FINAL SCRIPT: APPROVED FOR FILMING

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Title: Automated Analysis of *C. elegans* Fluorescence Images using SegElegans

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **10/20/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare

Current Protocol Length

Number of Steps: 18
Number of Shots: 38

Introduction

INTRODUCTION:

~~What technologies are currently used to advance research in your field?~~

1.1. **Konstantinos Kounakis:** *C. elegans* research makes routine use of *in vivo* imaging techniques to monitor processes and answer questions about cell biology.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.2.1*

~~What are the current experimental challenges?~~

1.2. **Konstantinos Kounakis:** The analysis of the imaging data often requires spending significant time designating Regions of Interest by making manual selections in the software.

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

CONCLUSION:

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

1.3. **Konstantinos Kounakis:** Existing options that could automate the process of generating individual worm ROIs are lacking in precision and often have difficulties distinguishing touching or overlapping worms.

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

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

1.4. **Konstantinos Kounakis:** SegElegans is a deep learning system that utilizes a special architecture that permits the accurate segmentation of individual worms even in crowded images.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:Figure 1*

~~How will your findings advance research in your field?~~

1.5. **Konstantinos Kounakis:** SegElegans is a potent and versatile tool that can help expedite the analysis of microscopy data without sacrificing accuracy.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.1.1*

2. Acquisition and Segmentation of *C. elegans* Images Using SegElegans Online

Demonstrator: Konstantinos Kounakis

2.1. To begin, image adult worms using a widefield microscope with a 4X objective lens **[1]**.

2.1.1. WIDE: Talent positioning a slide with adult worms under the widefield microscope and capturing brightfield images.

2.2. If the data is measured in brightfield images, acquire them normally **[1]**. If the data is measured in darkfield fluorescence images, acquire them simultaneously with matching brightfield guide images using the multichannel acquisition options in the microscope's software **[2]**. Ensure both image sets are saved with identical names but placed in separate folders **[3]**.

2.2.1. SCREEN: 69094_shot_12.mp4 00:07-00:17.

2.2.2. SCREEN: 69094_shot_13.mp4 00:08-00:37.

2.2.3. SCREEN: 69094_shot_14.mp4 00:05-00:20.

2.3. To run the online version of SegElegans *(Segg-Ele-guns)*, first, log in to a Google account using a web browser **[1]**. Enter Google Drive and upload the folder containing brightfield or guide images **[2]**.

2.3.1. SCREEN: 69094_shot_15.mp4 00:04-00:17.

2.3.2. SCREEN: 69094_shot_15.mp4 00:17-00:34, 00:45-00:47 .

2.4. Open the GitHub *(gitt-Hub)* page and click on the **SegElegans Body Prediction Interface.ipynb** *(Interface-dot-I-P-Y-N-B)* file **[1-TXT]**. Press the **Open in Colab** button at the top of the opened file **[2]**.

2.4.1. SCREEN: 69094_shot_16.mp4 00:04-00:16. **TXT: https://github.com/KonstantinosKounakis/SegEleganSOnline/tree/v1.0**

2.4.2. SCREEN: 69094_shot_16.mp4 00:17-00:29.

2.5. Ensure that Colab is running a CUDA *(Koo-duh)* compatible GPU runtime **[1]**. Press the play button to execute code block 1 and grant runtime permissions **[2]**.

2.5.1. SCREEN: 69094_shot_16.mp4 00:30-00:49.

2.5.2. SCREEN: 69094_shot_16.mp4 00:49-00:57

2.6. Next, execute code block 2 to load Google Drive into the runtime **[1].** Accept all confirmation dialogues and grant all requested permissions **[2]**. Then execute code blocks 3 and 4 in order, ensuring block 3 completes fully before running block 4 **[3]**.

2.6.1. SCREEN: 69094_shot_17.mp4 00:07-00:13 .

2.6.2. SCREEN: 69094_shot_17.mp4 00:13-00:30.

2.6.3. SCREEN: 69094_shot_17.mp4 00:38-00:45, 00:58-01:02

2.7. Open the folder tab icon on the left in the Colab interface and find the folder with your guide images **[1]**. Copy the path, then paste it into the **test_images** *(Test-images)* input form of code block 5 **[2]**. Also specify a separate path for the analysis output **[3]**. Execute code block 5 **[4]**.

2.7.1. SCREEN: 69094_shot_17.mp4 01:02-01:13.

2.7.2. SCREEN: 69094_shot_17.mp4 01:14-01:23 .

2.7.3. SCREEN: 69094_shot_17.mp4 01:23-01:32 .

2.7.4. SCREEN: 69094_shot_17.mp4 01:33-01:40.

2.8. Specify the exact image extension to be analysed in code block 6 **[1-TXT]**. Execute code block 6 and wait for completion **[2-TXT]**.

2.8.1. SCREEN: 69094_shot_18.mp4 00:05-00:18.
TXT: For the purpose of this input ".tif", ".TIF", and ".tiff" are treated as different extensions

2.8.2. SCREEN: 69094_shot_18.mp4 00:19-00:25, 00:39-00:42 . **TXT: Reduce the value of batch_crop_img to 4 if memory availability issues emerge**

2.9. Now, execute code block 7 without changing inputs unless memory issues occur **[1-TXT]**. In a separate tab or window, access Google Drive and open the output folder **[2]**.

2.9.1. SCREEN: 69094_shot_18.mp4 00:45-00:59
TXT: Reduce the number of subprocesses or outright disable parallel processing if memory availability issues emerge

2.9.2. SCREEN: 69094_shot_18.mp4 01:20-01:33 .

2.10. Choose one of three options. The first option is to use the ROIs *(R-O-Eyes)* from **1_all_rois_results** *(One-All-R-O-Eyes-Results)* and reject unwanted ROIs in ImageJ *(Image-J)* **[1]**. The second option is to accept the curated good masks from **1_complete_mask** *(One-Complete-Mask)* without any manual correction**,** although this will not include the segmentations of overlapping worms**[2]**.

2.10.1. *Reuse same footage as 3.3.1*.

2.10.2. SCREEN: 69094_shot_18.mp4 01:49-02:08 .

2.11. To manually adjust the curation, use code block 8 **[1].** Examine the results of the initial

curation from the summary graphs in the **0_summary results** folder **[2]**.

2.11.1. SCREEN: 69094_shot_19.mp4 00:04-00:18 .

2.11.2. SCREEN: 69094_shot_19.mp4 00:19-00:30 .

2.12. For each image that needs correction, input the full name of the original input image in the **name_image_change** *(name-image-change)* form **[1]** and the numbers of the masks to be kept, separated by commas in the **index_images** *(index-images)* form **[2].** Now, execute code block 8 **[3-TXT].**

2.12.1. SCREEN: 69094_shot_20.mp4 00:05-00:23.

2.12.2. SCREEN: 69094_shot_20.mp4 00:24-00:36 .

2.12.3. SCREEN: 69094_shot_20.mp4 00:38-00:46 . **TXT: Repeat this step for every image that needs correction**

2.13. After completing or skipping the curation correction, execute code block 9 to generate the **2_curated_rois_results** *(two-Curated-R-O-Eyes_results)* folder **[1]**. The folder includes all the final curated segmentations in the ImageJ format **[2].**

2.13.1. SCREEN: 69094_shot_21.mp4 00:09-00:27 .

2.13.2. SCREEN: 69094_shot_21.mp4 00:28-00:37 .

2.14. To run the pipeline on a new set of images, reset the runtime by navigating to **Runtime** then choose **Restart Session** and repeat the procedure from the beginning **[1]**.

2.14.1. SCREEN: 69094_shot_22.mp4 00:05-00:29.

2.15. It is also possible to run SegElegans without cloud computing on a local machine, using Jupyter *(Jupiter)* or a Python script.**[1].**

2.15.1. SCREEN: 69094_shot_23.mp4 00:06-00:12 .

3. ImageJ Segmentation Import and Processing

3.1. To import the segmentations, open one of the actual data images in ImageJ **[1]**.

3.1.1. SCREEN: 69094_shot_24.mp4 00:03-00:10 .

3.2. Open the corresponding zip file containing the ROIs of that image to load the selections

into the ImageJ ROI manager **[1]**. If they are from the **2_curated_rois_results** output, proceed with analysis using the desired methods, preferably automated with macros **[2]**.

3.2.1. SCREEN: 69094_shot_24.mp4 00:11-00:29.

3.2.2. SCREEN: 69094_shot_24.mp4 00:30-00:36, 00:40-00:43 .

3.3. If the ROIs originate from the **1_all_rois_results** output, remove unwanted segmentations from the ROI manager. Select them and press the **Delete** button on the ROI manager window **[1]**. Then proceed with analysis normally **[2].**

3.3.1. SCREEN: 69094_shot_25.mp4 00:17-00:40

3.3.2. SCREEN: 69094_shot_25.mp4 00:53-00:59, 01:03-01:07 .

3.4. If software other than ImageJ is required, import the segmentations as binary masks instead **[1-TXT]**.

3.4.1. SCREEN: 69094_shot_26.mp4 00:05-00:15. **TXT: Use the files from the 1_complete_mask and 1_overlap_mask folders**

Results

4. Results

4.1. In the validation and performance testing experiments, SegElegans significantly reduced the average time required per image compared to manual segmentation, cutting it from approximately 245 seconds **[1]** to under 60 seconds **[2]**.

4.1.1. LAB MEDIA: Figure 3. *Video editor: Highlight the tall black bar labeled "Manual"*

4.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight the short gray bar labeled "SegElegans"*

4.2. SegElegans achieved a segmentation Intersection over Union score above 93 percent, outperforming all alternative models listed at the time of publication **[1]**.

4.2.1. LAB MEDIA: Table 1. *Video editor: Highlight the row "SegElegans" and the value "0.9355" under "Whole Image".*

Pronunciation Guide:
□ *SegElegans*
Pronunciation link: No confirmed link found
IPA: /sɛg-ɛˈliːgænz/
Phonetic spelling: seg-ee-LEE-ganz
□ *C. elegans* (referring to the nematode species Caenorhabditis elegans)
Pronunciation link: https://www.merriam-webster.com/dictionary/elegans
IPA: /ˌsiː - ɛˈliːgænz/
Phonetic spelling: see- eh-LEE-ganz
□ *Colab* (as in Google Colab)
Pronunciation link: https://www.howtopronounce.com/google-colab (Note: site exists)
IPA: /ˈkoʊˌlæb/
Phonetic spelling: KO-lab
□ *ImageJ* (as in ImageJ)
Pronunciation link: No confirmed link found
IPA: /ˈɪmɪdʒ-dʒeɪ/
Phonetic spelling: IM-ij-JAY
□ *CUDA* (in "CUDA compatible GPU runtime")
Pronunciation link: https://www.merriam-webster.com/dictionary/CUDA
IPA: /ˈkuːdə/
Phonetic spelling: KOO-duh
□ *ROI* (Region Of Interest)
Pronunciation link: https://www.merriam-webster.com/dictionary/ROI
IPA: /ˌɑːr-oʊ-ˈaɪ/
Phonetic spelling: ar-oh-EYE
□ *Intersection over Union* (as in segmentation metric)
Pronunciation links:
- "Intersection": https://www.merriam-webster.com/dictionary/intersection
- "Union": https://www.merriam-webster.com/dictionary/union
IPA (for full phrase): /ˌɪntərˈsɛkʃən ˌoʊvər ˈjuːniən/
Phonetic spelling: in-ter-SEK-shun OH-ver YOO-nee-un