

# Distinguishing pericyte and smooth muscle cells in serial 2-photon tomography imaging of mouse brain blood vessels using convolutional neural networks

\*Note: Sub-titles are not captured in Xplore and should not be used

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## I. INTRODUCTION

Medical image segmentation and feature detection is arguably the most cutting-edge and important area in computerized image analysis. In fact, the first use of computerized image analysis was implemented to analyze cell microscopy images [1]. There is constant innovation in the field of medical imaging, whether it be in imaging for research, pathology, diagnosis, or one of many other possibilities. However, unlike many other image feature detection tasks, medical image data very rarely is labelled. In other tasks, such as identifying faces or animals in images, the manual labelling can be outsourced to the general population. However, in medical imaging, manual labelling must be done by a field expert, all of which have very valuable time and, often, better things to do with it.

This brings medical image segmentation in general to a predicament at this moment: there exists a massive amount of data, whose size and complexity increases more and more every day. Therefore, there is a large amount of room for automated segmentation and feature detection in medical images, on a large scale. Clearly, the algorithms used would be on a case-by-case basis, but techniques exist in the 2-dimensional plane and 3-dimensional plane to intelligently segment medical images with non-negligible results. Research in automatic image analysis has the potential to bridge the gap between professional fields, unlock insights about diseases [1], and save lives [4].

This paper outlines experiments performed on a dataset acquired by using PDGFR-CRE mice, which have an altered

genome that fluoresces neurovascular cells. This allows serial 2-photon tomography to be used to generate extremely microscopic images of the neurovascular cell structure; in particular, the pericytes and smooth muscle cells. If these cells could be distinguished effectively and efficiently, a large amount of data could be gained from these images. Experiments will be carried out in the following order: 2-dimensional automated image segmentation, 3-dimensional automated image segmentation, 2-dimensional CNN segmentation, 3-dimensional CNN segmentation. The 2-dimensional CNN architecture will be U-Net [2] and the 3-dimensional CNN architecture will be DeepMedic.

It is reasonable to hypothesize that using 2D or 3D automated segmentation techniques will yield a dataset that is 80% as accurate as human ground truth labelled data, because human ground truth labeling has a bit of variance due to differing expert opinion. This can then be used to train or augment a deep learning model that will make the segmentation accuracy comparable to an expert ground truth.

Initially, automated image segmentation techniques will be implemented in the 2D and 3D plane. Techniques including gaussian blur, mean-shift filtering, histogram thresholding, connected component analysis, shape analysis, and region growing will be experimented with in both the 2D and 3D space. These techniques are among the leading choices in automatic medical image segmentation [3] and combinations of them have had success in medical image segmentation in the past [4]. To benchmark, a small dataset of expert generated ground truth data will be compared with the output of the various algorithm combination strategies. Based on the results of these tests, the CNNs will ideally be trained using training data generated with these algorithms.

Because these medical images lack labelling, if the automatic labelling of the 2D and 3D algorithms is sufficiently close to human ground truth labelling, (because even experts have labelling variance), theoretically, the algorithms could be used to create training sets for deep learning. Thus, this paper

proposes the use of the aforementioned techniques to generate training data for deep learning. Further experiments will be conducted, using the aforementioned human ground-truth data as a test set, and a massive automatically labelled dataset as a training set. Ideally, these CNN models can bring the segmentation performance from slightly/moderately below human ground truth labelling up to comparable/indistinguishable from human ground truth labelling.

TODO: Insert results and model structure later

## II. LITERATURE REVIEW

This section will review related literature that aims to solve a similar medical image segmentation problem. In particular, this related literature either aims to solve a cellular image segmentation problem or provides techniques and knowledge concerning medical image segmentation that can be applied to cellular image segmentation. This section contains literature overviews concerning automated, image-based techniques that can be applied to cellular image segmentation, followed by overviews concerning specific automated cell segmentation pipelines and implementations, some of which include the use of deep learning. After this, this literature review provides literature concerning the biological context of the segmentation technique performed in this paper, followed by literature containing neurovascular segmentation, which the biological review suggests will be useful. Finally, this literature review concludes with literature concerning deep-learning approaches to medical image segmentation, which will be used at the end of this paper's approach and also as a benchmark technique. Ultimately, the goal of this section is to inform the reader of current, related works and build a context for the combined and new approaches explored in this paper.

One approach to cellular image segmentation is using entirely image-based transformational/analytical methods to isolate, detect, and separate cellular features. The ultimate goal is to create a binary mask from an image that contains cellular features, in which the mask contains only the cellular features. Many of these conventional techniques are explained by Sharma et al. in Automated medical image segmentation techniques [3]. These techniques include edge-based, region-based, and textural-based segmentation, along with thresholding algorithms, which include global thresholding, dynamic thresholding, and local thresholding. This work also explains the concept of pipelining techniques, that is, using multiple segmentation techniques or sub-techniques in a row in order to achieve a satisfactory result. This brings the addition of Separating touching and overlapping objects in particle images A combined approach, [5]. This paper outlines an approach for separating cellular structures in binary masks, which uses the changes in pixel density over the image to detect areas where overlap is possible, then removes them using the mask later. I propose the use many of the techniques outlined by Sharma et al. to form my own pipeline to detect cellular features, along with Korath et al.'s approach of region overlap removal, as an addition to my pipeline. This paper proposes that this combined pipeline approach will yield satisfactory

results in completing the proposed task of segmenting pericyte and smooth muscle cells in neurovascular images.

In terms of designing an automated cellular segmentation algorithm pipeline, it is intuitively useful to understand the biological context surrounding the images that the pipeline is processing. For this reason, this paper explores Establishment and Dysfunction of the Blood-Brain Barrier, by Zhao et al. [11]. The particular areas of significance in this paper surround the diagrams and explanations of pericytes and smooth muscle cells in the context of the blood-brain barrier. The incorporation of the knowledge of the shape and location of these cells in relation to one another is crucial to the design of the automatic segmentation process outlined in this paper. Zhao et al. explain that the smooth muscle cells are located surrounding much larger (by a factor of 2-3) blood vessels than those of pericytes. Knowing this, along with knowing how to isolate the location of blood vessels in the data images, could potentially facilitate the differentiation between the pericytes and smooth muscle cells with quite high accuracy. With vessel segmentation being a potentially important goal in relation to the biological context, it is important that the segmentation of vessels is an achievable goal by analyzing recent literature on the topic. In Blood vessel segmentation algorithms Review of methods, datasets and evaluation metrics, Moccia et al. discuss automated segmentation methods specifically tuned to vessel detection [8]. This paper discusses which methods are effective for which type of imaging, and what some common pipelines for vessel detection are. In addition, not only are these techniques useful for vessel detection, but some may be leveraged for cell segmentation as well. Specifically, Moccia et al. discuss accuracy evaluation metrics, such as positive predictive value, that will be invaluable in evaluating the methods in this paper, both for vessel and cell segmentation. Additionally, one work that implements some of these strategies is Retinal Blood Vessel Segmentation by Means of Scale-Space Analysis and Region Growing, by Martinez-Prez et al. [9]. In this work, analysis techniques incorporating vessel width, size, orientation, and other geometrical features (also included in the paper by Moccia et al.) are used in a scale-space analysis and region growing algorithm that achieves very favorable results in the segmentation of retinal blood vessels. As a result, it is a very feasible strategy, given the biological context, to include an investigation into segmenting the data in this experiment into blood vessel features, and use these blood vessel features to differentiate between cell types for the ultimate cell segmentation goal.

Development of the approach tested in this paper involved exploring current, related works that also tested implementations of medical or cellular image segmentation. One possible approach is to use image-based transformational/analytical methods, as outlined in the previous paragraph. One of these pipeline approaches was used by Zhou et al. to segment breast ultrasound images [4]. This approach involved cropping, gaussian filtering, histogram equalization, pyramid mean-shift filtering, and graph cuts segmentation, and the experiment yielded useful results. Additionally, another purely analytical

method was used by Liu et al. [6] to segment hematopoietic cells from blood smears. This approach introduced an iterative-based threshold algorithm, which used a variant of a simulated annealing algorithm. It also used median-filtering to remove noise and contour detection to isolate the cell shapes. Ultimately, the approach for the experiment outlined in this paper will use a combination ideas from Zhou et al. and Liu et al. to construct a pipeline, particularly median filtering, gaussian filtering, histogram equalization, and another thresholding algorithm variant. In addition, there are clearly uses for deep learning in medical image segmentation. The main drawback to this approach is that medical images lack a large enough ground-truth dataset to train models properly. However, in instances where there exists a large amount of ground-truth data, deep learning can have satisfactory results. In White blood cell classification and counting using convolutional neural network, by Macawile et al. [7], an approach known as transfer learning is used on a moderately sized classified dataset of different types of white blood cells. Macawile et al. used pretrained networks with a few extra training layers to train their data for a short time. In the majority of the pretrained networks tested, accuracy was above 95%. This paper also outlines preprocessing techniques and error metrics that will be useful in my ultimate approach, with or without deep learning. Ultimately, there is a plethora of literature containing implemented automatic medical image segmentation techniques, many of which have very satisfactory results.

As one of the goals for this research is to create training sets for deep learning automatically, it is paramount to explore current literature on deep learning for medical image segmentation. In fact, there are a few cutting-edge convolutional neural network architectures designed specifically for medical image segmentation. For the purposes of this experiment, a well-established architecture for a 3D convolutional neural network and a 2D convolutional neural network will be necessary for potential experimentation. In *Invited Talk: U-Net Convolutional Networks for Biomedical Image Segmentation*, [2], Ronneberger discusses the application, structure, implementation, and performance of U-Net, a 2D CNN designed for medical image segmentation. U-Net is designed to work with small datasets and to isolate complex features with low computational time. Additionally, Ronneberger discusses some augmentation techniques for small ground truth sets that can create larger sets of training and test data from small datasets. Furthermore, in *DeepMedic for Brain Tumor Segmentation*, [10], Kamnitsas et al. discuss the structure, implementation, and performance of DeepMedic, a 3D CNN architecture designed for medical image segmentation. DeepMedic uses a small kernel approach in its 3D convolutional architecture, which allows for relatively low computational time requirements, along with parallel convolutional pathways that allow for the maximum amount of context extraction in the network. Kamnitsas et al. also discuss preprocessing and postprocessing techniques for 3D CNNs in this network, along with the fact that DeepMedic has desirable performance on

medical image segmentation benchmarks. The experiments performed in this paper aim to generate training and test data to be fed to these networks. Ultimately, the hope is that the networks will achieve a greater performance than the automated segmentation algorithm can achieve alone, or alternatively that the performance achieved using DeepMedic and U-Net can be used as a benchmark for the automated segmentation techniques. Therefore, these two papers will be vital in understanding and implementing these networks for the experiments conducted for this research.

Thus, the current literature on cell image segmentation and medical image segmentation, both with deep learning and without, have yielded very promising results. In order to get the most out of these techniques, developing a pipeline of methods is a crucial step. In this paper, the biological context of the brain processes being imaged is crucial to the success of the approach. Therefore, the approach proposed in this paper will include a combination of techniques that has not been proposed in the past, particularly for the rare images that are being analyzed. Particularly, the approach in this paper will combine automatic cell image segmentation methods and vascular structure segmentation methods, and use them, respectively, to extract cell masks from images and differentiate between these cells. Then, this technique can be used to train a CNN to achieve even greater (3 dimensional) context learning.

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### ACKNOWLEDGMENT

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