Tissue Thickness Estimation

A project report submitted in partial fulfillment of the requirements for the award of the degree of

B.Tech. in Computer Science

by

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under the guidance of
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Declaration of Authorship

I, **Jalagam Sindhuja**, declare that the work presented in "**Tissue Thickness Estimation**" is my own. I confirm that:

- This work was completed entirely while in candidature for B.Tech. degree at Indian Institute of Information Technology, Lucknow.
- Where I have consulted the published work of others, it is always cited.
- Wherever I have cited the work of others, the source is always indicated. Except for the aforementioned quotations, this work is solely my work.
- I have acknowledged all major sources of information.

Signed: Jalagam Sindhuja

Date: May 2024

CERTIFICATE

This is to certify that the work entitled "**Tissue Thickness Estimation**" submitted by **Jalagam Sindhuja** who got her name registered on **Jul 2020** for the award of B.Tech. degree at Indian Institute of Information Technology, Lucknow is absolutely based upon his/her own work under the supervision of **Dr.Abhinesh Kaushik**, Department of $Information Technology$, Indian Institute of Information Technology, Lucknow - 226 002, U.P., India and that neither this work nor any part of it has been submitted for any degree/diploma or any other academic award anywhere before.

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Lucknow
May 2024

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ABSTRACT

This project presents an automated algorithm for estimating tissue thickness by analyzing images taken at different focal planes. Accurate tissue thickness determination is crucial in histopathological analysis for comprehensive sampling and reliable diagnostic outcomes.

Our method captures a series of images at varying focal planes and employs a two-step process: FOV-thick tissue classification and stack size estimation. Initially, the algorithm examines the biopsy ratio and the focus content distribution at stack boundary indices. The tissue is classified as thick if the biopsy ratio exceeds a threshold and the focus content at any boundary index is significant, or if the estimated stack size is large.

The core of our approach is stack size estimation, where the focus content distribution across the stack is assessed. The process begins by initializing the focus content percentage sum and stack size counter. The algorithm identifies the maximum focus content index and iteratively adjusts the focus content sum and stack size counter. If achieving the desired focus content requires images from more than five focal planes, the tissue is categorized as thick. This iterative process continues until the final stack size, indicating the number of focal planes needed to achieve the desired focus content threshold, is determined.

This technique offers a precise and efficient method for evaluating tissue thickness, enhancing the preparation and analysis of biopsy samples. By automating the classification and quantification of tissue thickness, the algorithm improves the accuracy of histopathological examinations, contributing to better diagnostic precision and patient care outcomes.

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Chapter 1

Introduction

1.1 Overview

Pramana is a health technology company from Nference, is leading the digital transformation in pathology. Offering a Digital Pathology as a Service (DPaaS) solution, Pramana integrates advanced whole slide imaging systems with a scalable software platform to enhance clinical workflows and support AI-enabled decision-making.

In this context, our project focuses on developing a robust and automated algorithm for tissue thickness estimation. Accurate tissue thickness measurement is crucial in histopathological analysis, as it directly impacts the quality and reliability of diagnostic outcomes. By analyzing images taken at different focal planes, our method aims to precisely determine the thickness of tissue samples, thereby ensuring comprehensive and accurate sampling.

1.2 Motivation

The motivation behind this project is to enhance diagnostic precision and efficiency in pathology. Accurate tissue thickness estimation ensures pathologists have a clear view of tissue architecture, reducing diagnostic errors and improving reliability. Automation streamlines workflows, integrates with Pramana's software platform, and leverages AI for better decision support, ultimately improving patient care through timely and accurate diagnoses.

Additionally, the increasing complexity and volume of pathological data necessitate more sophisticated tools to aid pathologists. By automating tissue thickness estimation, we not only reduce the manual effort required but also enable pathologists to focus on more critical aspects of diagnosis and treatment planning. This project represents a significant step towards integrating advanced technologies in routine pathology practice, fostering a future where digital tools enhance human expertise to deliver superior healthcare outcomes.

1.3 Objectives

Through this project, we aim to set a new standard in digital pathology, driving innovation and excellence in histopathological analysis. Our automated tissue thickness estimation algorithm is a significant step towards achieving this goal, ensuring that pathologists have the tools they need to deliver precise and reliable diagnoses in a rapidly evolving digital landscape.

Chapter 2

Literature Review

2.1 Objectives and Techniques

The primary objective of tissue thickness estimation is to quantify the number of stacked images required to achieve 90 percent focus within the field of view (FOV) of a tissue region. This distinction helps classify tissues as 'thin' or 'thick,' depending on the number of images needed for optimal focus. For example, a thin tissue may only require two images, whereas a thick tissue could necessitate seven images.

Key methodologies employed in this estimation include:

- **AOI (Area of Interest) Level Focus Content Distribution**: This technique analyzes the distribution of focus across different AOIs, differentiating between normal and thick tissues.
- **Slide-Level Thickness Heatmaps**: These visual representations depict the thickness of tissue across entire slides, aiding in the identification of regions that may need specialized handling or further analysis.
- **Focus Content Distribution Histograms**: Histograms illustrate the distribution of focused content, providing insights into the variability and consistency of focus across tissue samples.

The documents provide extensive data and visual aids to support these methodologies. For instance, slide images paired with thickness heatmaps offer a comprehensive visual depiction of tissue thickness, highlighting areas that may require more detailed examination. These visual tools are essential for both qualitative and quantitative analysis, facilitating a deeper understanding of tissue structure and consistency.

Additionally, histograms showing focus content distribution are crucial for comparing the sharpness of edges in stack images versus fused images. This comparison is important for evaluating the effectiveness of image fusion techniques in maintaining clarity and focus.

2.2 Challenges and Solutions

One significant challenge in tissue thickness estimation is the issue of high content distribution across stacks in faint regions. This problem can lead to inaccuracies in thickness measurements. To address this, several refinements are proposed:

- **Blob Level Focus Content Distribution**: A shift from FOV level calculations to blob level focus content distribution is suggested. This method increases the resolution of thickness metrics, providing more detailed and accurate measurements.

- **Grid-Based Analysis**: Implementing a grid-based analysis, where the AOI is divided into smaller grids (e.g., 4x4 blocks), can enhance precision. This localized analysis allows for a more granular examination of focus content distribution, improving the overall accuracy of thickness estimation.

Chapter 3

Methodology

Data collection begins with the acquisition of tissue samples, which are then subjected to imaging using high-resolution microscopy. The imaging process captures multiple layers (stacks) of the tissue at different focal depths.

3.1 Tissue Sample Preparation

- **Tissue Sample Preparation**: Tissue samples are prepared and placed on microscope slides.
- **Imaging Equipment**: High-resolution microscopes capable of capturing multiple focal planes are used.
- **Image Stacking**: Multiple images (stacks) are captured at different focal depths to encompass the entire thickness of the tissue.

3.2 Image Processing

The captured images are processed to identify the regions of interest and assess the focus quality.

- **AOI (Area of Interest) Identification**: Specific regions within the tissue sample are selected for detailed analysis.
- **Focus Assessment**: The focus quality of each image in the stack is evaluated using image processing algorithms. Metrics such as edge sharpness and contrast are used to determine the focus level.

3.3 Focus Content Distribution

The focus content distribution is analyzed at both the AOI and slide levels to determine the variation in focus across the tissue.

- **AOI Level Analysis**: The focus distribution within each AOI is measured. This involves calculating the proportion of the AOI that is in focus for each image in the stack.
- **Slide-Level Heatmaps**: Heatmaps are generated to visualize the distribution of focused content across the entire slide. These heatmaps help identify thick and thin regions of the tissue.

3.4 Thickness Measurement

The number of images required to achieve 90 percent focus in the FOV is used to classify the tissue thickness.

- **Calculation of Focus Percentage**: For each stack, the percentage of the AOI that is in focus is calculated. This is repeated for all images in the stack.
- **Threshold Determination**: A threshold of 90 percent focus is set to determine the minimum number of images required to classify the tissue as thin or thick.
  - **Thin Tissue**: If 90 percent focus is achieved with fewer images, the tissue is classified as thin.
  - **Thick Tissue**: If more images are needed to achieve 90 percent focus, the tissue is classified as thick.

3.5 Analysis and Visualization

Detailed analysis and visualization techniques are employed to present the results of the thickness estimation.

- **Histograms**: Histograms are created to display the focus content distribution for each AOI. These histograms help in understanding the variability and consistency of focus across different regions.

- **Edge Sharpness Comparison**: The sharpness of edges in stack images is compared to that in fused images to evaluate the effectiveness of the fusion process.
- **Error Analysis**: Potential errors in focus measurement and image fusion are analyzed and documented.

3.6 Refinement and Optimization

To address challenges and improve accuracy, several refinements are proposed.

- **Blob Level Focus Distribution**: Instead of calculating focus content at the FOV level, a more detailed blob level analysis is conducted. This increases the resolution and accuracy of thickness estimation.
- **Grid-Based Analysis**: The AOI is divided into smaller grids (e.g., 4x4 blocks), allowing for more localized focus content analysis.
- **Error Correction**: Techniques are developed to correct issues such as high content distribution in faint regions, improving the reliability of thickness measurements.

Chapter 4

Simulation and Results

In this chapter, we present the simulation and results of our tissue thickness estimation methodology. The data is analyzed to generate visual representations, such as histograms, which illustrate the distribution of tissue thickness across various Areas of Interest (AOIs).

4.1 Simulation Process

Data Acquisition: High-resolution images of tissue samples are captured using a microscope. Multiple images at different focal depths are obtained to form a stack for each sample.

Image Processing:

- **Focus Assessment**: Each image in the stack is processed to determine the focus quality. Metrics such as edge sharpness and contrast are calculated.
- **AOI Identification**: Specific regions within each image are selected for detailed analysis.

Focus Content Distribution:

- For each AOI, the proportion of the area that is in focus is calculated for every image in the stack.
- The distribution of focused content is analyzed to identify the number of images required to achieve 90 percent focus within the AOI.

Thickness Classification:

- The thickness of the tissue is classified based on the number of images required to achieve 90 percent focus.
- Tissues requiring fewer images are classified as thin, while those needing more images are classified as thick.

4.2 Results

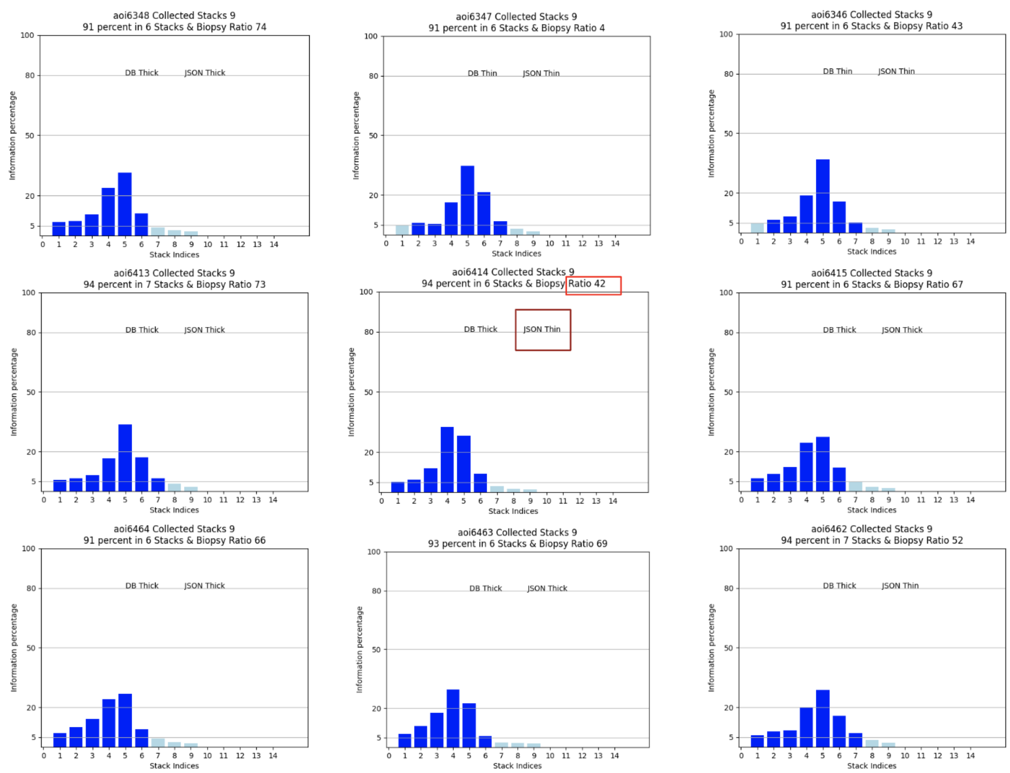

Figure 4.1: Example histogram illustrating the distribution of AOIs across different stack indices.

4.2.1 Analysis of Histogram

The histogram generated illustrates the thickness distribution of various AOIs. Key observations include:

- **Peak Distribution**: The histogram shows distinct peaks, indicating the number of AOIs with specific thickness levels.
- **Variability**: The spread of the histogram reflects the variability in tissue thickness across different samples.
- **Focus Content**: The histogram helps visualize the proportion of AOIs that fall within different thickness categories (thin vs. thick).

4.3 Overview of Histograms

The provided histograms illustrate the distribution of Areas of Interest (AOIs) collected at each stack index, where each stack represents an image taken at a different focal plane. These histograms are generated using proprietary algorithms that analyze the focus content and thickness of tissue samples. The algorithms themselves are confidential, but the overall process and interpretation of the histograms can be explained.

4.4 Key Components of the Histograms

- **Stack Indices**: Represent different focal planes in the image stack. Each index corresponds to an image taken at a specific focal depth.
- **Information Percentage**: Indicates the percentage of information (or focus content) at each stack index for the collected AOIs.
- **Collected Stacks**: The total number of stacks (images) collected for analysis.
- **Percentage in Stacks**: The percentage of AOIs that achieve a certain level of focus content within a specified number of stacks.
- **Biopsy Ratio**: A measure of the proportion of thick vs. thin tissue areas identified in the sample.

4.5 Conclusion

The simulation and resulting histogram demonstrate the effectiveness of our tissue thickness estimation methodology. By analyzing the focus content distribution across AOIs, we can accurately classify tissue thickness, which is crucial for histological analysis and diagnosis. The visual representation provided by the histogram offers clear insights into the distribution of tissue thickness, highlighting areas for further investigation and potential improvements in imaging techniques.

This analysis underscores the importance of accurate tissue thickness estimation and provides a robust framework for ongoing research and clinical applications.

Chapter 5

Conclusion and Future Work

5.1 Enhanced Accuracy and Resolution

5.1.1 Improved Algorithms

Development and implementation of more advanced algorithms to enhance the precision and resolution of tissue thickness measurements.

5.1.2 Machine Learning Integration

Utilizing machine learning and artificial intelligence to automatically identify and correct errors in focus content distribution, potentially leading to more accurate and efficient thickness estimations.

5.2 Automation and Scalability

5.2.1 Automated Analysis Pipelines

Creating automated workflows that can handle large volumes of tissue samples, reducing manual intervention and increasing throughput.

5.2.2 Scalability

Adapting the methodology to handle a wider variety of tissue types and sample sizes, making it applicable to diverse biological and medical research areas.

5.3 Imaging Techniques

5.3.1 3D Imaging

Integrating 3D imaging techniques to provide a more comprehensive understanding of tissue structure and thickness.

5.4 Integration with Other Technologies

5.4.1 Histological Staining Techniques

Combining thickness estimation with advanced histological staining techniques to provide more detailed and informative tissue analyses.