Achieving Spatial Transcriptomic Data from Colonic Mucosal Compartments using FFPE Tissue and Laser Capture Microdissection

Gunnar A. Walaas[1], Lusie Frostvoll Kuraas[1], Ann-Therese Chattergoon Ali[1], Tone Christensen[1], Vidar Beisvåg[1,2], Torunn Bruland[1,3], Ann Elisabet Østvik[1,4], Ingunn Bakke[1,3]

[1]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway, [2]Central Staff, St. Olav's Hospital, St. Olav's University Hospital, Trondheim, Norway, [3]Department of Gastroenterology and Hepatology, Clinic of Medicine, St. Olav's University Hospital, Trondheim, Norway, [4]Clinic of Laboratory Medicine, St. Olav's University Hospital, Trondheim, Norway

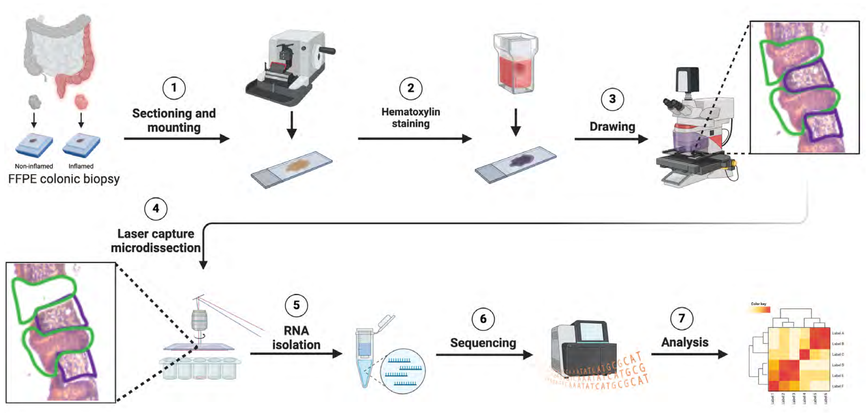
INTRODUCTION

Laser capture microdissection is a powerful tool for histology-driven multiomics analyses, as it allows for the precise extraction of targeted tissue regions. Although formalin-fixed paraffin-embedded (FFPE) tissues provide superior morphological quality, they also cause substantial RNA degradation because of chemical interactions between formaldehyde and the nucleic acids compared to frozen sections[1]. The technique has been effectively utilized to profile distinct tissue compartments[2].

While traditional bulk RNA-sequencing provides transcriptomic insights, it lacks cell-to-cell resolution, a limitation addressed by single-cell RNA-sequencing (scRNA-seq). However, scRNA-seq can lose spatial context[3], emphasizing the need for techniques like LCM that maintain spatial integrity even at single-cell resolution.

Despite the potential of LCM, there is a lack of protocols for extracting quality RNA from microdissected FFPE colonic biopsies. To bridge this gap, we present an optimized protocol for LCM-based transcriptomic analysis of FFPE colonic biopsies.

METHODS



- FFPE colonic biopsies from Ulcerative Colitis patients were sectioned at 8 µm and mounted on PEN 2,0 µm membrane slides
- Sections were stained in hematoxylin solution
- Epithelium and lamina propria compartments were captured using Leica LMD7
- RNA quality was measured using Bioanalyzer and Qubit
- Total RNA was sequenced on NovaSeq6000 S2 flowcell (Illumina)

CONCLUSION

We have optimized a laser capture microdissection protocol to collect colonic material for RNA sequencing. Despite the RNA exhibiting substantial degradation, the RNA sequencing yielded successful results. Notably, the analysis revealed intact biological information within our samples, demonstrating that even degraded RNA can provide valuable insights into the underlying molecular profiles of colonic tissues. This underscores the potential of LCM in facilitating transcriptomic studies, particularly in challenging samples from clinical contexts.

RESULTS

1. Increasing section thickness distorts morphology. **A**: Representative images of sections stained with hematoxylin. Thicker sections were associated with increased chatter frequency. **B**: Sections stained with hematoxylin. Thicker sections were associated with darker coloring.
2. Inflamed and non-inflamed sections were mounted on the same glass slide. The sections were exposed to Cresyl violet or hematoxylin for one second. Hematoxylin displays the different cellular compartments with the best contrast.
3. Correlation between the area collected and RNA concentration was most prominent in inflamed lamina propria. **A**: The correlation between RNA concentration and area collected were less impactful than anticipated. **B**: The scatterplot from A, divided based on the material and inflammation status of the sample, revealed different correlation curves. The curve with 95% confidence interval was constructed using a linear model with y ~ x.
4. Quality control of samples for RNA-sequencing. **A-B**: Distribution of RIN values and DV200 suggest low concentration and poor quality samples from LCM.
5. **A**: Total RNA-seq revealed a good separation between tissue compartments and inflammation status. **B**: Immune cell marker gene CD4 and epithelial cell marker gene EPCAM demonstrated similar distinction between lamina propria and epithelial derived samples.

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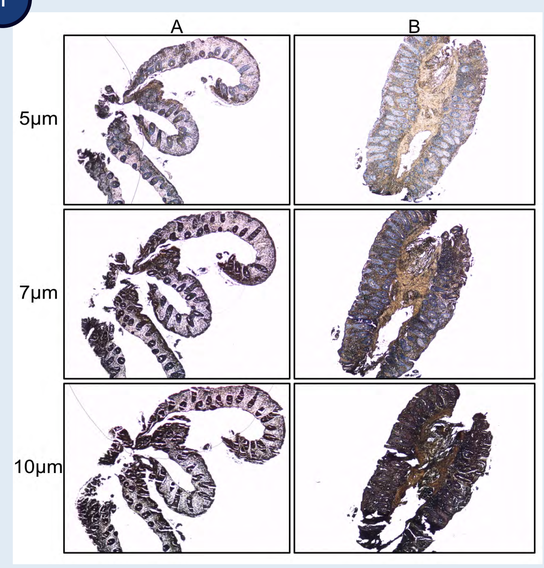

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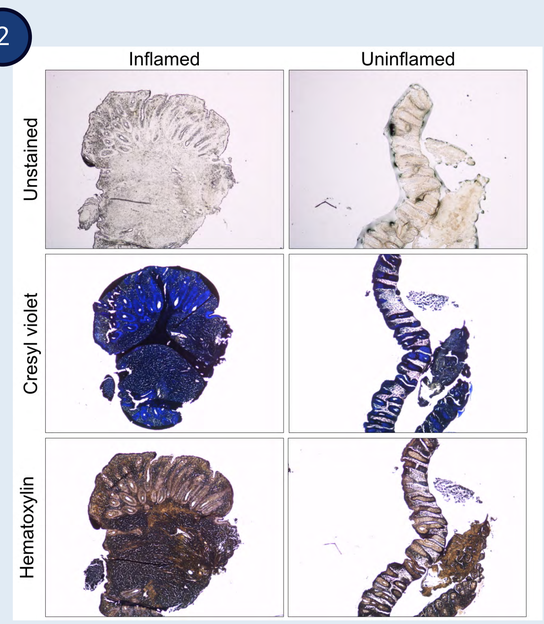

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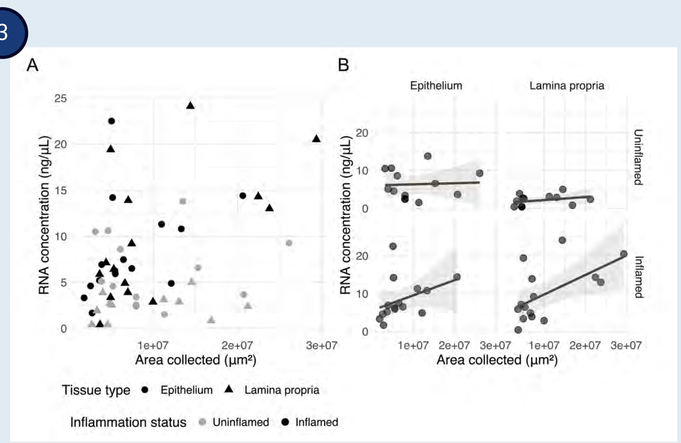

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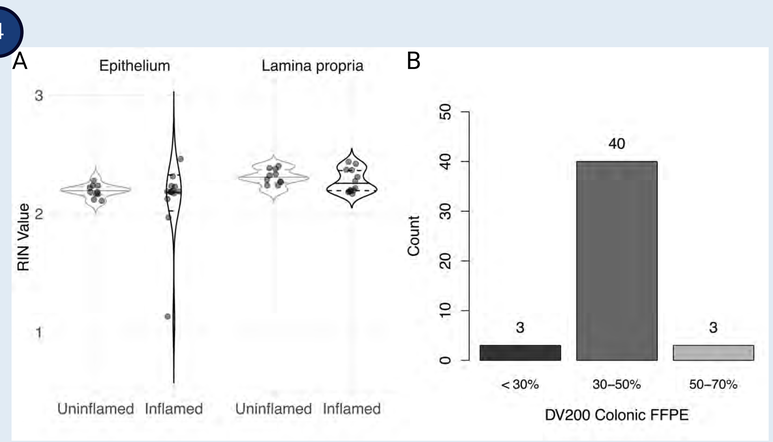

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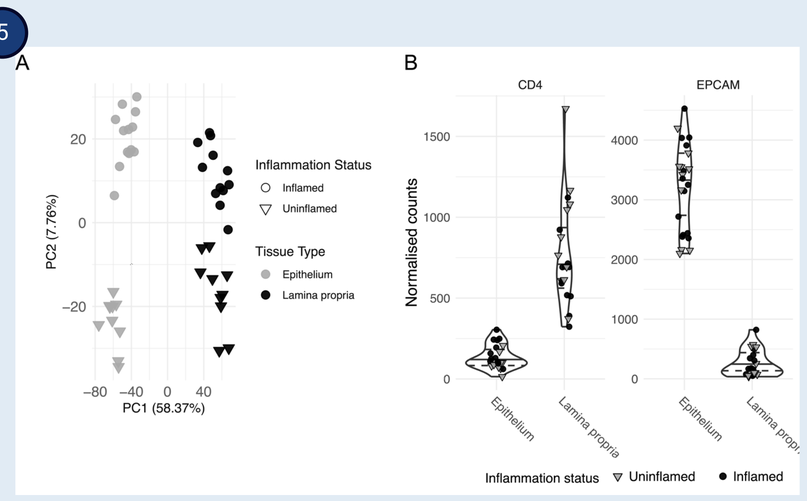

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References:
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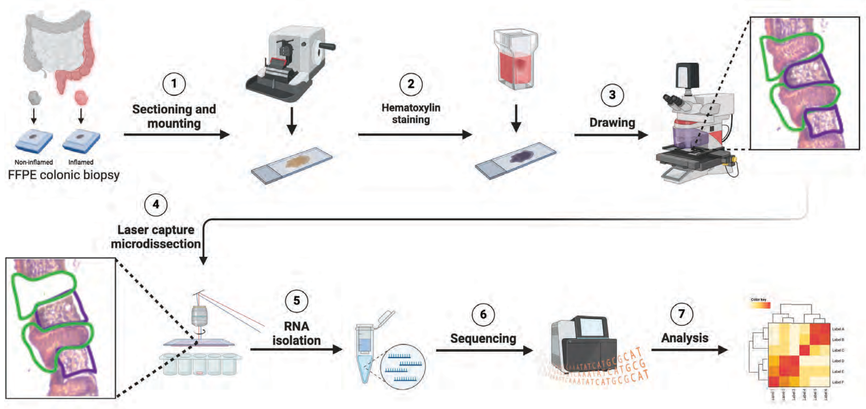

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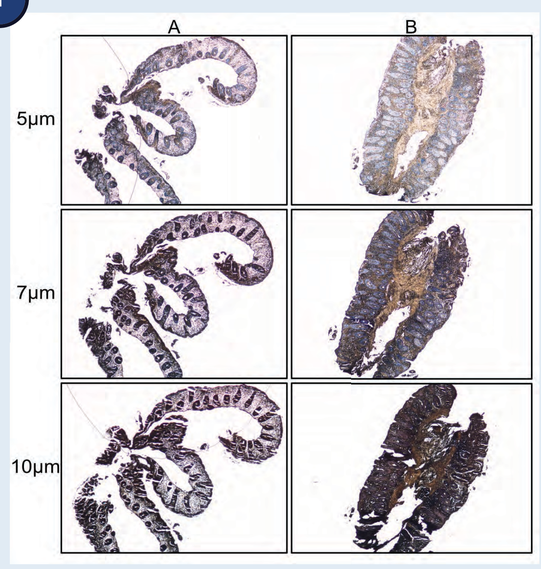

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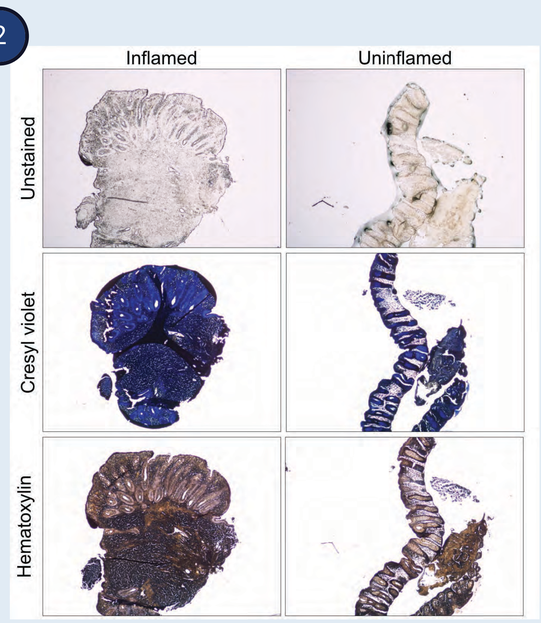

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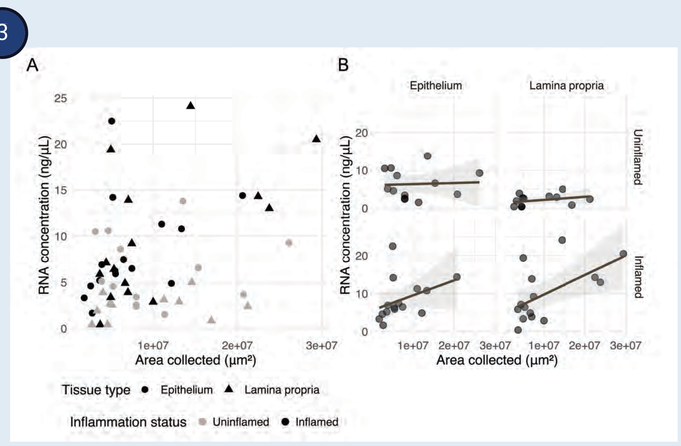

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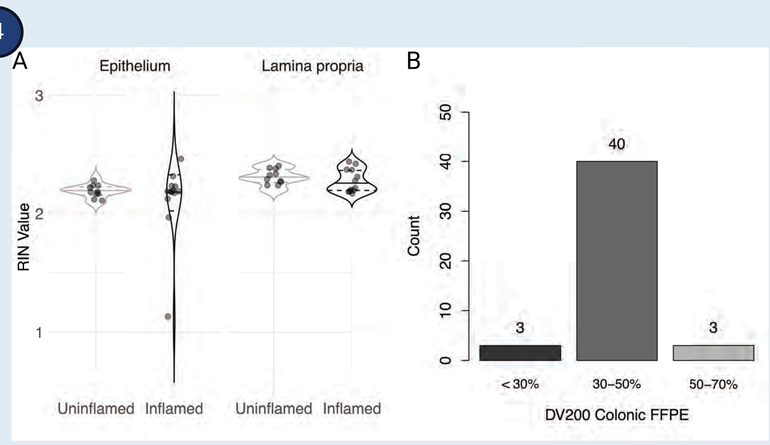

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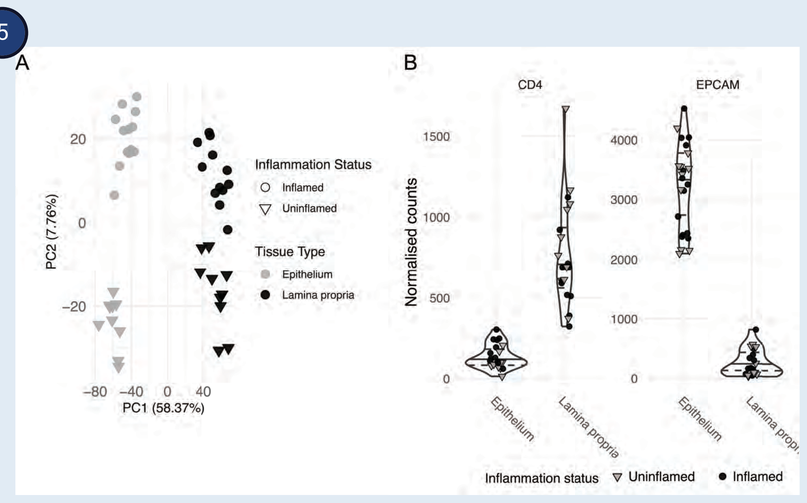

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