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Geometric Characterization of Mouse Intestines Under Applied Loading

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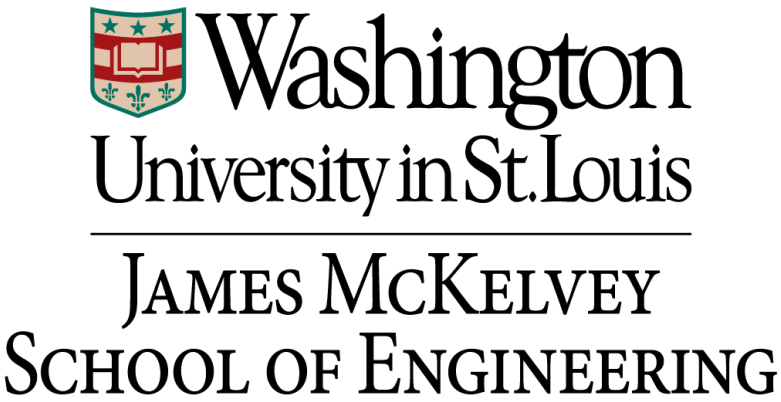

Mechanical Engineering and Material Science

Geometric Characterization of Mouse Intestines under Applied Loading

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Abstract

The purpose of this experiment was to apply bench-top imaging (optical coherence tomography or OCT) to intestinal and pelvic floor tissues from mice, focusing on the visualization of these anatomical structures and geometric characterization under applied loading. Quantifying such properties of the intestine will allow for further understanding of this tissue's response to pressure and its mechanical properties. To supplement the images gathered from the OCT imaging (loaded and unloaded tissue samples), the histology was also examined to further understand the structure of these tissues.

Introduction

According to the American Cancer Society, "…excluding skin cancers, colorectal cancer is the third most common cancer diagnosed in the United States" [1]. This, coupled with the myriad of other diseases (IBS, Crohn's disease, and diverticulitis) that can affect the gastrointestinal (GI) tract, makes further exploration about the properties of these tissues so pertinent.

The main tissue examined was the intestinal tissue of the mouse. There were three main methods employed to learn more about these intestinal structures which were OCT imaging, histology, and cannulation. These methods helped identify further possible studies that could be performed to learn more about the structures of the intestine and its mechanical properties.

Methodology

The intestines were extracted and stored in phosphate buffered solution (PBS) at 25 degrees Celsius. To prepare the tissue for OCT imaging, it was first flushed with PBS solution to remove the digestive enzymes secreted by intestine cells during digestion. Then the white outer connective tissue was surgically removed using a scalpel. The OCT imaging system utilizes reflected light to produce an image, in which "the echo time delay of the reflected light" is measured, "using low-coherence interferometry" [2]. Both steps ensured better light penetration of the intestine walls to produce clearer OCT images. Images were taken throughout different regions of the GI tract, but special emphasis was placed on the regional differences of the intestine.

To look at the histology of the intestine, the intestinal tissue was flushed, and connective tissue was removed. It was soaked in a 20% sucrose solution until the intestine sank to the bottom of the vial (approximately ten minutes). It was then left in 10% Formalin for 24 hours to preserve the tissue while undergoing the histology process. The tissue was then placed in OCT gel (an

embedding medium used for freezing tissue), frozen, then cut into 12 μm thick slices with a cryostat. The intestines were shaped into two formations then placed in the freezer. The first was laid down in the gel horizontally and the second was a spiral configuration where the intestine was rolled up with space left between each layer of the spiral to prevent breakage during slicing: the OCT gel helped stabilize the tissue.

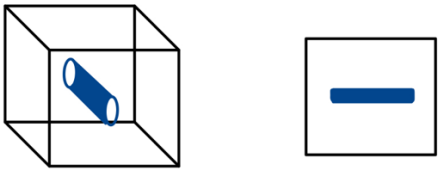


Figure 1. First configuration of intestine, 3D view (left) and side view (right)

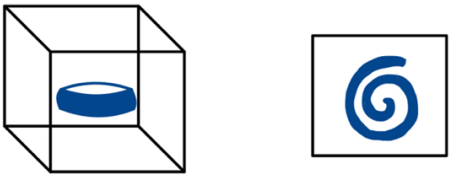


Figure 2. Spiral configuration of intestine, 3D view (left) and top view (right)

The slices of tissue were placed on slides and then hematoxylin and eosin (H & E) staining was carried out. Once the slides were properly stained, they were examined under the microscope. The final methodology used was cannulation. The tissue was prepped in a similar manner as it was for the OCT imaging and mounted on to metal rods.

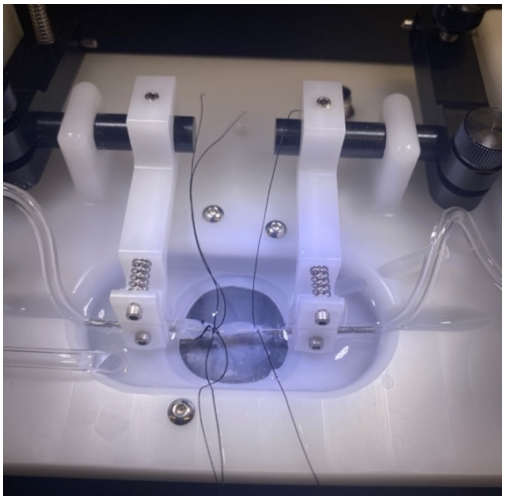


Figure 3. Bath used for cannulation

Each side was tied down with suture and fluid was pumped through the intestine. The pressure was increased in increments of 10 mmHg and OCT images were taken after each pressure increase.

Results

As discussed before, the first method explored the intestinal tissue under the OCT machine. There were visible differences in different parts of the GI track (example stomach versus intestine).

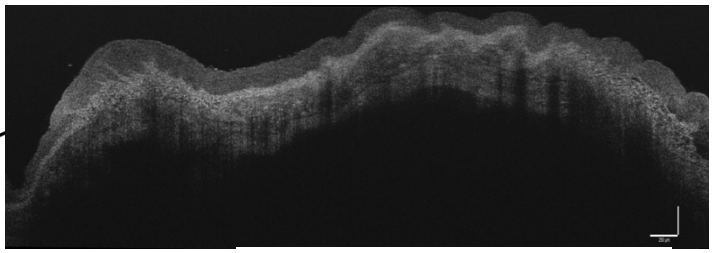


Figure 4. Stomach

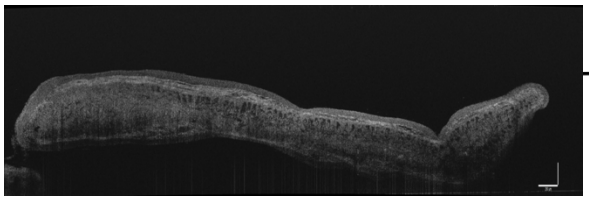


Figure 5. Cecum

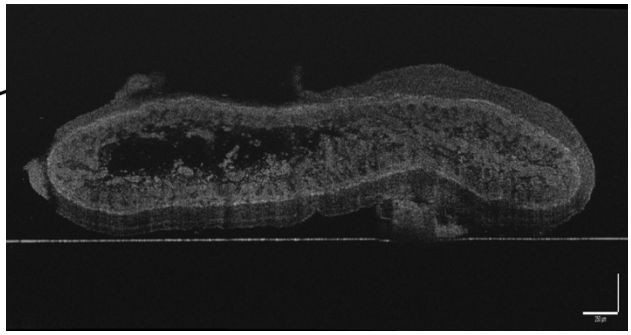
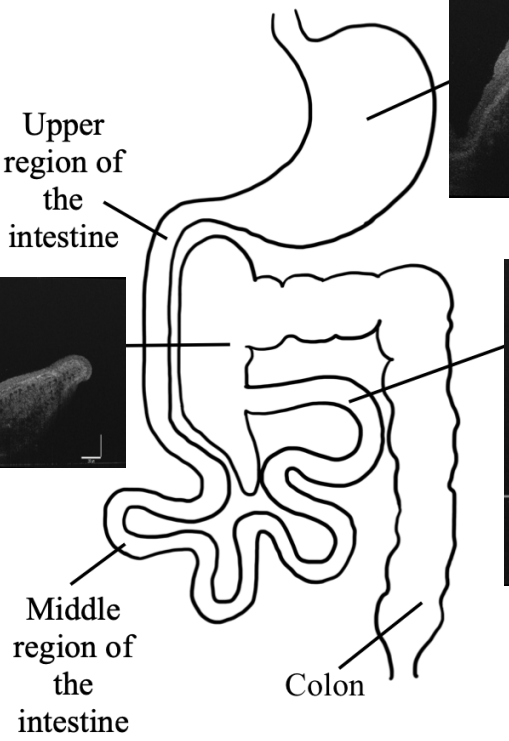


Figure 6. Lower region of the intestine



However, the intestine itself had no noticeable differences in wall thickness along the upper, middle, and lower regions of the intestinal track. Each region had the distinct bright line indicating the layer that separated the outer and inner layers of the intestinal wall. The OCT images also showed the villi projecting downwards. Villi are "tiny hair-like projections that line the inside of the intestine," [3] that increase the surface area of the intestine maximizing the nutrients that can be absorbed.

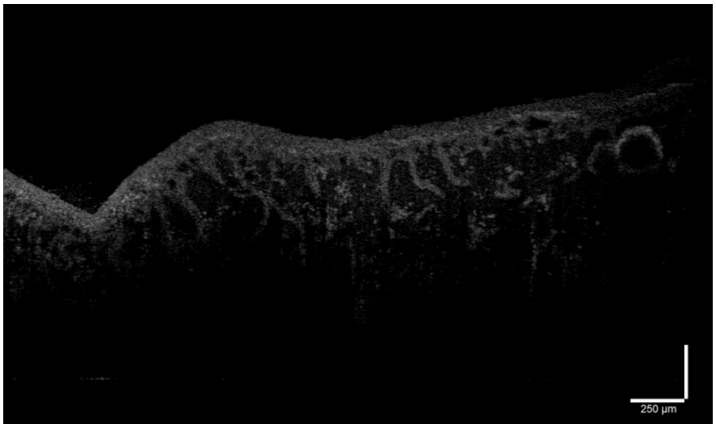


Figure 7. Image of intestine villi

While the OCT images revealed the distinctive pattern of the villi projecting downwards as well as the bright layer within the intestinal wall across the entirety of the intestine, it did not reveal information about the actual structure of the intestine wall. To further explore these properties, cryo-sectioning was used to slice the tissue samples to look at the histology. The tissue was sliced in two different orientations.

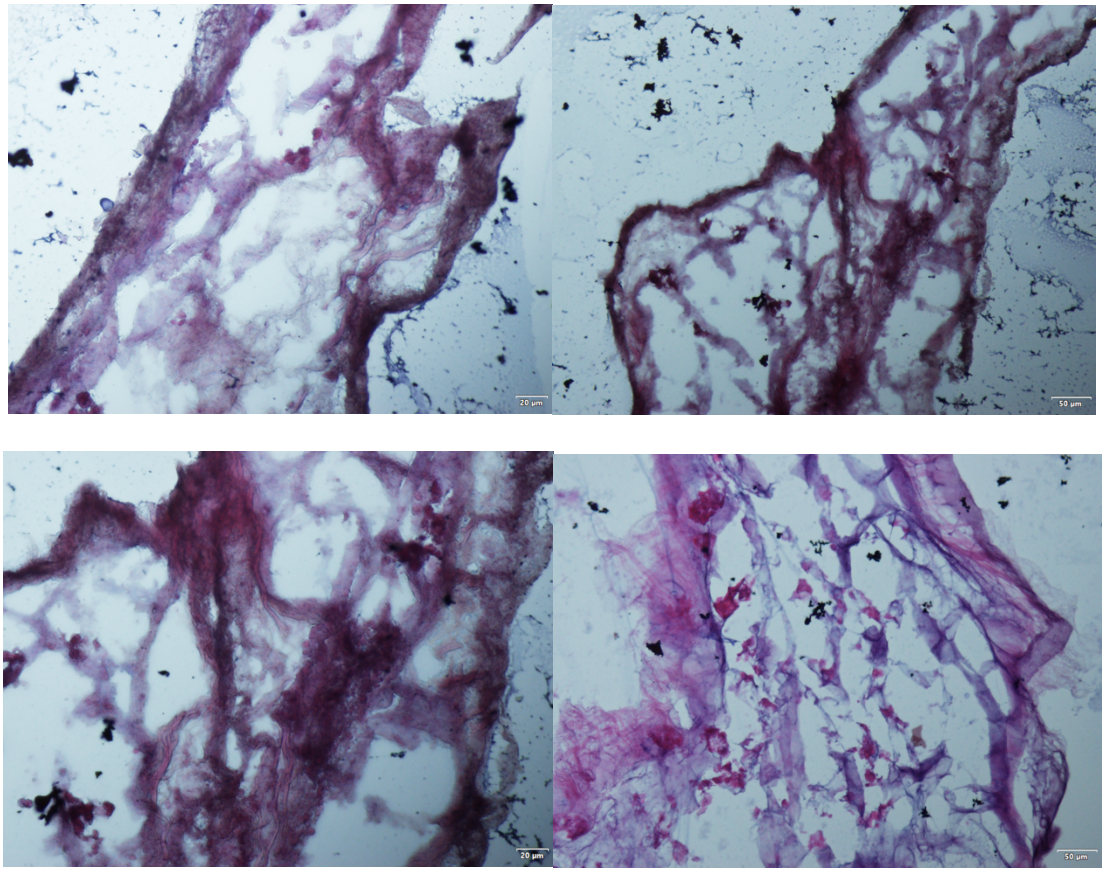


Figure 8. Round one of H & E staining

As seen in the images above, during the first round of H & E staining, most of the tissue was destroyed in the ethanol washes which were meant to "dry" out the slide. In subsequent H & E staining, the sucrose and formalin procedures were implemented and the amount of time the slides were dipped in ethanol was decreased from six minutes to one minute and thirty seconds (broken up into three different washes) to preserve more of the tissue.

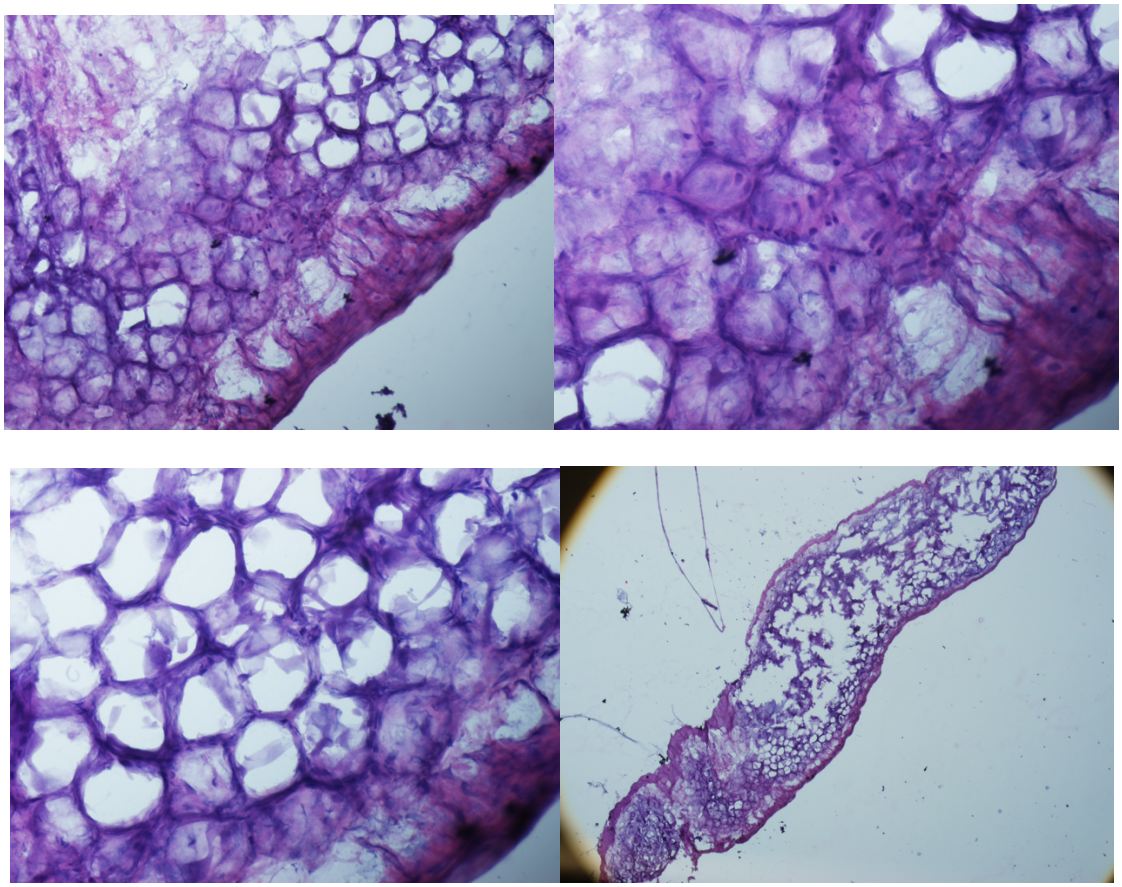


Figure 9. Round two of H & E staining

As seen in the images above, once the modifications to the procedure were made, a distinct honeycomb pattern was evident throughout the edges of the cross-sectional image of the intestine. The intestinal wall was well defined in the lighter shade of purple and noticeable in all the images. Originally, it was thought that this honeycomb pattern was the structure of the intestinal wall. However, the same prepped tissue (soaked in the 20% sucrose solution and left in Formalin) was re-examined under the OCT and the same honeycomb pattern was seen only in the connective tissue surrounding the intestine itself.

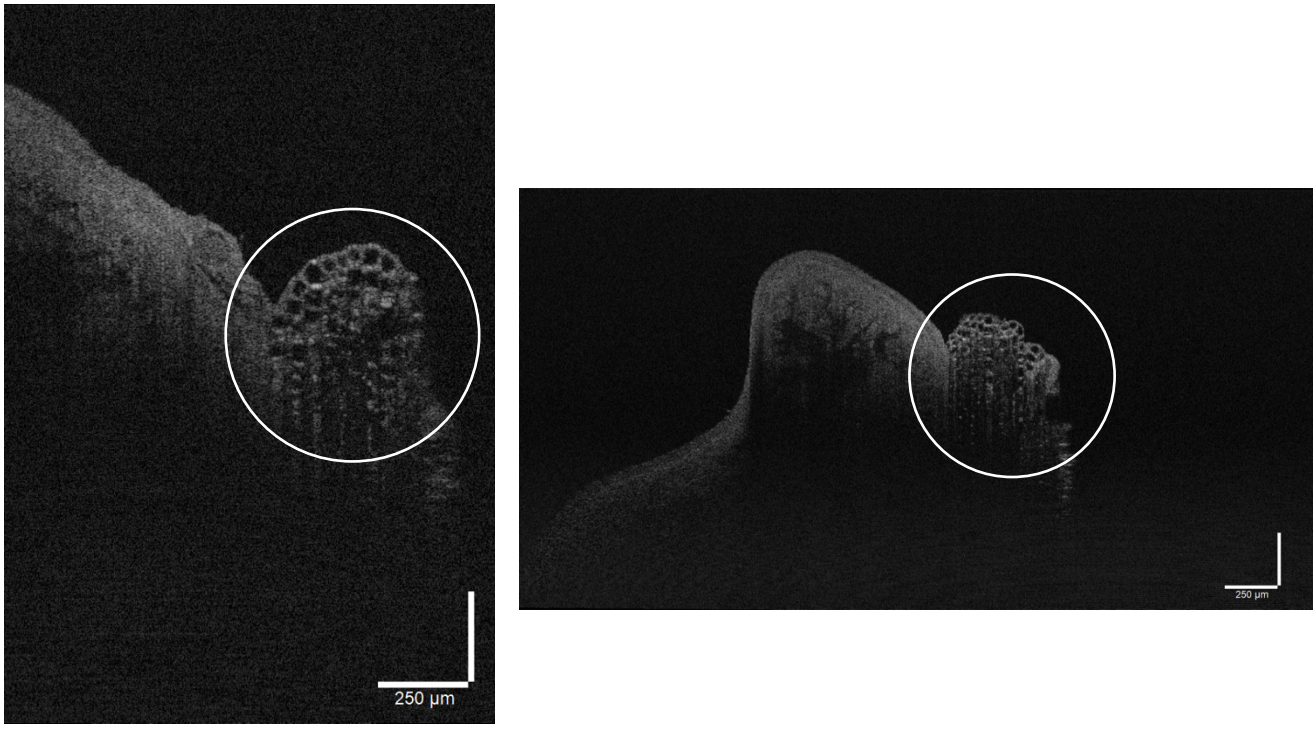


Figure 10. Image of connective tissue soaked in Formalin

These images showed the lack of uniformed structure within the intestine itself. It is thought that the Formalin worked to preserve the connective tissue and enhance the honeycomb pattern, as this defined pattern did not show up in the original OCT images. In the original OCT images, the connective tissue showed up as a fuzzy white blur alongside the more defined intestinal wall, and no honeycomb pattern was seen.

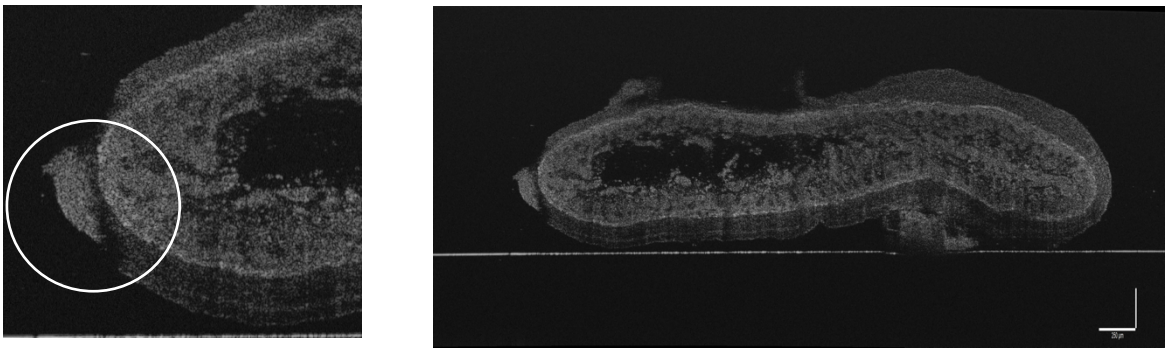


Figure 11. Image of connective tissue not in Formalin

While it was determined that the honeycomb structure was not connected to the intestinal wall

itself, it was useful to learn more about the uniformity of the connective tissue along the GI tract. A further study could explore the different regional differences of the connective tissue and see if the pattern would vary along the intestine in diseased and healthy tissue. This could lead to a better understanding in how diseases such as colon cancer and ulcerative colitis affect the mechanical properties of the intestine and change its ability to properly function. In terms of uncovering the actual pattern of the intestinal wall, a different method of stain could be tried. Paraffin-embedded sectioning would permeate the tissue with wax, which might keep the delicate tissues intact during the H & E staining. However, one downside of this type of staining is that it is challenging to remove the wax in a way that would not disrupt the expression of protein targets of interest.

The last method used was cannulation. Tissue was flushed, and the connective tissue was removed. Once mounted, OCT images were taken at different increments of pressure (measured in mmHg). The highest amount of pressure applied to the tissue was 300 mmHg; under this pressure the tissue did not tear. However, the pre and post cannulation images demonstrated inelasticity, as the tissue did not recoil back to its original diameter. Further experiments could be performed to see at what pressure the tissue goes from being elastic to inelastic.

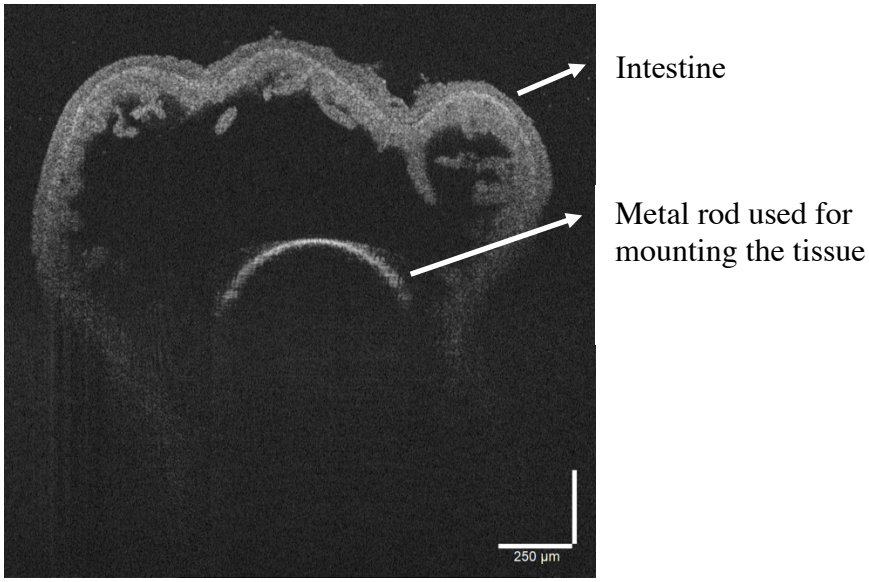


Figure 12. Tissue pre cannulation (0 mmHg)

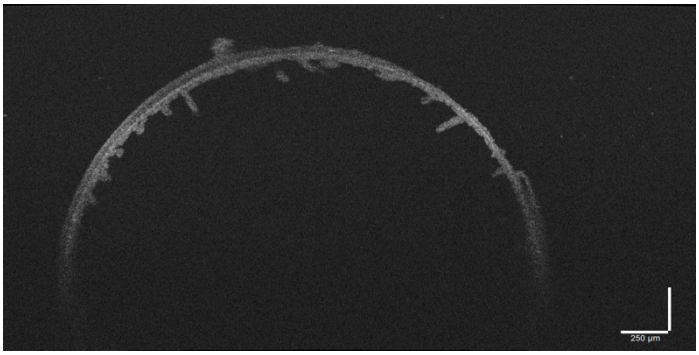


Figure 13. Pressure of 5 mmHg

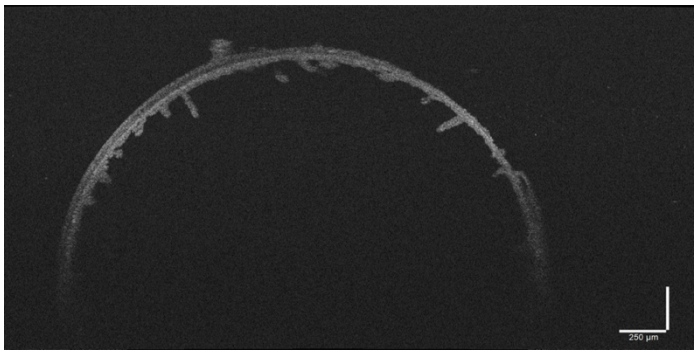


Figure 14. Pressure of 10 mmHg

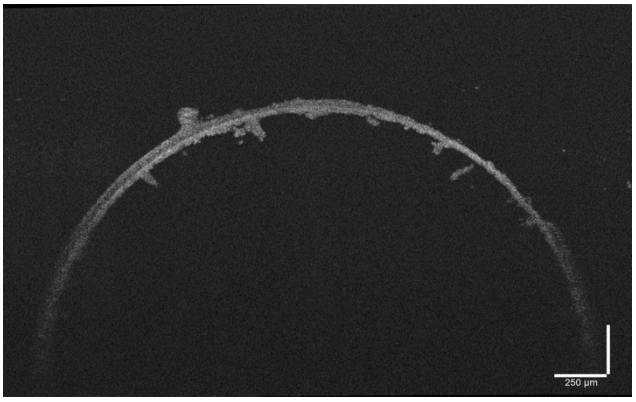


Figure 15. Pressure of 50 mmHg

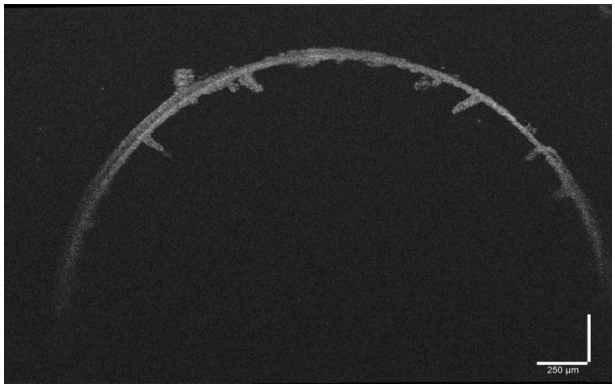


Figure 16. Pressure of 100 mmHg

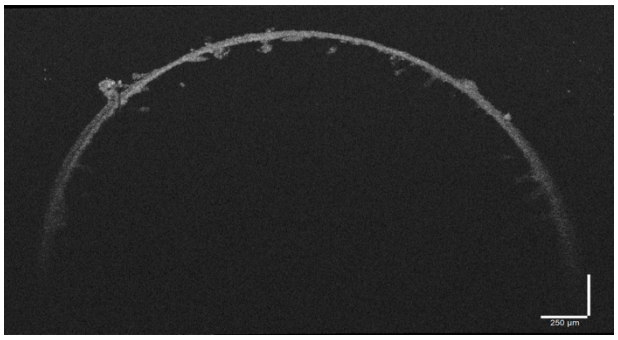


Figure 17. Pressure of 160 mmHg

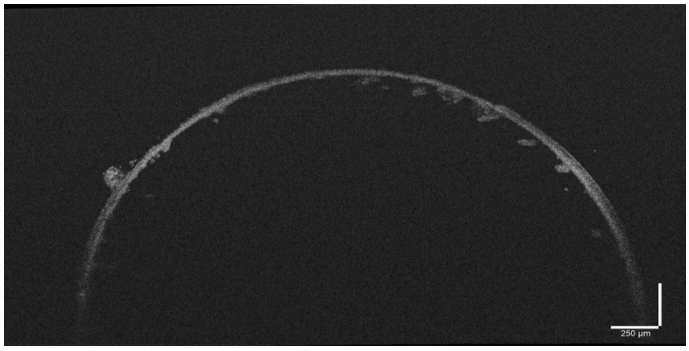


Figure 18. Pressure of 300 mmHg

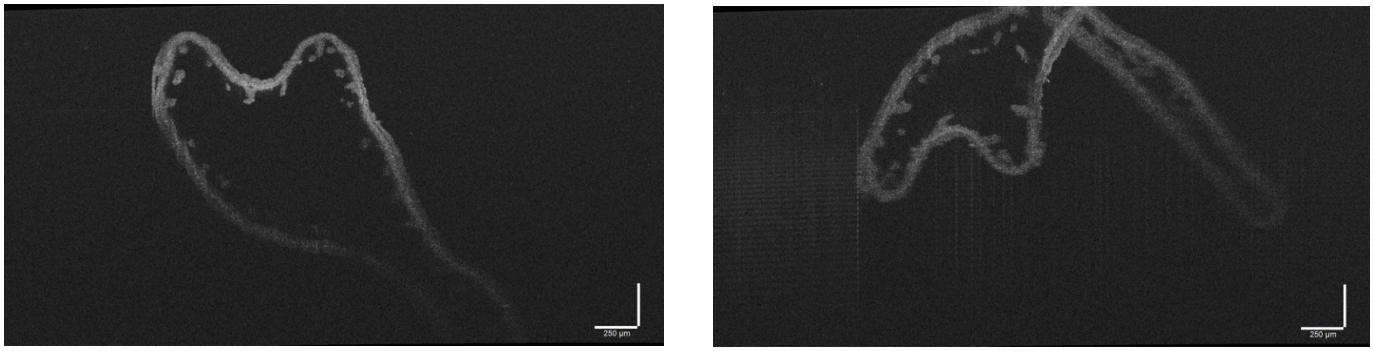


Figure 19. Tissue post cannulation (300 mmHg)

Conclusion

Three main methods were utilized to geometrically characterize mouse intestines. The OCT images revealed the characteristic bright white line of the intestinal wall, and structure of the villi throughout the various regions of the GI tract. The histology demonstrated the uniformity in the connective tissue around the intestine and further studies could explore the regional differences. The cannulation also demonstrated the intestine's ability to function under pressure and how the pressure deformed the intestinal walls. Each of the different techniques revealed more information about structure of the intestine and its properties. From this work, further studies can be performed to deepen the understanding of the mechanical properties of these tissues and how they relate to diseases such as colon cancer or ulcerative colitis.

References

[1] American Cancer Society. Colorectal Cancer Facts & Figures 2020-2022. Atlanta, Ga: American Cancer Society; 2020. https://www.cancer.org/cancer/colon-rectal-cancer/about/key-statistics.html#:~:text=Excluding%20skin%20cancers%2C%20colorectal%20cancer,new%20cases%20of%20rectal%20cancer

[2] Fujimoto, J. G., Pitris, C., Boppart, S. A., & Brezinski, M. E. (2000). Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy. Neoplasia (New York, N.Y.), 2(1-2), 9–25. https://doi.org/10.1038/sj.neo.7900071

[3] NCI Dictionary of Cancer terms. National Cancer Institute. (n.d.). Retrieved December 23, 2022, from https://www.cancer.gov/publications/dictionaries/cancer-terms/def/intestinal-villi