# On the pitfalls of deep image segmentation for lightsheet microscopy

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## Abstract

Fluorescence light sheet microscopy (LSM) of tissue cleared samples enables holistic 3D imaging of the human brain and the full murine body. While this novel imaging method creates high resolution scans and has led to an abundance of high-profile publications in the last 5 years, analysing them is not trivial and comes with complex obstacles. In this paper we present a review and discussion of our groups previous works to present best practices on both animal and human scans and guidelines to overcome these obstacles.

**Keywords:** image segmentation, light sheet microscopy, neurons, blood vessels.

## 1. Introduction

The recent advances in tissue clearing, staining and light-sheet microscopy push the boundaries of biological imaging for both whole body imaging of mice (Cai et al., 2018) as well as human organs (Zhao et al., 2020). By being able to capture full 3D volumetric images, even with open source devices (Voigt et al., 2019), one can capture a holistic view of organisms at a cellular resolution. This enables the study of systems like the peripheral nervous system, cancer metastasis and drug distribution (Pan et al., 2019) or brain vasculature (Todorov* et al., 2020) down to cell level. While novel deep learning methods provide researchers with powerful tools for rapid and accurate 3D image analysis, the application of deep learning to tissue cleared LSM images comes with its own challenges different from more generic computer vision tasks. For example, unique artifacts of the imaging technique and other pitfalls make this anything but a trivial task. In this paper we discuss multiple recent publications from our group which developed and applied deep learning concepts and pipelines to light sheet microscopy images and how one can deal with aforementioned pitfalls.

## 2. Light-sheet microscopy analysis in practice

The complexity of signal in light-sheet microscopy makes a strong case for the use of deep learning for analysis, as traditional methods like thresholding or filters fail to capture the following data sets in its entirety. The following publications did all make use of CNNs.

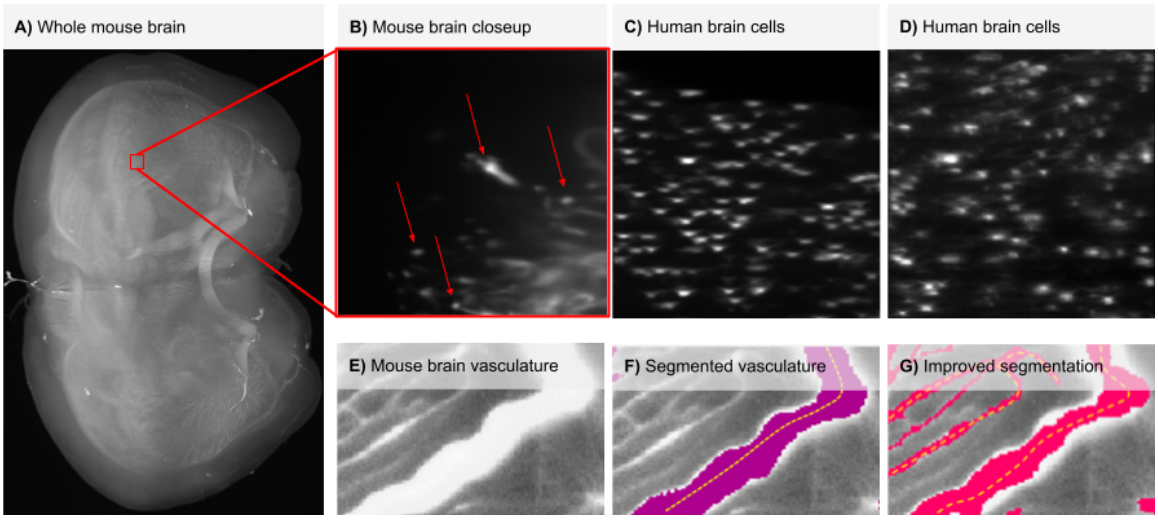


Figure 1: A)Maximum Intensity Projection of DISCO-MS mouse brain B) Closeup single slide containing structures susceptible of being detected as FP (in this area are no plaques) C) - D) SHANEL cleared human brain cell variations in single slices E) - G) VESSAP Small vessels are overlooked with conventional methods.

### 2.1. Human brain cell analysis

The *SHANEL* protocol (Zhao et al., 2020) allows for the clearing of human tissue and the subsequent analysis of neuronal cell bodies by CNNs. Here the main challenge is the heterogeneity of the scan - light-sheet artifacts, permeabilization issues and drop of light intensity deeper in the tissue make the same foreground take on different shapes throughout the selected brain regions. This poses a challenge for human annotators, too, and the heterogeneity translates to the manual annotation. To leverage this, a binary cross entropy loss is weighted with the inverse of the class frequency for each training image to ensure a consistent segmentation throughout the whole scan.

### 2.2. Amyloid-beta plaque analysis for Alzheimer's Disease research

*Disco-MS* is a pipeline that can analyse the distribution of indicators for Alzheimer's disease in tissue cleared mouse brains using CNNs (Bhatia et al., 2021). The main concern here is the sparsity of the foreground, with only a handful of plaques in a whole brain. Preliminary trainings yielded many false positives in structures remotely resembling spherical blobs. The solution here is to include large numbers of expert vetted image samples containing nothing but empty background to improve segmentation precision.

### 2.3. Whole brain vasculature analysis

*Vessap* (Todorov* et al., 2020) is a deep learning-based framework to quantify and analyze the entire brain vasculature using a CNN and with a transfer learning approach for seg-

mentation, it achieves human-level accuracy. The main concern was that small vessels are overlooked in the segmentation, because traditional loss functions optimize the loss toward the total number of correctly classified pixels, and do not account for the highly relevant network structure and topology of vessels. Similar to vessels, many biological structures' most important property is their topology. Therefore, dedicated approaches such as the cldice loss for vessels (Shit et al., 2021) are required to optimize the segmentations towards a biologically optimal goal, which is beyond pixelwise accuracy.

## 3. Discussion and Outlook

New methods such as vision transformers (Dosovitskiy et al., 2020) promise improvements in accuracy over current traditional convolutional networks. We foresee that some of the issues and challenges, which are network independent, will prevail. As labeling is crucial for all tasks, a big potential that has yet to be fully embraced is the inclusion of VR labeling such as Syglass (Pidhorskyi et al., 2018) to annotate 3D structures in their entirety.

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