Capillary Histology Imagery Visualization and Exploration

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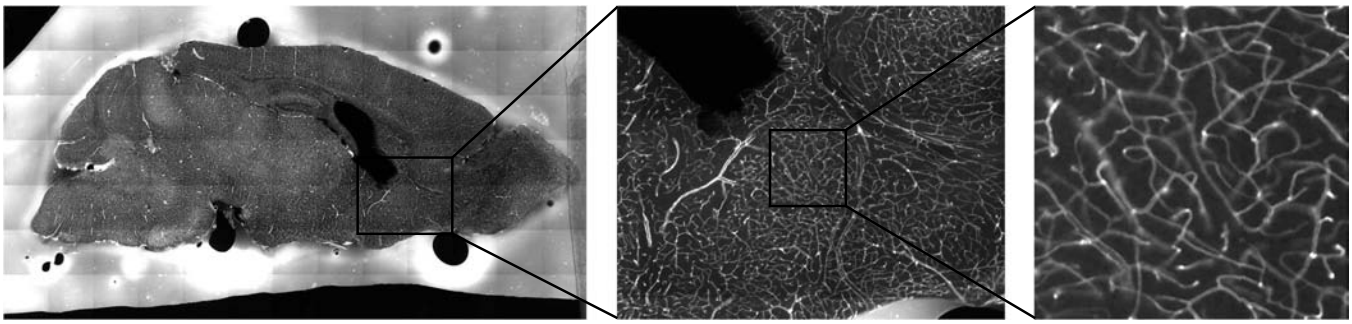


Figure 1: A section of a mouse brain. The overall image is 6656x3584, yet the vessels of interest are 2-3 pixels wide.

1 Introduction

In this poster, we describe the Capillary Histology Imagery Visualization and Exploration (CHIVE) project that is developing new tools for studying the vascular system in the mouse brain. Our ultimate goal is to understand the micro-vascular architecture of the brain and its role in development and disease. The size and complexity of brain vasculature demands a new methodology for study. We are developing such a methodology for imaging and examining the micro-vasculature of the mouse brain.

To study the vascular system in the brain, we need an imaging modality capable of seeing the capillaries, the smallest vessels that ultimately deliver oxygen and nutrients from the blood. We are interested in the patternings of these vessels throughout the brain. This requires an imaging modality capable of extremely high resolution: we must resolve individual capillaries that are a few microns wide, over the entire brain. At present, the only method capable of this resolution is histology and montage-microscopy: physically sectioning a specimen and imaging it under a light microscope.

The histological process produces a unique dataset containing an immense amount of small details over a large volume. To make use of this data, we have needed to develop a new suite of tools for visualization and analysis. The result is a visualization system that allows a scientist to work with the large image sets that result from histology in a way that supports exploration and analysis that is tuned to the unusual features of the image sets.

In the poster summarized in this abstract we will outline our protocol for data collection and the types of data it creates. We will describe how the features of the data lead to challenges for visualization and analysis. We will describe our preliminary system for working with the data, CHIVE, and describe how it responds to some of these challenges. We conclude by discussing some of the issues CHIVE does not presently address.

2 Protocol and Data

The vascular system of the brain consists of a variety of vessels. The smallest are capillaries are 5-7 microns across. Such features are too small to resolve by non-invasive imaging such as computerized tomography (CT) and magnetic resonance (MR). Three dimensional microscopy techniques, such as confocal or multi-photon microscopy, can only image small regions of tissue, and are therefore inappropriate for studying the overall architecture across the brain. Therefore, in order to study the vasculature across the brain, we use a histological approach (physical sectioning).

2.1 Imaging

To image the vasculature, we perfuse the vessels with a fluorescent dye. The brain is then prepared for sectioning by embedding it in a rigid block. The block is then sectioned into slices which are approximately 50 microns thick. The slices are then photographed under a bright-light microscope. Automatic montage-microscopy allows the entire slice to be photographed at high resolution. Typically, imaging is done at a resolution of two microns per pixel.

The histological process provides a method for imaging the microvasculature over the entire mouse brain. For each specimen, it produces a set of between 50-150 images. Each image itself is large (averaging 5000x3000 pixels). Another benefit of the histological process is that the slices are available for the application of additional stains that can provide more information. For example, slices are typically stained with a Nissl stain that highlights cell bodies, allowing biologists to correlate vasculature with a standard atlas.

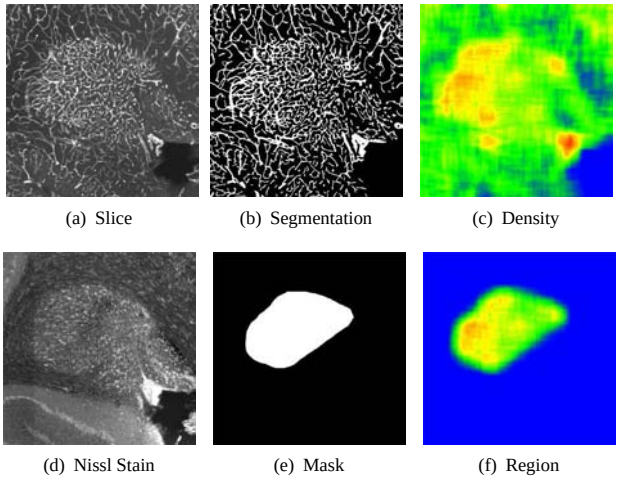
The histological procedure does have drawbacks. For instance, despite efforts to provide a rigid embedding of the brain, we cannot prevent all deformations during the physical sectioning. The evident deformations that occur are influenced by local brain structure and hence are non-linear. The lack of landmark features, as well as the resolution required to fit vessels together, make registration challenging. We therefore focus our efforts on analysis of the 2D slices, and reserve full 3D exploration for future work.

The result of the histological data collection process is, therefore, a collection of large images. We typically have several per slice: one of the vasculature, and others taken using alternate stains. We also record other "meta-data" such as mouse age, environmental or genetic factors, or notes about the sectioning process. Initially, this data was simply stored in the file system, however, we are moving toward the use of a centralized database server (using PostgreSQL) to record all information.

2.2 Image Analysis

While the fluorescent stains make vessels appear bright relative to the surrounding brain, the process is imperfect. Not all vessels receive adequate amounts of stain, and some artifacts such as air bubbles can appear bright in the images. To handle these cases, we have created a segmentation tool that classifies pixels that are likely to be vessels. The segmenter is designed to account for brightness variation due to tiling artifacts from the montage microscopy.

The segmenter is heuristic and therefore imperfect. We have validated its effectiveness by comparing the results of its use ver-



(a) Slice (b) Segmentation (c) Density

(d) Nissl Stain (e) Mask (f) Region

Figure 2: The Nissl stain view of the slice allows an anatomist to identify the cerebellar nucleus. Computations can be performed in this region.

sus having biologists manually identify vessels in the raw images. The use of segmented images provided more consistency and lower variance, yet produced the same results. At present, the segmenter often misclassifies larger structures such as air bubbles and large vessels, so we allow manual identification of these structures.

For analysis, our goal is to make estimates of biologically meaningful quantities from the 2D images, such as the amount of vascularization in a particular region. The science of such estimation is known as Stereology. Historically, this has been done by a set of manual methods that do not scale to the large datasets that we have. We have not yet developed a theoretically unbiased stereological technique for large-scale vessel measurement. Presently, we use a simple scheme of using pixel densities (percentage of pixels in a region classified as vessel by the segmenter) as an estimator of vascularization. We have demonstrated empirically that this provides similar results to the manual methods.

Pseudo-color images map pixel density to varying color, allowing biologists to identify regions with large degrees of vascularization. Interactive control over the color map enables the user to focus on particular ranges of interest.

2.3 Regions and Masks

Often, biologists are interested in specific regions of the brain, such as functional units. These regions are typically difficult to identify, requiring a trained anatomist to look at a specially stained slice. To facilitate such analysis, we provide tools for identifying regions as masks, which are manually drawn on slices. For example, a biologist can encircle a functional unit such as the amygdala on the image of the Nissl-stained slice, and then determine the vessel density within this region.

These manually defined masks serve two significant purposes. First, the masks allow our computations, such as density, to filter out other sections of the brain that are not relevant to that region. Second, we provide a visualization of the mask in a transparent color determined by the user to provide the user with a means for quickly locating a region at a later time. We therefore provide facilities for managing sets of masks to be associated with each image.

3 The CHIVE Visualization Tool

Our process for imaging the mouse brain provides large image sets that are difficult to register and therefore must be handled as 2D images, have sub-region information that must be defined manually, and contain vast amounts of detail that is more useful in a statistically summarized form. We have therefore built a custom visualization tool called CHIVE for working with this data.

At the base level, CHIVE is a tool for viewing sets of large images. To provide interactive performance where a user can browse through the brain, pre-computation and caching are used. By storing low resolution versions of images, and by using the graphics hardware to perform image blending and zooming operations, we are able to provide the performance desired by the users.

CHIVE coordinates the various types of images that exist for each slice, including the fluorescent microscopy image, the results of vessel segmentation, other stains, masks, and pseudo-color images for visualizing statistics. These layers can be dynamically toggled and blended together to allow the user to gather information from each simultaneously. This feature provides the biologist with the ability to focus their analysis on as many or as few of the representations of the data as needed. Masks can be drawn or displayed over any other image layer in CHIVE. CHIVE can connect directly to a database server, allowing access to data stored by multiple users and from multiple machines.

4 Status and Directions

The current version of the CHIVE visualization tool has been deployed in a biology laboratory and has been instrumental in the research of brain vascularization. Using CHIVE, biologists have been able to identify and quantify relationships between behavior, genotype, and vascularization in affected brain regions.

While the current version of CHIVE is already a useful tool, we feel that there is great potential for developing better tools that will enhance our ability to study the vasculature. Some directions that we plan to explore include:

3D visualization — even if a high-resolution registration, sufficient to align individual vessels between slices, is impractical, a coarse registration will allow 3D views to help in understanding the spatial context of anomalies.

Better Analysis — the pixel density provides one method for providing a statistical summary that can be used for comparison. We hope to design new stereological methods that can provide more sound metrics that can consider vessel shape and quantity.

Automatic Artifact Rejection — presently, artifacts such as air bubbles must be manually identified. Better automation will allow for higher-throughput analysis.

Better use of the Database — a larger library of specimen provides opportunities for analysis over sets of images. This will require tools for searching for the right images to use and for aggregating the results.

Performance — as we begin to put more of the data on database servers, there will be new challenges in providing for interactive viewing on remote clients.

The study of the vascular system of the brain has required a new methodology for imaging, and a corresponding new set of tools for working with these images. Our initial efforts at building these tools have already provided a useful platform for biological discovery, and provide a range of opportunities for further development.

5 Acknowledgements

This work was supported in part by NSF grants CCR-9984506 and IIS-0097456, and equipment donations from NVidia and Intel. TB was supported in part by an NLM training grant to the Computational and Informatics in Biology and Medicine Training Program (NLM T15 LM 07359).