlates of colony size in ants lacking morphological castes". In: *Proceedings of the Royal Society B: Biological Sciences* 282.1801 (2015), p. 20142502. DOI: 10.1098/rspb.2014.2502. eprint: https://royalsocietypublishing.org/doi/pdf/10.1098/rspb.2014.2502. URL: https://royalsocietypublishing.org/doi/abs/10.1098/rspb.2014.2502.

[31] Jiawei Han, Micheline Kamber, and Jian Pei. *Data Mining: Concepts and Techniques*. 3rd. San Francisco, CA, USA: Morgan Kaufmann Publishers Inc., 2011. ISBN: 0123814790.

[32] Geert Litjens et al. "A survey on deep learning in medical image analysis". In: *Medical Image Analysis* 42 (2017), pp. 60–88. ISSN: 1361-8415. DOI: https://doi.org/10.1016/j.media.2017.07.005. URL: https://www.sciencedirect.com/science/article/pii/S1361841517301135.

[33] Diogo M. Camacho et al. "Next-Generation Machine Learning for Biological Networks". In: *Cell* 173.7 (2018), pp. 1581–1592. ISSN: 0092-8674. DOI: https://doi.org/10.1016/j.cell.2018.05.015. URL: https://www.sciencedirect.com/science/article/pii/S0092867418305920.

[34] Martha M Muñoz and Samantha A Price. "The Future is Bright for Evolutionary Morphology and Biomechanics in the Era of Big Data". In: *Integrative and Comparative Biology* 59.3 (June 2019), pp. 599–603. ISSN: 1540-7063. DOI: 10.1093/icb/icz121. eprint: https://academic.oup.com/icb/article-pdf/59/3/599/30041327/icz121.pdf. URL: https://doi.org/10.1093/icb/icz121.

SUPPORTING INFORMATION

Additional supporting information can be found in the Supporting Information section.