

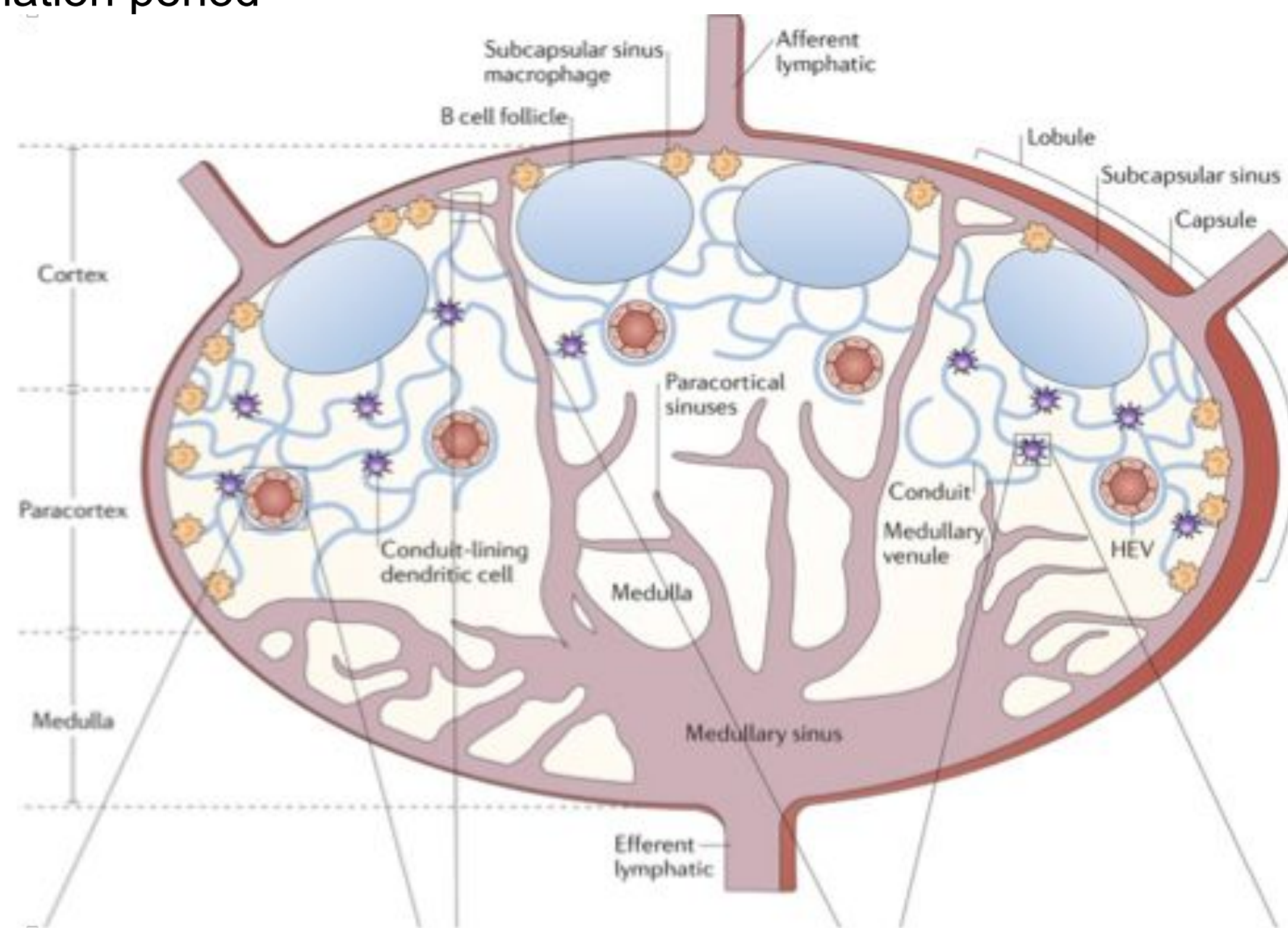
Understanding the Extracellular Matrix Structure of Lymph Nodes During Immune Response

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Introduction

- Lymph nodes (LN) are integral in facilitating lymphocyte movement throughout the body's immune system
- LNs can expand up to 10x their original volumetric size to accommodate lymphocytes during immunological challenges¹
- LN extracellular matrix (ECM) contributes greatly to cell structure, proliferation, and migration
- The ECM experiences stages of stress and relaxation during the 14-day LN inflammation period

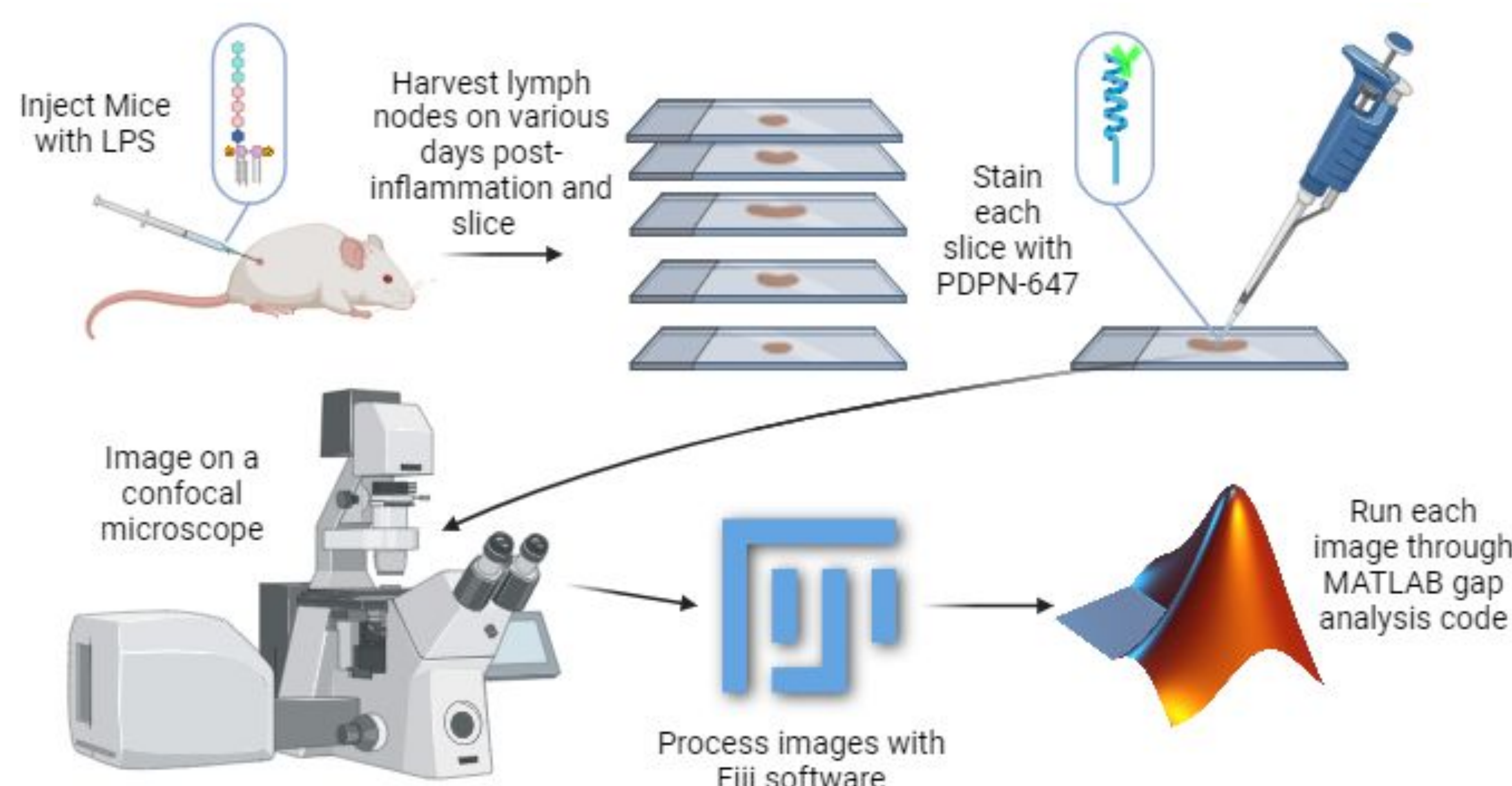


Schudel et al. *Natur Rev Mater* 4, 2019.

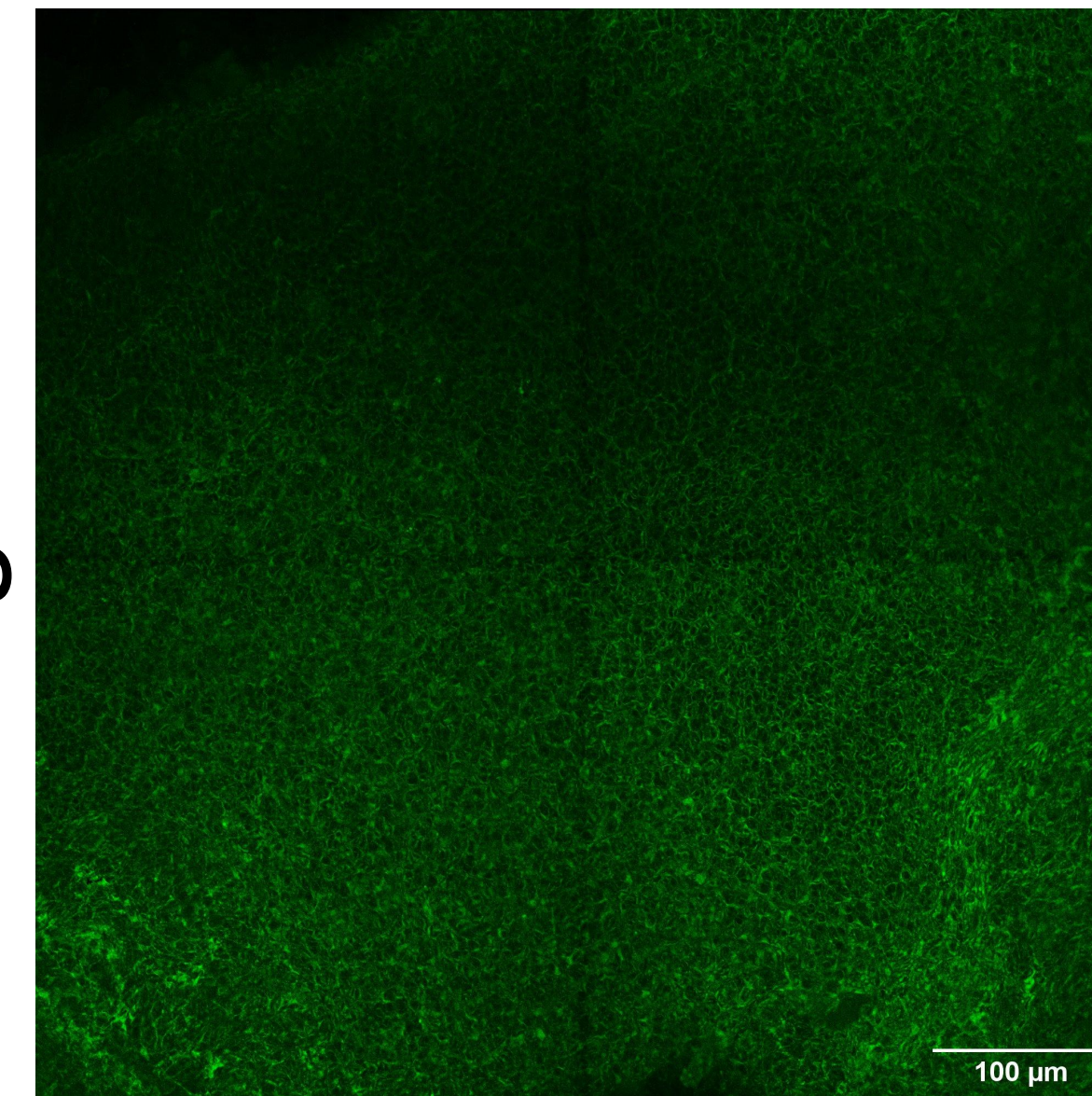
Objectives

- Optimize an immunofluorescence procedure to visualize the ECM throughout the stages of inflammation
- Use confocal microscopy to develop z-stack images of inflamed LN tissue slices
- Quantify the interstitial spacing within the ECM using MATLAB software and gap analysis
- Investigate how the ECM changes throughout inflammation

Methods

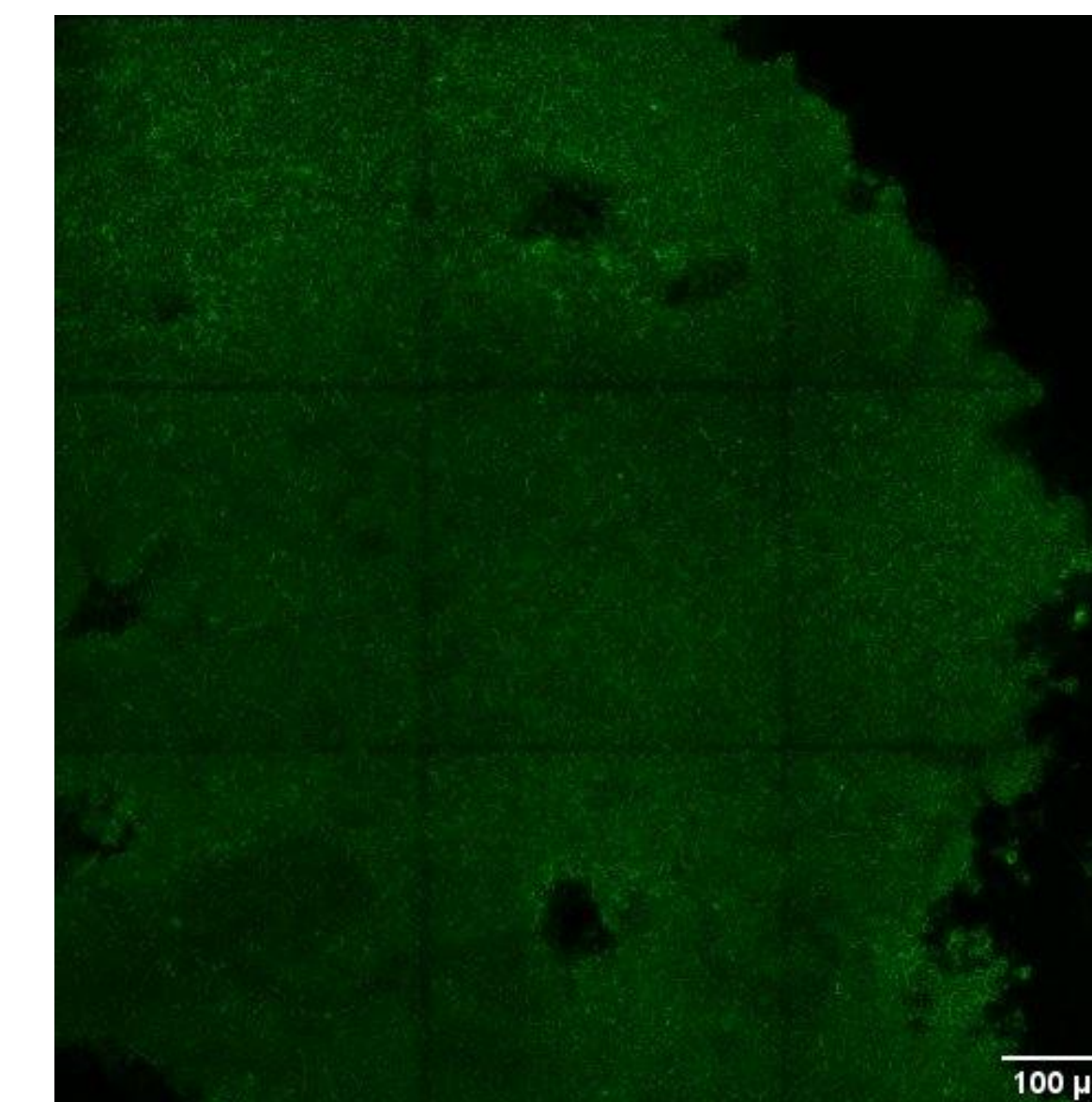


Day 1: Slice 13

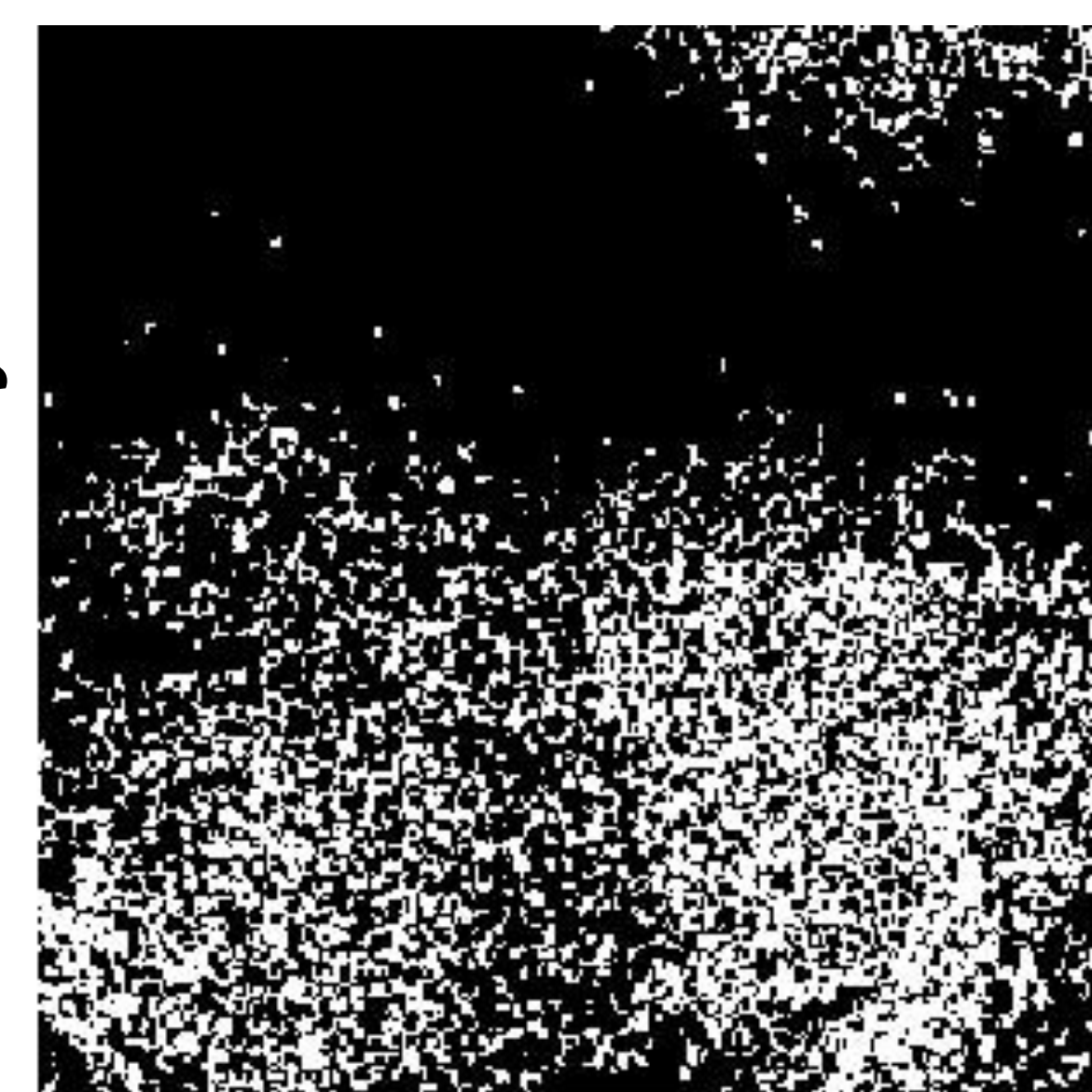


Original

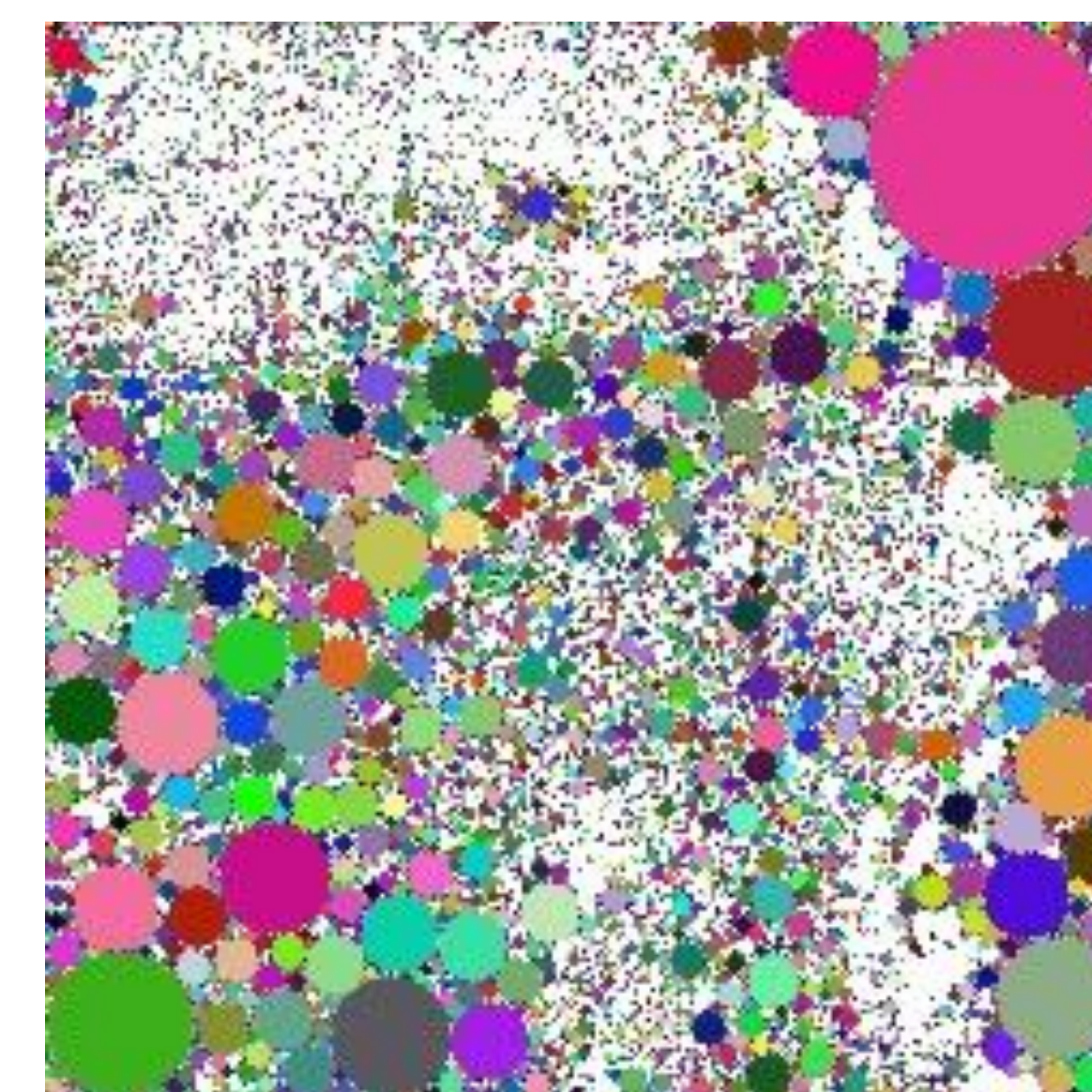
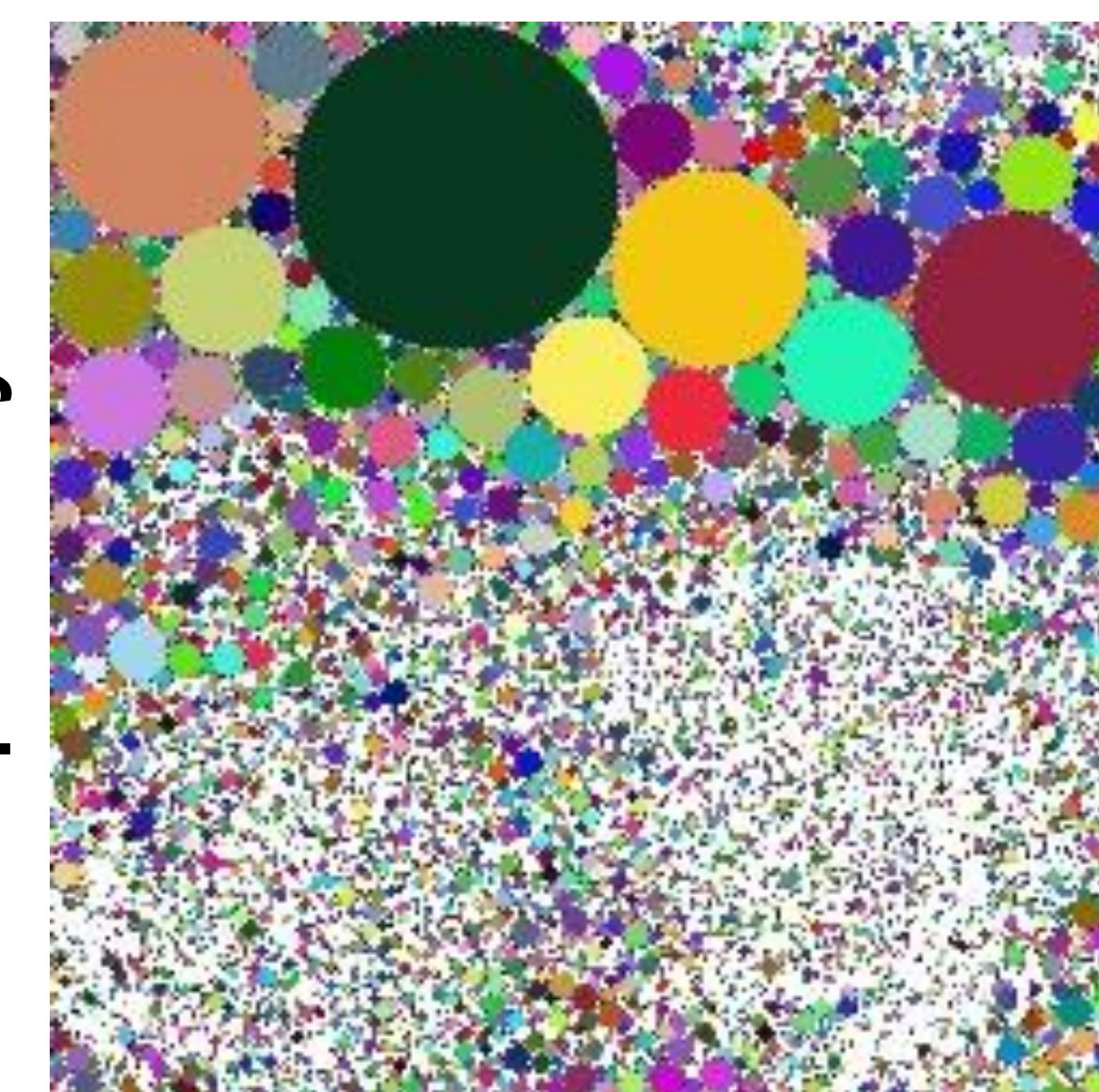
Day 3: Slice 11



Binary



Gap Analysis



Results

Figure 2. LN slices stained with Podoplanin-647 antibody (PDPN-647). Composition of fibroblastic reticular cells (FRCs) surrounding extracellular collagen. Day 3 shows an increase in collagen deposits, specifically in the paracortex (T cells) region of the node.

Figure 3. Conversion of the original LN image to binary with a threshold level of 0.001 using MATLAB (gap analysis) software. Gaps are present in areas where the original image does not meet the threshold. Day 1 shows more gaps in the paracortex region, whereas day 3 shows gaps throughout the tissue slice.

Figure 4. The maximum sized circles fit into the gaps of the Binary image. Circle radii dictate the relative size of the gaps present within the ECM. The circle radii within the day 1 LN have a larger range when compared to the day 3 LN.

LN pore sizes increase between day 1 and day 3

Pore Size

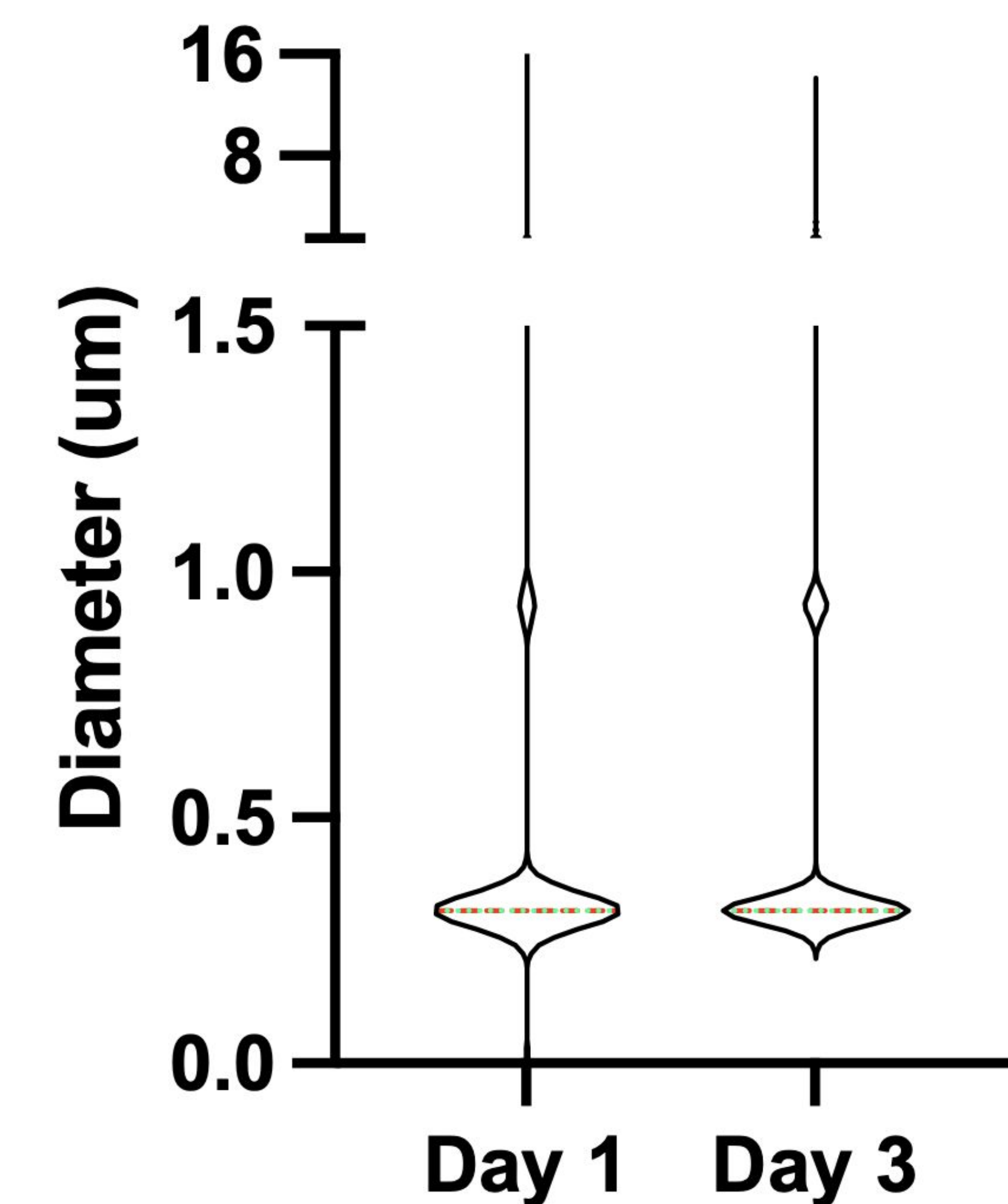


Figure 5. Pore sizes of LN tissue slices using Gap analysis. Pore sizes are roughly 0.5 μm in diameter. However, both day 1 (mean = 0.42 μm) and day 3 pores (mean = 0.49 μm) are smaller than the expected, indicating the beginning of inflammation and immune cell proliferation.

Conclusions

- PDPN marks for FRCs surrounding collagen so during initial inflammation, spacing between PDPN should appear larger. FRCs deposit more collagen at peak inflammation so, staining should appear 'dense.'
- Day 1 can be considered "initial inflammation." These LN slices have a larger range of pore sizes in preparation for cell proliferation during immune response.
- It is predicted that during peak inflammation, cell infiltration will crowd the LN causing a decrease in mean pore size.
- Small increase in pore size for day 3 could be attributed to the large gaps in the binary image - further optimization of gap analysis is required.
- LN ECM adapts to the changing needs of the immune system when there is an influx of immune cells in the region.
- In the future, several other tissue samples from each time point will be analyzed to confirm how the ECM changes.

References

- Assen F, Hons M, Hauschild R, et al. *Multi-Tier Mechanics Control Stromal Adaptations in Swelling Lymph Nodes*. Cold Spring Harbor Laboratory; 2021. Accessed December 4, 2023. <http://dx.doi.org/10.1101/2021.07.28.454217>
- Schudel, A., Francis, D.M. & Thomas, S.N. Material design for lymph node drug delivery. *Nat Rev Mater* 4, 415–428 (2019). <https://doi.org/10.1038/s41578-019-0110-7>

Acknowledgements

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Figure 1. Mice were injected with *E. coli* lipopolysaccharide. Inflamed lymph nodes were embedded in Tissue-Tek® O.C.T. compound and sliced using a cryostat machine. The tissues were imaged at 1024 x 1024 resolution and combined into a tiled, stacked image.