

Segment AnyNeuron

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Abstract

Image segmentation is critical in neuroimaging for analyzing brain structures and identifying biomarkers associated with disorders. Deep learning models have shown exponential success in computer vision tasks over the years, including image segmentation. However, to achieve optimal performance, these models require extensive annotated data for training, which is often the bottleneck in expediting brain-wide image analysis. For segmenting cellular structures such as neurons, the annotation process is cumbersome and time-consuming due to the inherent structural, intensity, and background variations present in the data caused by genetic markers, imaging techniques, etc. We propose an Active Learning-based neuron segmentation framework (Segment AnyNeuron), which incorporates state-of-the-art image segmentation modules - Detectron2 and HQ SAM, and requires minimal ground truth annotation to achieve high precision for brain-wide segmentation of neurons. Our framework can classify and segment completely unseen neuronal data by selecting the most representative samples for manual annotation, thus avoiding the cold-start problem common in Active Learning. We demonstrate the effectiveness of our framework for automated brain-wide segmentation of neurons on a variety of open-source neuron imaging datasets, acquired from different scanners and a variety of transgenic mouse lines.

1. Introduction

Recent advancements in Deep Learning (DL) have revolutionized computer vision, demonstrating tremendous success in tasks such as object detection [32] and image segmentation [15]. However, despite these successes, a significant challenge persists: DL models require large quantities of annotated data for training, which often proves to be a bottleneck [20]. When sufficient labeled data is available, DL models for image segmentation and object detection exhibit remarkable performance on downstream

tasks and are actively utilized in medical image analysis [25]. While DL offers substantial benefits for numerous medical applications, including disease diagnosis, treatment planning, and biological research, the requirement for extensive data remains a limiting factor due to the high cost and time involved in annotation [25]. This challenge is particularly pronounced in neuron segmentation, where the small and intricate structures make manual annotation exceptionally laborious and time-consuming.

To address this issue, Active Learning (AL) is a widely adopted approach designed to minimize the time and resources required for manual annotation [21]. AL strategically selects the most representative and informative samples from a pool of unlabelled data. These samples, once manually annotated, are used to train or fine-tune the model, yielding significantly better results in less time. Given that AL is a well-studied solution in the context of image segmentation [18] and object detection [13, 14], it is increasingly being leveraged to enhance deep learning models in medical imaging [22]. Although AL-based medical image segmentation models have seen significant advancements over the past few years [3, 22], the application of AL to neuron segmentation remains rare [13].

We propose a novel Active Learning-based framework for neuron segmentation that leverages state-of-the-art (SOTA) image detection and segmentation models, specifically Detectron2 [32] and HQ-SAM [15]. While Detectron2 is one of the most commonly used detection models for medical images [1, 7, 28], HQ-SAM is also being integrated into medical applications [33]. We chose Detectron2 for its proven performance in instance detection on medical images, including the ability to produce high-quality region proposals even for small objects (thanks to its multi-scale Feature Pyramid Network (FPN) architecture [17]). Meanwhile, HQ-SAM was selected because it addresses the challenge of accurately segmenting fine structures by incorporating high-resolution feature refinement layers, thus preserving small-scale object details—crucial for neurons. This ensures that our framework is designed to operate with

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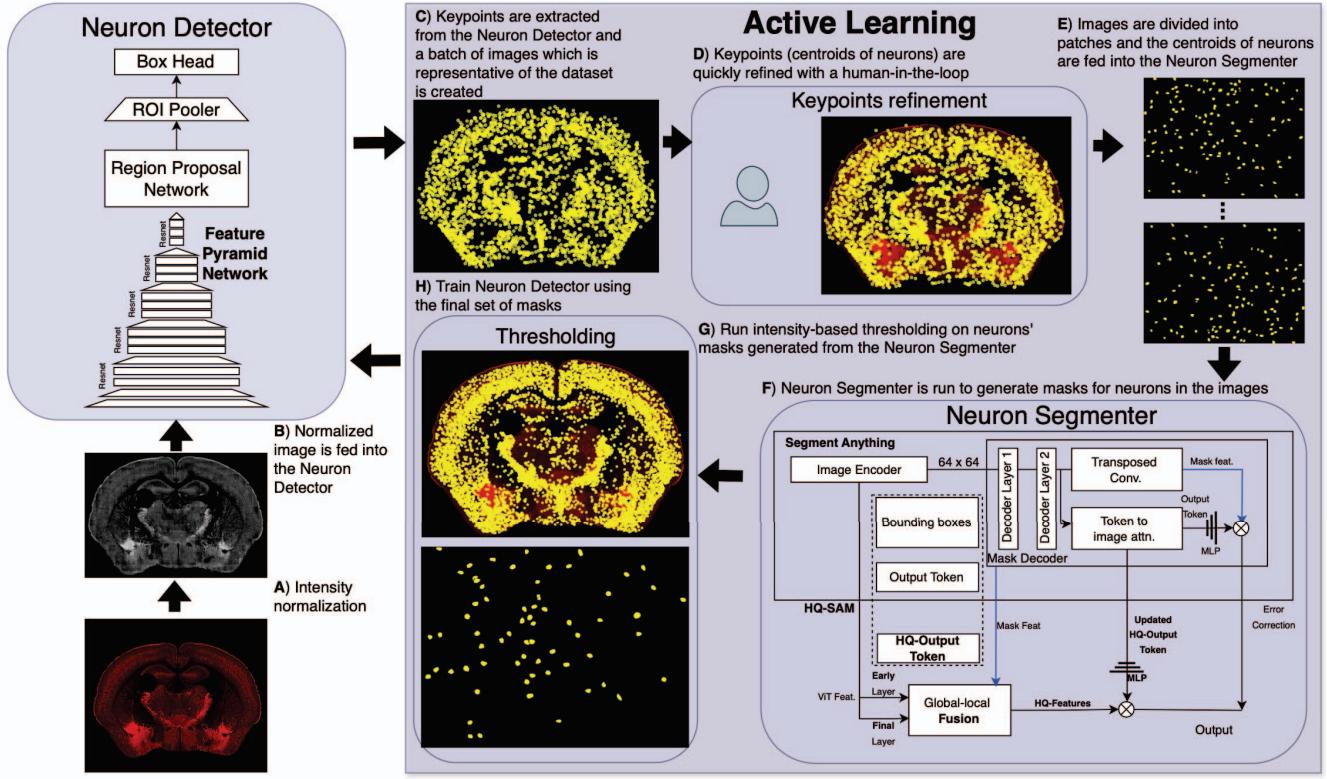


Figure 1. Block diagram for Segment AnyNeuron. A,B) An intensity-normalized version of the input unlabelled image is generated and fed into the Neuron Detector to generate keypoints (dirty ground truth). C) Representative samples from the entire unlabelled dataset are selected and fed into the Active Learning pipeline. D,E) After manual annotation fixing, the refined keypoints are F) processed by the neuron segmenter to generate masks, which are further G) refined through thresholding. H) The data is then used to train/fine-tune the Neuron Detector.

minimal ground truth annotation, significantly reducing the annotation burden while maintaining high performance on unseen neuronal data. The key innovation of our approach lies in the integration of instance detection and Active Learning to iteratively refine the centers of the outputted bounding boxes (key points) and enhance segmentation accuracy. By using Detectron2 to generate initial key points on unseen, unlabelled data, we provide a strong baseline that can be corrected with minimal manual intervention. These corrected key points are then used by HQ-SAM to generate precise segmentation masks. Additionally, our framework includes an intensity-based thresholding feature that allows users to control the segmentation output by adjusting the intensity of detected neurons, providing flexibility and customization based on specific requirements. Our methodology also incorporates advanced preprocessing steps such as intensity normalization and patch-based image segmentation, ensuring that our model receives the cleanest and most relevant data inputs. We demonstrate the effectiveness of our approach through analyses of a disease dataset, showcasing its adaptability and superior

performance compared to existing methods. We aim to open-source our framework and provide a comprehensive guide on applying our Active Learning framework to novel datasets.

2. Methods

2.1. Intensity normalization

Medical images, especially fluorescent images, often exhibit varying intensities, posing challenges for DL object detection and image segmentation models. Therefore, our pipeline incorporates essential preprocessing steps, including intensity normalization, to address this issue effectively. The input image is divided into smaller patches that undergo intensity normalization. This process enables efficient handling of high-resolution images, while intensity normalization adjusts the pixel values to significantly reduce overall intensity variability.

The image I is split into patches (of size $x_1 \times x_2$) and for each patch, an intensity threshold θ is calculated

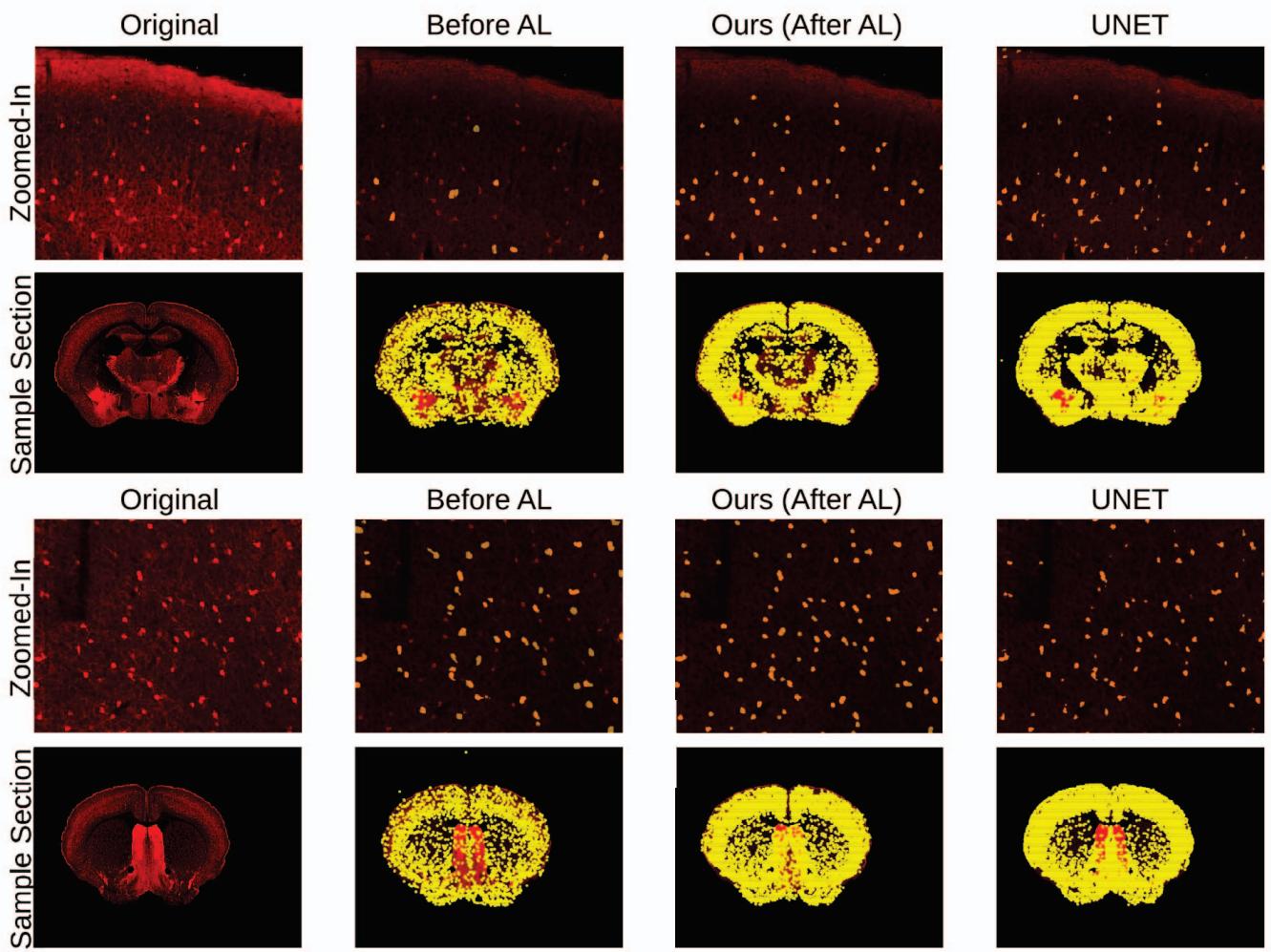


Figure 2. Qualitative results on the unseen Allen Brain dataset. The bottom rows (2nd and 4th) show the original sample, our model’s segmentation mask before Active Learning overlaid, our model’s segmentation masks after Active Learning overlaid, and UNets segmentation masks, from left to right. A zoomed-in subsection, following the same order, is shown in the top rows (1st and 3rd).

which is used for the normalization process.

$$\theta = \min(\max(I_{\text{patch}}) - \text{sorted}(I_{\text{patch}})[-k], T) \quad (1)$$

We set $k = 5$ to ignore the top 5 pixel intensities within each patch, which typically correspond to extreme outliers in our fluorescent images, and $T = 10$ to prevent excessively bright pixels from dominating the normalization. In practice, these constants were determined by testing a range of values (e.g., $k = 1$ to $k = 10$) on a subset of images and selecting those that minimized over-contrast or under-contrast artifacts. Furthermore, for the purposes of our experiments, we set $x_1 = x_2 = 256$. Using θ and its mean intensity (μ), the patch is normalized, followed by gamma correction. The final image patch intensities are then rescaled between the 0.1 and 0.99 percentiles.

$$I'_{\text{patch}} = \frac{(I_{\text{patch}} - \mu)}{\max(I_{\text{patch}}) - \theta} \times 255 \quad (2)$$

$$I''_{\text{patch}} = (I'_{\text{patch}})^\gamma \quad (3)$$

Intensity normalization enhances the contrast of the image leading to a more accurate and robust segmentation. All the intensity-normalized patches are stitched together to reconstruct the original image, which is then fed as input to the neuron detector.

2.2. Neuron Detector

The object detection model we employ as part of our neuron detector is Detectron2, chosen for its widespread use and efficacy in medical image analysis [28], [1]. Building upon Mask-RCNN [12], Detectron2 uses a Feature Pyramid

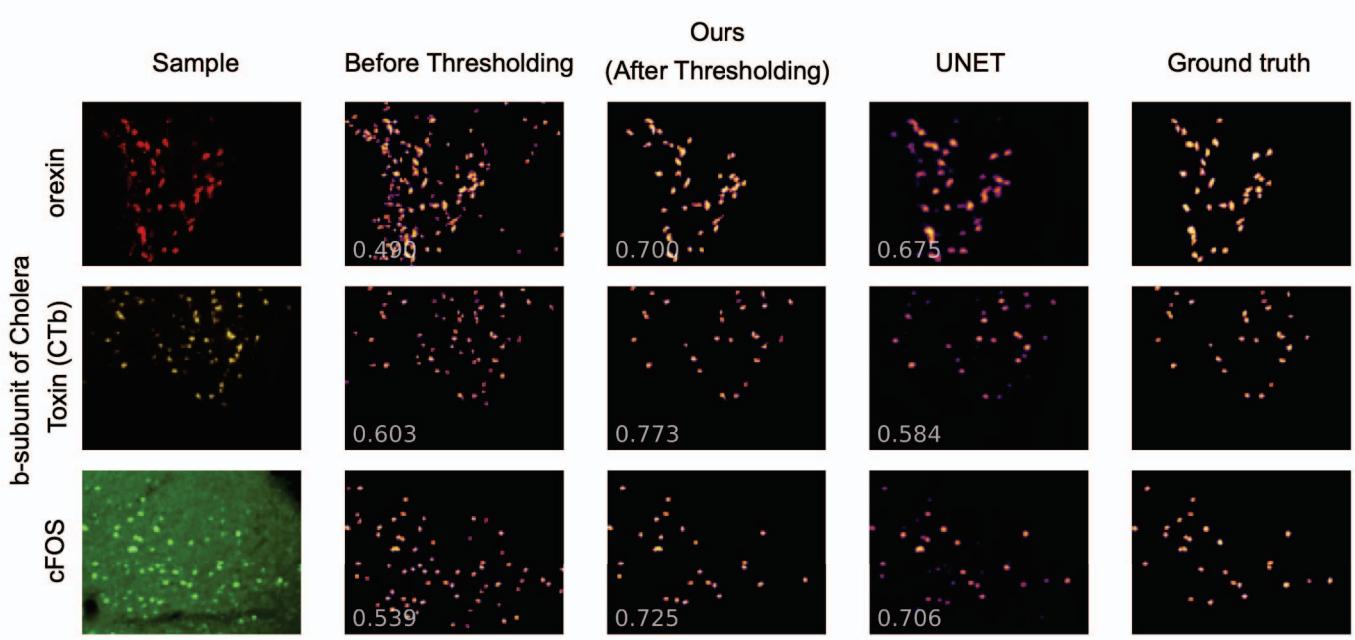


Figure 3. Qualitative results on the unseen Fluocells dataset. The original sample for the 3 stains in the dataset is shown on the left, followed by the masks generated by our framework before intensity thresholding, the masks after applying intensity thresholding, masks generated by UNet, and the actual ground truths. The DICE score between the model’s masks and ground truth is mentioned in the bottom left corner of the masks.

Network (FPN) [17] with ResNet [11] blocks to downsample images and extract hierarchical features. Detectron2’s FPN-based architecture [17] has been shown to be effective at multi-scale object detection, generating bounding boxes that often align well with neuron centers even under challenging intensity conditions. This baseline reduces the annotation effort required for small structures by minimizing gross localization errors, hence allowing experts to focus only on fine corrections. Furthermore, the Region Proposal Network (RPN) [23] processes these features to generate top-scoring bounding boxes, which are refined through the BoxHead for the final output. In our framework, Detectron2 detects neurons in normalized images by using the centers of bounding boxes as keypoints, crucial for accurate neuron identification. While Detectron2 can produce segmentation masks, it is less effective for small neurons, often merging multiple neurons into a single mask. Thus, we rely on the object detection head for precise neuron identification and segmentation. Moreover, during finetuning the neuron detector, we use individual neuron masks within each image to further minimize the possibility of multiple neurons being assigned a single mask. Detectron2 also plays a pivotal role in generating an initial, albeit imperfect, ground truth for our Active Learning pathway. Using keypoints over segmentation masks significantly speeds up the ground truth correction process, as annotating key points is more

straightforward and expedient, enhancing annotation efficiency.

2.3. Active Learning

To optimize our model for any neuron data, we employ Active Learning, which allows fine-tuning with minimal ground truth and fewer training iterations. This human-in-the-loop approach involves experts correcting the initial, “dirty” ground truth generated by our neuron detector for the most representative samples. To ensure comprehensive coverage of the feature space during sample selection for manual annotation, we first embed all unlabeled images using UMAP [19] and then partition the projection space into equally sized clusters (e.g., 5–10 clusters based on cluster density). From each cluster, we randomly select 5% of its points, ensuring both dense and sparse regions are sampled. We then select representative samples from both sparse and dense clusters for use in the active learning pipeline.

Starting with the neuron detector’s output provides an initial baseline, reducing manual labeling effort and circumventing the cold-start problem commonly associated with Active Learning [6]. This iterative process of refining the ground truth and continuously updating the model enhances its generalization capabilities and enables rapid

convergence to a highly accurate state.

2.4. Neuron Segmente

The refined keypoints from the Active Learning step are fed into the Neuron segmenter. We use HQ SAM [15], a state-of-the-art segmentation model as part of our pipeline as it excels at processing keypoints to generate high-quality segmentation masks, even in complex and noisy images, achieving precise neuron segmentation. Compared to the standard Segment Anything Model (SAM) [16], HQ-SAM incorporates additional feature refinement modules and multi-resolution attention, which better preserve small object details—critical in neuron segmentation where structures can be just a few pixels wide. We fine-tuned HQ-SAM on a small subset of neuron data to adapt its learned priors to domain-specific intensity distributions, improving its mask quality for neuronal boundaries. The process begins with the refined keypoints, which are corrected through minimal ground truth annotation during the Active Learning phase. These keypoints serve as crucial landmarks, guiding HQ SAM to focus on specific regions of interest within the image patches. The accurate reference points provided by these keypoints significantly enhance the precision of the segmentation. This approach helps mitigate issues of overlapping and closely packed neurons. The precise masks generated by HQ SAM are more reliable and accurate. Once the masks are generated, intensity-based thresholding is applied to filter out low-intensity neurons, enhancing the overall segmentation accuracy.

2.5. Intensity-based thresholding

We apply intensity-based thresholding to the masks generated by HQ-SAM since it allows us to filter out low-intensity neurons, which are often false positives. By adjusting the intensity threshold, users can control the inclusion of neurons in the final segmentation mask, optimizing the results based on their specific requirements. The user-controlled intensity knob provides flexibility and customization, ensuring that the segmentation meets the desired accuracy and specificity. Post-segmentation, the intensity of each neuron is measured using the original image to ensure accurate intensity values. These values are then normalized for consistency. Users can adjust the intensity threshold, which allows them to filter out neurons that do not meet the desired intensity criteria. This step facilitates in removing false positives and improving the overall accuracy of the segmentation by giving users the freedom to exclude neurons based on their specific intensity requirements. This flexibility is essential for tailoring the segmentation to different applications and datasets, enhancing the framework’s effectiveness.

2.6. Performance evaluation

To quantify our model’s performance, we compare our results with U-Net [27], a well-established model in medical image segmentation. U-Net serves as a benchmark in the domain, particularly for medical datasets, and is widely used by recent models to demonstrate segmentation efficacy [30], [5]. Its architecture, featuring a contracting path for context capture and an expansive path for precise localization, makes it exceptionally effective for tasks such as neuron segmentation. U-Net’s ability to work well with limited annotated data and produce high-resolution segmentation maps has led to its widespread adoption and significant success in various medical imaging applications. This makes it an ideal model for benchmarking and comparing new segmentation algorithms [2].

Prior to experimentation, we finetuned UNet on the pre-defined training set of Fluocells for approximately 100 epochs and used that checkpoint for performance comparisons. We demonstrate that our model performs marginally better than UNet on both the Fluocells and SOM-Cre Mouse Line datasets.

3. Results & Discussion

We propose Segment AnyNeuron, a multi-step framework designed to optimize segmentation performance on novel neuron data. The framework consists of a neuron detector and segmenter, which, in conjunction with the Active Learning module, deliver benchmarking performance on unlabeled neuron datasets with minimal manual annotation. The overall pipeline of our framework, Segment AnyNeuron, is shown in [Figure 1](#).

3.1. Image datasets

SOM-Cre mouse line dataset: To evaluate the performance of our Active Learning pipeline, we employ open-source data from the Allen Brain Data Repository, focusing on transgenic somatostatin-Cre (SOM-Cre) mouse strains (Sst-IRES-Cre;Ai14). The SOM-Cre strain is extensively studied to elucidate the physiological role of somatostatin-expressing neurons in the mouse brain [29] and its association with Alzheimer’s disease [24, 31], thus providing a pertinent dataset for our experimental validation. The dataset comprises detailed neuronal mouse brain sections, from which we strategically select a few for our Active Learning pipeline.

Fluocells v2 dataset: Since our (pre-trained) model was primarily fine-tuned on an in-house neuron dataset, we sought to demonstrate its effectiveness on similar datasets by selecting the Fluorescent Neuronal Cells v2 dataset [8]. Fluocells comprise three fluorescence microscopy

Model	cFOS			Orexin			CTb			LIVECell			
	Precision	Recall	DICE Score	mAP									
Ours (After AL)	0.81	0.78	0.71	0.32	0.39	0.29	0.85	0.66	0.63	0.88	0.76	0.77	0.560
Baseline (Before AL)	0.43	0.77	0.54	0.12	0.22	0.14	0.43	0.57	0.47	0.55	0.33	0.24	0.290
Cell ResUNet [8]	0.79	0.62	0.69	0.33	0.25	0.28	0.67	0.63	0.65	–	–	–	–
UNET [27]	0.51	0.43	0.41	0.18	0.13	0.15	0.41	0.54	0.37	0.82	0.27	0.36	0.1420
Cascade Mask RCNN [4]	–	–	–	–	–	–	–	–	–	–	–	–	0.4790

Table 1. Performance comparison of our AL framework with different models, on the Fluocells dataset (cFOS, Orexin, and CTb stains) and the LIVECell dataset.

image collections, where rodent neuronal cell nuclei and cytoplasm are stained with cFOS, the b-subunit of Cholera Toxin (CTb), and orexin markers, highlighting their anatomical and functional characteristics. Ground truth annotations for these images are publicly available.

LIVECell dataset: For performance validation, we compared our pre-trained model and UNET on the LIVECell dataset [10], a comprehensive, high-quality dataset of phase-contrast images that have been manually annotated and validated by experts. It includes over 1.6 million cells, encompassing a wide range of cell morphologies and culture densities. Prior to model input, all images underwent standard pre-processing procedures, including intensity normalization and patching.

3.2. Active Learning performance on SOM-Cre mouse line dataset

To evaluate the effectiveness of our Active Learning pipeline on novel data, we employ unseen SOM-Cre mouse line samples from the Allen Brain Data repository, which differ in neuron size and structure from our training set. As a result, the current model checkpoint shows suboptimal performance on this new dataset. To address this domain shift, we generate preliminary (dirty) ground truth using our neuron detector on a strategically chosen subset of samples, then refine these annotations manually via the Active Learning loop. These corrected samples, along with their key points, are used by the segmentation model to generate the corresponding masks. The generated masks are further refined using an intensity thresholding parameter, which enables the elimination of extraneous neurons, thereby producing a more accurate and cleaner ground truth mask. After pre-processing, the images and their masks are converted into patches and fed into our model for fine-tuning. We conduct minimal fine-tuning (approximately 10 epochs) and present the qualitative results of our model before and after Active Learning as seen in [Figure 2](#). It presents distinct sections of the mouse brain, accompanied by the masks generated by our pipeline before and after the application of Active Learning. In addition to the full section masks, the top rows

(1st and 3rd rows) display zoomed-in subsections with their corresponding masks overlaid.

Before Active Learning, our model struggled to accurately capture neurons, often producing blob-like masks with a significant number of false positives. However, in post-active learning, our model demonstrates an enhanced capability to precisely identify and generate individual masks for specific neurons. By employing a detection model followed by image segmentation, we effectively address the issue of multiple neurons being amalgamated under a single mask. As illustrated in the zoomed-in subsections in [Figure 2](#), our model successfully generates distinct masks for neurons even in close proximity. Additionally, the intensity-based thresholding significantly reduces false positives, resulting in cleaner and more accurate segmentation.

We compare our model’s performance with UNET [26] using the same data samples. As shown in [Figure 2](#), UNET exhibits a higher occurrence of false positives and often misses smaller-sized neurons. The zoomed-in sections reveal that the segmentation masks generated by UNET contain significant broken masks as well.

3.3. Evaluation on Fluocells v2 and LIVECell dataset

To establish the baseline performance of our AL pipeline, first we use the Fluocells v2 dataset and compare the results with those obtained using UNET. We utilize the existing test set for qualitative and quantitative evaluation. The pre-processed, normalized images are passed through the neuron detector and segmenter, and the DICE score [9] is computed between the model’s output and the corresponding ground truth. [Figure 3](#) and [Table 1](#) illustrate the quantitative and qualitative results of our model and UNET on samples corresponding to the three stains present in the dataset. Initially, we observe that our model’s results include the actual neurons (true positives) but also a significant number of false positives, resulting in a low DICE score. To address the issue of excessive false positives, we apply intensity thresholding to the generated masks. As

demonstrated in **Figure 3**, this process effectively removed the false positives, leading to a significant increase in the DICE score and producing a cleaner output mask. This improvement was consistently observed across all three stains — cFOS, CTb, and orexin.

While UNET achieves decent overall performance and a comparable DICE score, our model demonstrates superior performance, particularly in handling samples with varying intensity levels. UNET struggles to capture low-intensity neurons, resulting in missed detections and a lower DICE score in such cases. In contrast, our model is better equipped to handle these variations, leading to more accurate segmentation and higher overall performance. A similar trend is observed for the LIVECell dataset (**Table 1**), where our model achieves superior performance across all metrics. Notably, it outperforms Cascade Mask R-CNN [4], the current state-of-the-art for cell segmentation, along with the other models.

Using the Fluocells, LIVECell and SOM-Cre Mouse line datasets, we demonstrate the performance of our framework. While the improvements in DICE score are sometimes modest, these small gains can be crucial for large-scale brain-wide analyses where even a slight reduction in false positives or missed neurons can substantially influence downstream cell counting or morphology assessments. Moreover, the active learning component accelerates annotation, offsetting the complexity of the pipeline. Furthermore, it is important to note that once the intensity parameter is adjusted, the segmentation results closely match the ground truth, leading to near-perfect ground truth masks.

4. Conclusion

We present Segment AnyNeuron, an active learning-based framework for neuron segmentation using Detectron2 and HQ-SAM. This approach reduces manual annotation needs by iteratively refining the model with minimal ground truth correction while maintaining high performance. Advanced preprocessing, including intensity normalization and patch-based segmentation, ensures clean inputs, and intensity-based thresholding further enhances accuracy. We validate our framework on the Fluocells, LIVECell, and SOM-Cre mouse line datasets, showing high accuracy and robustness. Active Learning on the SOM-Cre dataset further improves performance, mitigating the cold-start problem and optimizing manual annotation of key samples.

While our approach adds overhead from the two-stage detection and segmentation pipeline, the Active Learning loop ultimately reduces total manual annotation effort compared to a fully supervised approach, making the

added compute cost worthwhile for large datasets. Looking ahead, automating intensity-threshold selection and further refining HQ-SAM for smaller neuronal structures are promising directions to explore. Despite these limitations, Segment AnyNeuron offers a robust and adaptable solution for neuron segmentation, combining state-of-the-art models with Active Learning efficiency. This method enhances segmentation accuracy and provides a scalable approach for complex medical imaging datasets, paving the way for future innovations in medical image analysis.

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