Fibrostenosing Crohn's Disease histological changes quantified by computational pathology image analysis using QuPath

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Introduction

Inflammatory Bowel Diseases (IBDs) such as Crohn's Disease are estimated to affect up to 1% of the European population over the next 5 years and that number is expected to increase. Although well-established single nucleotide polymorphisms (SNPs) influence risk, the effects of Crohn's Disease are mostly due to increased and prolonged inflammation involving immune-response cells in the intestinal wall, leading to progressive fibrosis, which may lead to wall thickening with partial luminal obstruction. Although immune cell populations present in these fibrostenosing lesions have been largely identified, accurate quantification of such cell populations within each intestinal wall layer has not yet been reported.

Methods

Archival formalin-fixed, paraffin-embedded terminal ileal resections from 30 control and 30 fibrostenosing Crohn's Disease patients were stained using immunohistochemistry (IHC) to identify specific cell types: CD3, CD4 and CD8 T-Cells and Smooth Muscle Actin-positive cells. Collagen deposits were visualised using Picro-Sirius Red (PSR) staining. The sections were captured digitally and analysed with QuPath, an open-source whole-slide imaging and quantification software, to quantify the fibrosis and cell populations within each ileal wall layer. Specific tissue regions were annotated and labelled. The physical changes in collagen deposits were quantified using a pre-trained machine-learning classifier, while the DAB-positive IHC-detected cells were counted using the built-in positive-cell detection function.

Results

1. Crohn's Disease shows abnormal collagen depisition throughout the ileum

- QuPath allows for annotation and visualisation of ileal layers.
- In-built pixel classifier in QuPath allowed for quantification of the changes in collagen between healthy and Crohn's Disease samples.
- There is a loss of mucosa due to ulcerations and marked expansion of serosa.
- A significant collagen expansion was detected in serosa and some in muscularis propria.

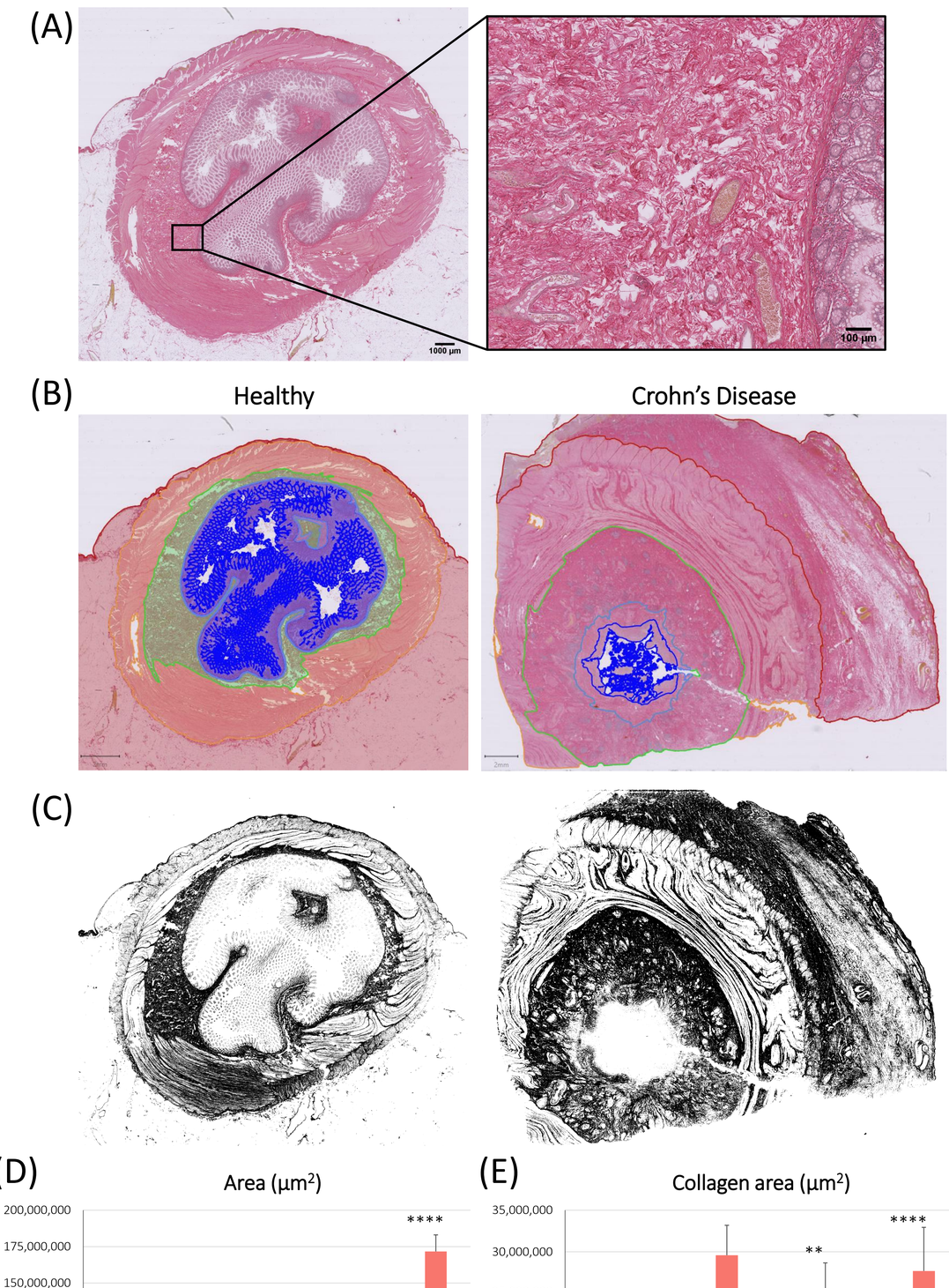

Figure 1. Quantification of collagen in ileal layers. (A) Picro-Sirius Red (PSR) staining on healthy ileum showing collagen fibres stained in pink. **(B)** QuPath allows for annotating ileal layers in healthy (left, filled in layers) and Crohn's Disease (right, just layer outline) ileum. From the interior – mucosa (blue), muscularis mucosae (light blue), submucosa (green), muscularis propria (orange) and serosa (red). **(C)** Image generated from pixel classifier trained on a series of images from stained tissue. Black pixels indicate detected collagen fibres. Left – healthy ileum; right – Crohn's Disease ileum. **(D)** Quantification of total tissue area (µm²) per condition. **(E)** Quantification of collagen area within ileal regions.
Statistical significance using Wilcoxon non-parametric test: p>0.05 ns, p≤0.05 *, p≤0.01 **, p<0.001 ***, p<0.0001 ****. Error bars represent SEM.

2. Crohn's Disease shows elevated immune cell number throughout the ileum

- In-built positive-cell detection function in QuPath allowed for quantification of the IHC-stained cells
- There is an increased infiltration of $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes in submucosa, muscularis propria and serosa
- Smooth Muscle $Actin^+$ cells presence was elevated in muscularis propria and serosa.

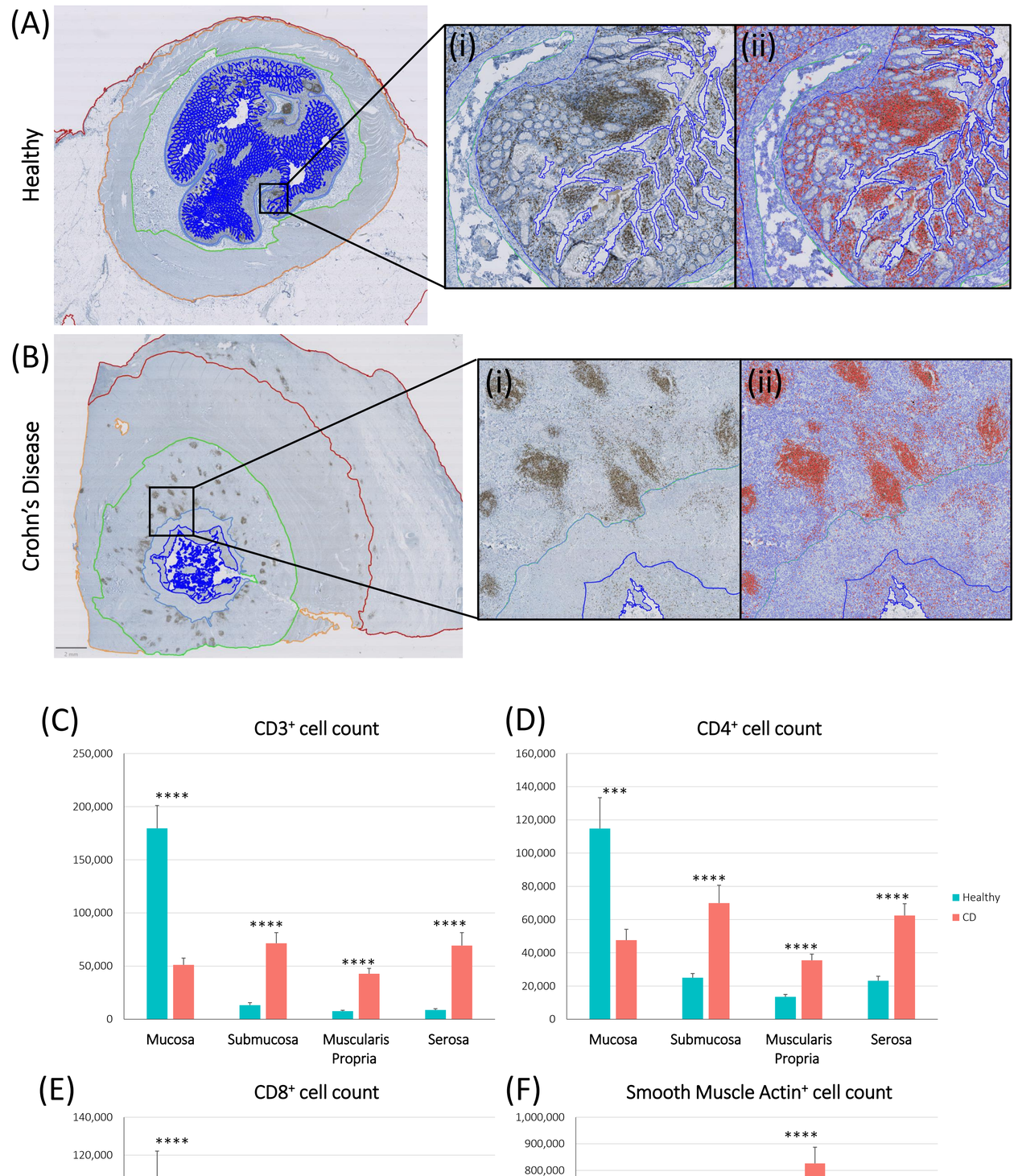

Figure 2. Quantification of positive cells stained with IHC. IHC staining on **(A)** healthy and **(B)** Crohn's Disease ileal sample. Positive cells have been stained brown (i) and QuPath detected them and highlighted them red (ii). Quantification of **(C)** $CD3^+$ cells, **(D)** $CD4^+$ cells, **(E)** $CD8^+$ cells, **(F)** Smooth Muscle $Actin^+$ cells in healthy and Crohn's Disease samples.
Numbers represent raw cell number. Statistical significance using Wilcoxon non-parametric test: p>0.05 ns, p≤0.05 *, p≤0.01 **, p<0.001 ***, p<0.0001 ****. Error bars represent SEM.

Conclusions and Future Plans

The combination of IHC and QuPath enabled accurate identification and quantification of changes in tissue areas, fibrosis and specific cell populations. The software's capabilities enabled the determination of cell type localization in each of the layers. The quantification and imaging showed loss of mucosa layer due to prevalent ulcerations and a marked expansion of serosa due to increased fibrosis. The quantification also showed increased infiltration by $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes in all layers of the ileum, as well as increased Smooth Muscle $Actin^+$ cells in muscularis propria and serosa.
Accurate quantification allows for better understanding of immune cell involvement in these disease processes and targeted treatment of the disease. The next steps involve expansion to more cell types such as CD20+ (B-cells), CD31 (endothelial cells), CD68 (macrophages) and fibroblasts, and incorporation of single-cell RNA-seq data for further insight into cell type and subtype composition, altered states and mechanistic involvement in inflammation and fibrosis.

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References and contact

References: **QuPath:** https://qupath.github.io/

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