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Title: Automated Joint Space Detection Improves Bone Segmentation Accuracy

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes , all done**

Videographer: Please film the computer screen if the authors wish to film any of the protocol shots

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 16

Number of Shots: 29 (25 SC)

Introduction

NOTE: 1.1, 1.2 and 1.5 were self-filmed by authors

Videographer: Obtain headshots for all authors available at the filming location.

Videographer: Please film section 1.3 and 1.4 and the talent shots in section 2 (2.1.1).

~~What are the most recent developments in your field of research?~~

- 1.1. **Mark Kenney:** Recent high-throughput image processing algorithms have provided numerous gold-standard bone segmentations of complex joints with the potential to train deep learning models
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **NOTE: Use Kenney_1.1FINAL.mov on project page**

~~What are the current experimental challenges?~~

- 1.2. **Mark Kenney:** The current challenge is the limited ability to utilize prior segmentation strategies in future experiments, given structure-specific algorithms and significant manual corrective processes.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **NOTE: Use Kenney_1.2FINAL.mov on project page**

~~What research gap are you addressing with your protocol?~~

- 1.3. **Remi Blanc:** We address the need for reproducible, automated image analysis that enables quantitative assessment of bone health and osteoarthritis progression while minimizing manual labor and variability.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Videographer's NOTE: Shot 3.0 The talent walks into the building**

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.4. **Remi Blanc:** Our protocol improves segmentation accuracy and automation while still allowing manual corrections and continuous performance enhancement through added expert annotations.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~What questions will future research focus on?~~

- 1.5. **Mark Kenney:** Our future research aims to incorporate multiple unique structures and disease models in automated algorithms to continue enhancing flexibility and application.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **NOTE: Use Kenney_1.5FINAL.mov on project page**

NOTE: The interviews may have been slated as 1.1, 2.1, 3.1, 4.1 and 5.1, no clear notes from the videographer

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the University Committee for Animal Resources at the University of Rochester Medical Center

Protocol

NOTE: This project has many exceptions (example, figures and flowcharts in protocol and video in results, long on-screen texts etc). The **flow** of the protocol is also entirely different from that of the highlighted manuscript. The authors are very particular about what they want. This is a special case and has been approved by Swati and Anastasia

2. Applying the Deep Learning-Assisted Bone Segmentation Workflow

Demonstrators: Remi Blanc and Rosa Pipitone

2.1. To begin, view the micro-computed tomography dataset to observe that bone and soft tissue are clearly distinguishable [1]. Inspect regions where adjacent bones are closely packed to identify areas where limited image resolution and minimal gaps make their boundaries difficult to detect [2].

2.1.1. WIDE: Talent taking a seat at the computer table and viewing the micro-computed tomography dataset. **NOTE:** An extra B roll of talent walking in was shot as 2.1.4

2.1.2. SCREEN: 2.1.1-v2.mp4 00:08-00:23

2.2. Note the baseline where the supervised watershed-based segmentation method produces an overall accuracy of approximately 80 percent [1].

2.2.1. LAB MEDIA: Figure 2 E and F. *Video editor: Show the data points for “semi-automated” method.*

2.3. To improve segmentation accuracy, apply an updated workflow that combines a pre-trained deep learning joint space prediction model with a pre-built image-processing and watershed-based bone separation recipe in the Amira software environment [1 and 2]. **Authors:** This is a new step showing the workflow chart that I shared in the email. The flow chart may be edited by our video team to improvise it

2.3.1. Talent opening the Amira software on the computer. **NOTE:** This was filmed as 2.1.3

2.3.2. MEDIA: Screenshot 2026-01-12 164649 (Flow chart picture suggested by Poornima showing the workflow) *Video editor: If this is blur, then please contact Poornima*

- 2.4. Load the micro-computed tomography dataset into Amira [1]. Connect the Deep Learning Prediction module, select the trained model, and press Apply [2] to generate an initial joint space segmentation [3]. Then, load the recipe in the Image Recipe Player module [4], connect the micro-computed tomography dataset and the preliminary joint segmentation, and apply the recipe [5] to produce the final segmentation with fully individualized bones [6 and 7].

2.4.1. SCREEN: 2.4.1---2.4.6.mp4 00:00-00:10.

2.4.2. SCREEN: 2.4.1---2.4.6.mp4 00:11-00:20.

2.4.3. SCREEN: 2.4.1---2.4.6.mp4 00:21-00:24.

2.4.4. SCREEN: 2.4.1---2.4.6.mp4 00:25-00:30.

2.4.5. SCREEN: 2.4.1---2.4.6.mp4 00:31-00:36.

2.4.6. SCREEN: 2.4.1---2.4.6.mp4 00:37-00:41.

- 2.5. Finally, evaluate the resulting output by adjusting the colormap to Labels256 [1] and inspecting orthogonal slices and volume renderings to confirm successful separation of individual bones throughout the hind paw [2].

2.5.1. SCREEN: 2.4.1---2.4.6.mp4 00:43-00:51.

2.5.2. SCREEN: 2.4.1---2.4.6.mp4 01:10-01:25.

3. Detailed Workflow Construction and Model Development

- 3.1. To inspect or edit the image processing recipe, open the **Image Recipe** workroom in Amira and load the bone-separation recipe [1-TXT]. Connect the required input data and adjust parameters in the Properties window to customize the workflow for the current dataset [2-TXT].

3.1.1. SCREEN: 2.4.1-v2.mp4. **TXT: The following steps describe model development; They are not required to apply the workflow**

3.1.2. SCREEN: 2.5.1-v2.mp4. 00:00-00:15 **TXT: Fully edit the recipe structure as needed**

- 3.2. To review the workflow structure, sequentially visualize the intermediate outputs generated by the recipe [1].

3.2.1. SCREEN: 2.5.1-v2.mp4. 00:16-00:27.

~~3.3. To generate the joint mask, apply a combination of classical algorithms and a Deep Learning component available in Amira [1] and use the resulting joint mask to identify interfaces between adjacent bones [2].~~

~~3.3.1. SCREEN: Show the run in progress in Amira.~~

~~3.3.2. SCREEN: Display the initial joint mask overlaid on the bone image.~~

3.4. Then, apply a mathematical morphology **Black Top Hat** method implemented through closing, arithmetic subtraction, and thresholding and use this step to emphasize voxels that are darker than their surroundings and correspond to joint regions [1].

3.4.1. SCREEN: 2.7.1-v2.mp4 00:00-00:10.

3.5. To improve specificity, apply the **Structure Enhancement Filter** to enhance dark, thin planar structures corresponding to joint spaces. Further reinforce joint continuity by applying the **Membrane Enhancement Filter**, which incorporates a tensor voting stage [1].

3.5.1. SCREEN: 2.7.1-v2.mp4 00:11-00:20

3.6. Then, integrate the joint mask generated by a convolutional neural network trained to identify peri-articular negative space with the results from the previous image processing filters to generate a final joint mask [1].

3.6.1. SCREEN: 2.7.1.-v2.mp4 00:20-00:24

3.7. To complete bone separation, subtract the final joint mask from the bone mask and treat each remaining connected component as a marker for an individual bone [1]. Apply the watershed algorithm to grow each marker within the original bone mask, reconstructing individual bones and placing boundaries at optimal positions based on image intensities [2].

3.7.1. SCREEN: 2.5.1-v2.mp4 00:22-00:24

3.7.2. SCREEN: 2.5.1-v2.mp4 00:25-00:27.

~~3.7.3. Talent opening the Amira software on the computer.~~

~~Added shot 3.11.4: B-roll~~

4. Training the Deep Learning Model

- 4.1. To prepare training data for the Deep Learning model, select 20 wild-type datasets representing 40 hind paws with balanced sex distribution and ages ranging from 2 to 6 months [1-TXT].
 - 4.1.1. LAB MEDIA: Figure 1D. **TXT: Segment individual bones using the supervised method and apply the joint-extraction recipe**
- 4.2. Extract six subvolumes from each dataset, with three subvolumes per hind paw [1]. Use subvolumes measuring 200 by 200 by 200 voxels to cover ankle regions, digits, and representative background areas [2].
 - 4.2.1. SCREEN: 2.9.1.mp4. 00:00-00:06 *Video editor: Highlight the 6 white boxes*
 - 4.2.2. SCREEN: 2.9.1.mp4 00:15-00:35
- 4.3. Configure a three-dimensional U-Net architecture with a ResNet-18 backbone. Optimize the model using the Adam algorithm with an initial learning rate of 0.0001 and randomly initialized weights [1].
 - 4.3.1. SCREEN: 2.10.1-v2.mp4 00:04-00:09.
- 4.4. Train the model using the Dice loss function and Monitor validation performance using the intersection-over-union metric [1].
 - 4.4.1. SCREEN: 2.10.1-v2.mp4 00:10-00:18.

Results

5. Results

- 5.1. Using deep learning to identify joint spaces in micro-CT datasets enabled clear and consistent separation of individual hind paw bones. By focusing on the peri-articular negative space, the model accurately delineated bone boundaries even in densely packed regions [1].

5.1.1. LAB MEDIA: 3.1.1.mp4

- 5.2. Compared with the semi-automated workflow, which achieved about 80 percent accuracy [1], the deep learning approach delivered a marked improvement for wild-type mice, reaching 98 to 99 percent accuracy with minimal manual correction [2].

5.2.1. LAB MEDIA: Figure 2E and 2F *Video editor: Highlight the curves for “WT” in the semiautomated sections*

5.2.2. LAB MEDIA: Figure 2E and 2F *Highlight the curves for “WT” in the Deep-learning sections*

- 5.3. In tumor necrosis factor or TNF–transgenic mice, the deep-learning method maintained high performance despite progressive inflammatory-erosive disease [1], with the accuracy of around 98 percent during the early disease progression stages [2].

5.3.1. LAB MEDIA: Figure 2E and 2F, *Video editor: Highlight the curves for “TNF” in the “deep learning” sections.*

5.3.2. LAB MEDIA: Figure 2E and 2F, *Video editor: Highlight the curves for “TNF” in the “deep learning” sections only covering 2m, 3m, 4m 5m marks on the X-axis.*

- 5.4. Although the model was trained exclusively on hind paws, it could be applied directly to forepaws without retraining to obtain anatomically coherent segmentations, demonstrating strong generalization to complex structures [1]. It was found that the progression of severe erosive disease and joint destruction was accelerated in TNF-Transgenic female forepaws [2].

5.4.1. LAB MEDIA: Figure 4 A, B, C D

5.4.2. LAB MEDIA: Figure 4 F *Video editor: Highlight the red curve in “TNF” group corresponding to the “forepaw”*

6. Micro-computed
Pronunciation link: <https://www.merriam-webster.com/dictionary/microcomputed>
IPA: /ˌmaɪkroʊkəmˈpjuːtɪd/
Phonetic Spelling: my·kroh·kuhm·pyoo·tid
7. Tomography
Pronunciation link: <https://www.merriam-webster.com/dictionary/tomography>
IPA: /təˈmɑːɡrəfi/
Phonetic Spelling: tuh·mah·gruh·fee
8. Watershed
Pronunciation link: <https://www.merriam-webster.com/dictionary/watershed>
IPA: /ˈwɔːtərˌʃɛd/
Phonetic Spelling: waw·ter·shed
9. Segmentation
Pronunciation link: <https://www.merriam-webster.com/dictionary/segmentation>
IPA: /ˌsɛgmənˈteɪʃən/
Phonetic Spelling: seg·muhn·tay·shuhn
10. Semi-automated
Pronunciation link: <https://www.merriam-webster.com/dictionary/semi-automated>
IPA: /ˌsɛmaɪˈɔːtəˌmɛtɪd/
Phonetic Spelling: seh·my·aw·tuh·may·tid
11. Amira
Pronunciation link: <https://www.howtopronounce.com/amira>
IPA: /əˈmɪrə/
Phonetic Spelling: uh·meer·uh
12. Orthogonal
Pronunciation link: <https://www.merriam-webster.com/dictionary/orthogonal>
IPA: /ɔːrˈθɑːɡənəl/
Phonetic Spelling: or·thah·guh·nuhl
13. Colormap
Pronunciation link: <https://www.merriam-webster.com/dictionary/colormap>
IPA: /ˈkɒlərˌmæp/
Phonetic Spelling: kuh·lur·map
14. Hind paw
Pronunciation link: <https://www.merriam-webster.com/dictionary/hind>
IPA: /haɪnd/
Phonetic Spelling: hind
15. Morphology
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>
IPA: /mɔːrˈfɑːlədʒi/
Phonetic Spelling: mor·fah·luh·jee
16. Thresholding
Pronunciation link: <https://www.merriam-webster.com/dictionary/thresholding>

IPA: /'θreʃˌhoʊldɪŋ/

Phonetic Spelling: thresh·hohld·ing

17. Voxels

Pronunciation link: <https://www.merriam-webster.com/dictionary/voxel>

IPA: /'vɔːksəl/

Phonetic Spelling: vok·suhl

18. Convolutional

Pronunciation link: <https://www.merriam-webster.com/dictionary/convolutional>

IPA: /ˌkɒːnvəˈluːʃənəl/

Phonetic Spelling: kon·vuh·loo·shuh·nuhl

19. Peri-articular

Pronunciation link: <https://www.howtopronounce.com/periarticular>

IPA: /ˌpəriɑːrˈtɪkjələr/

Phonetic Spelling: pair·ee·ar·tik·yuh·ler

20. U-Net

Pronunciation link: <https://www.howtopronounce.com/u-net>

IPA: /'juːˌnet/

Phonetic Spelling: yoo·net

21. ResNet

Pronunciation link: <https://www.howtopronounce.com/resnet>

IPA: /'rezˌnet/

Phonetic Spelling: rez·net

22. Algorithm

Pronunciation link: <https://www.merriam-webster.com/dictionary/algorithm>

IPA: /'ælgəˌrɪðəm/

Phonetic Spelling: al·guh·ri·thuhm

23. Intersection-over-union

Pronunciation link: <https://www.howtopronounce.com/intersection-over-union>

IPA: /ˌɪntərˈsekʃən ˈoʊvər ˈjuːnjən/

Phonetic Spelling: in·ter·sek·shuhn oh·ver yoo·nyuhn

24. Transgenic

Pronunciation link: <https://www.merriam-webster.com/dictionary/transgenic>

IPA: /trænzˈdʒɛnɪk/

Phonetic Spelling: tranz·jen·ik

25. Necrosis

Pronunciation link: <https://www.merriam-webster.com/dictionary/necrosis>

IPA: /nɪˈkroʊsɪs/

Phonetic Spelling: nih·kroh·suhs

26. Inflammatory

Pronunciation link: <https://www.merriam-webster.com/dictionary/inflammatory>

IPA: /ɪnˈflæmətɔːri/

Phonetic Spelling: in·fla·muh·tor·ee

27. Erosive

Pronunciation link: <https://www.merriam-webster.com/dictionary/erosive>

IPA: /ɪˈroʊsɪv/

Phonetic Spelling: ih·roh·siv

28. Anatomically

Pronunciation link: <https://www.merriam-webster.com/dictionary/anatomically>

IPA: /ˌænəˈtɑːmɪkli/

Phonetic Spelling: an·uh·tah·mik·lee

29. Generalization

Pronunciation link: <https://www.merriam-webster.com/dictionary/generalization>

IPA: /ˌdʒɛnərələˈzeɪʒən/

Phonetic Spelling: jen·uh·ruh·luh·zay·shuhn

30. Dataset

Pronunciation link: <https://www.merriam-webster.com/dictionary/dataset>

IPA: /ˈdeɪtəˌset/

Phonetic Spelling: day·tuh·set