KEYSTONE SYMPOSIA

Fibrosis Pathogenesis and Resolution: From Mechanisms to Therapies
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Contribution of Digital Pathology and AI to the quantification of fibrosis in Crohn's disease

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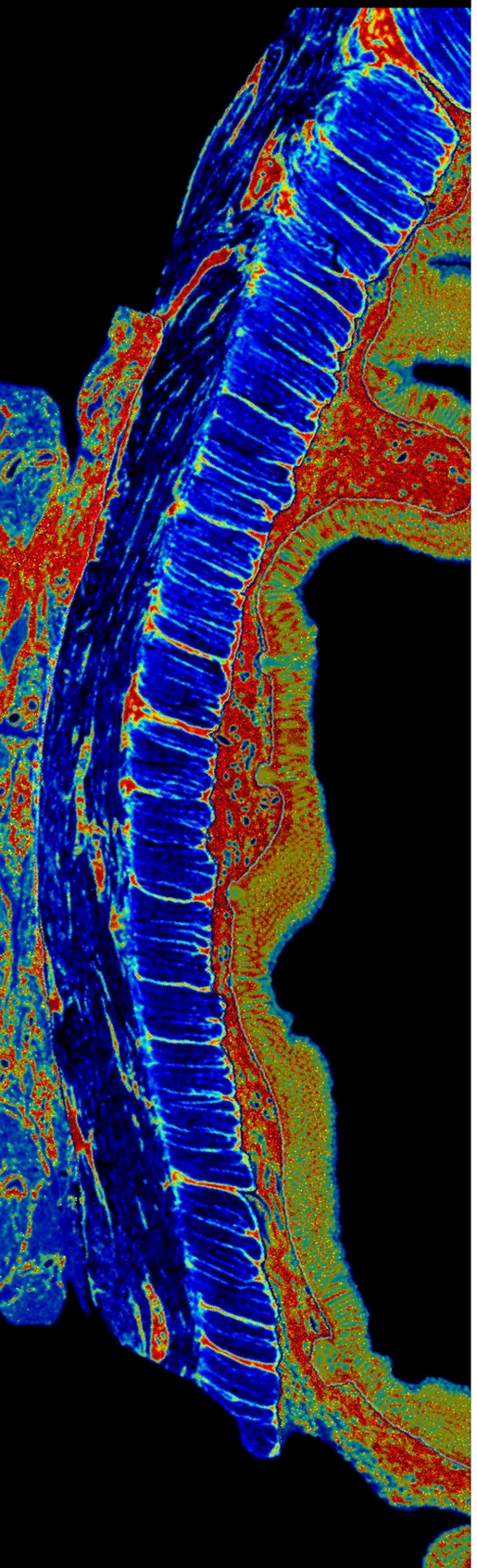


1 Introduction

Despite significant progress in the research of fibrosis in various organs, fibrosis remains a poorly understood complication of inflammatory bowel diseases (IBD), particularly Crohn's disease (CD). Pathologic studies of fibrosis in CD are relatively rare as the phenotype of fibrosis severity varies across the bowel tissue layers and is easier to perform in the deep subserosa layer, thus requiring surgical intervention to obtain bowel resection tissues. Despite progress and tentative of normalization, there are no standardized histopathological methods to score fibrosis in Crohn's disease.

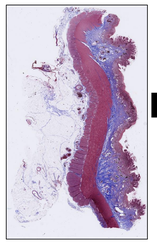
2 Aim

We used single-fiber and quantitative Digital Pathology and Artificial Intelligence (AI), to quantify pathologic phenotypes of fibrosis in each of the tissue layers forming the bowel wall, and compared them with the normal bowel, and ulcerative colitis (UC) aiming to quantify the phenotype of fibrosis severity in CD and across tissue layers.

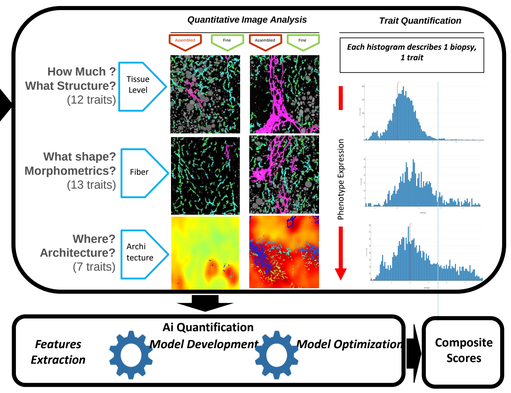
3 Method

The study included 40 patients in total who had undergone bowel resection and provided consent to the research protocol.

Condition	Control Normal Bowel	UC Ulcerative Colitis	INF-CD Inflammatory CD	FIB-CD Fibrotic CD
N Patients	10	10	10	10



FFPE sections were stained with Masson's Trichrome and imaged at 40X (0.23 mm/pixel) with a Hamamatsu NanoZoomer and uploaded to FibroNest.



The histological phenotype of fibrosis was described for its collagen features (12 traits), the morphometric traits of the collagen fibers (12), and fibrosis architecture traits (7). Each trait was quantified with 7 parameters (qFTs) to account for severity, distortion, and variance, resulting in a total of 448 qFTs. The qFT dataset was automatically surveyed to identify traits (principal qFTs) that would exhibit a significant ($p<0.05$) and meaningful (>20%) relative difference (group average) between the control and CD-Fibrosis groups. The principal qFT are assembled into a normalized **Phenotypic Fibrosis Composite Score (Ph-FCS)**. The principal qFT related to the collagen, morphometric and architectural sub-phenotypic dimensions are combined into sub-composites cores. The architecture of the non-fibrotic tissue was quantified using 7 texture phenotypes, 49 quantitative tissue parameters that yielded to 23 principal tissues traits later combined into a normalized Tissue Architecture Score.

The approach was performed for each tissue layer: the **mucosa**, the **submucosa**, the **muscularis propria** and **a 2 mm deep subserosa.**

4 Representative Images and Results

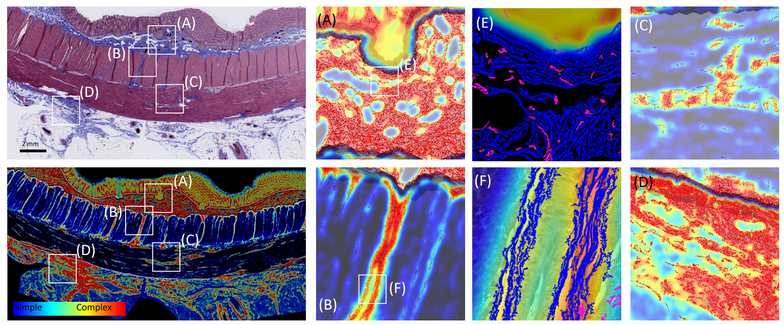


Fig. 1: Representative images (CD-Fibrosis) illustrating the quantitative Image analysis:
(i) architecture (Heat chart, simple to complex architecture) and
(ii) Single fiber analysis including fine (pink) and Assembled fibers (blue): (A) Sub mucosa with detail showing fibers in (E), (B) and (C) Muscularis Propria with details showing single fibers in (F) and (D) subserosa

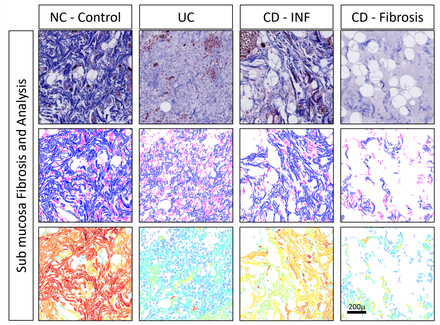


Fig. 2: Representative Images Submucosa. Top: Masson Trichrome at 10X. Middle: Collagen fibers: Fine (pink) and Assembled (blue). Bottom: Collagen Fibers: Optical Density color scale (red | dense, Blue | faint) . The scale bar is the same for all the images

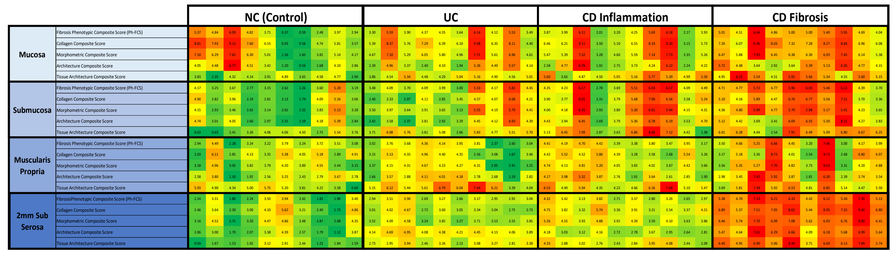


Fig. 3: Phenotypic Heat chart. Fibrosis and Tissue composite score values (for each bowel tissue layer, from Top to bottom: Phenotypic Fibrosis composite score, Collagen composite score, Fiber Morphometric composite scores, Fibrosis architecture composite score and Tissue Architecture composite score) for each patient in the study cohort. Values are normalized to their maximal value in each layer to be compared. Severity increases from green to red.

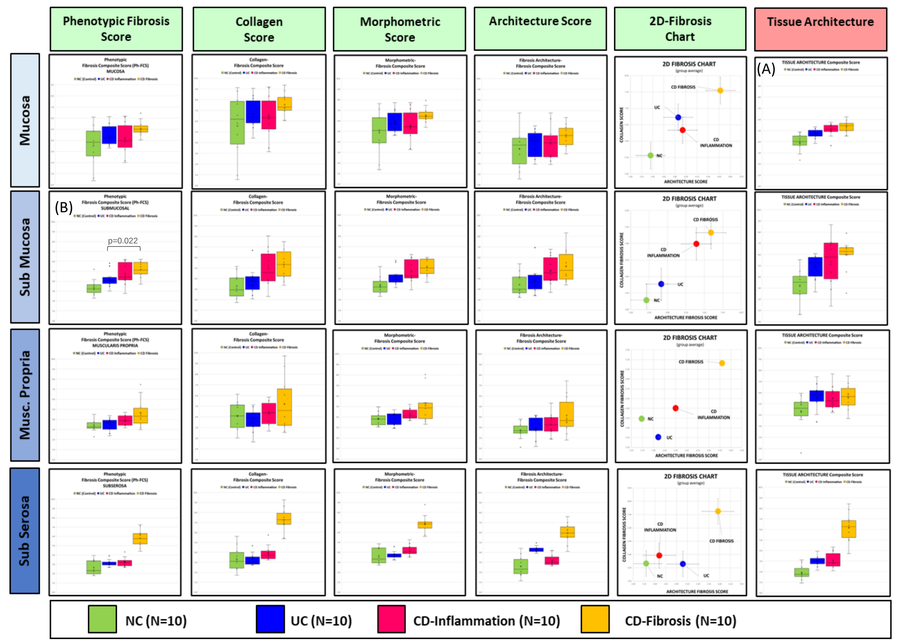


Fig.4: Box and whisker plots for the automated and continuous fibrosis composite scores as the disease condition progresses from Normal to UC, CD with inflammation and CD with fibrosis, as diagnoses by pathologists. The two 2D- Fibrosis Chart combines the collagen content and fibrosis architecture in one view, which augments the classification performance of the method, as observed in other fibrotic conditions. The Tissue architecture score enriches the assessment, in particular in the mucosa layer.

Discussion:
Fig. 4. provides a mapping of the fibrosis and non-fibrotic tissue histological changes for each patient group and across tissue layers. Remarkably:

(i) Panel (A): the tissue composite score reflects the onset of the inflammation in the mucosa ($p=0.018$)

(ii) Panel (B): from the submucosal tissue layer, the Ph-FCS stratifies UC from CD patients ($p=0.022$) and correlates with the Ph-FCS measured in the subserosa layer which reflects the highest phenotypic changes between CD with and without fibrosis.

5 Conclusions

The high-resolution (FibroNest) quantification of the histological phenotype of fibrosis and non-fibrotic tissue in each layer of the bowel wall provides significant insights into the histological hallmarks and pathogenesis of fibrosis in IBD, particularly of fibrostenosing CD. The severity scores could be used to distinguish among various forms of IBD, such as UC, CD with inflammatory stenosis and CD with fibrostenosis in the surgical specimens of IBD patients.

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