KEYSTONE SYMPOSIA

Fibrosis Pathogenesis and Resolution: From Mechanisms to Therapies
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# Evaluation of the performance of a novel Digital Pathology score for the evaluation of Fibrosis in patients with Sjögren's syndrome

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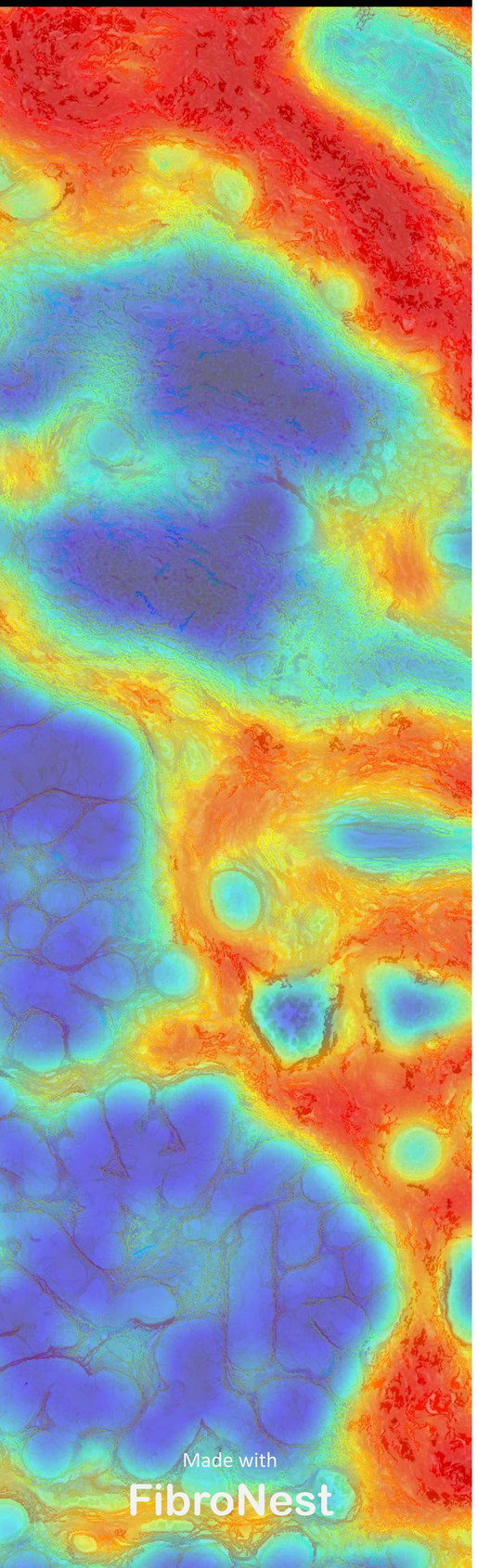



## 1 Introduction

Sjögren's syndrome (SS) is a systemic rheumatic autoimmune disorder with cardinal features of chronic, severe dry eyes and mouth and focal lymphocytic infiltrates in salivary and lacrimal gland tissue. The etiology of SS includes genetic risk, epigenetic, environmental and stochastic factors. Causative pathogenic mechanisms remain unclear but involve dysregulation of innate and adaptive immunity and epithelial cell defects. Fibrosis is a common consequence of tissue damage and inflammation and often complicates rheumatic diseases. However, whether these fibrotic changes merely reflect ageing or are a feature of disease pathology is unclear. Studies in this field are hampered by the lack of established histological quantification of SS-related fibrosis.

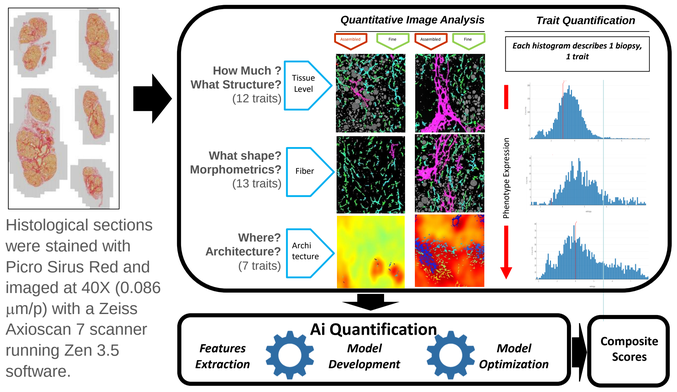
## 2 Aim

In this study, we use FibroNest™, a cloud-based, single fiber quantitative image analysis platform, to provide a continuous score to describe the severity of the histological phenotype of fibrosis in Salivary gland biopsies from patients with SS. Our long-term goal is to understand patient heterogeneity in Sjogren's including the accrual and type of fibrotic damage. To meet that long term goal and to develop a fibrosis score, we compared tissues from verified healthy controls vs. Sjogren's and non-Sjogren's sicca cases that were selected for the presence of fibrosis in minor salivary gland tissue

## 3 Method

FFPE (~4 microns) sections containing 5-6 minor salivary glands per subject were studied from:

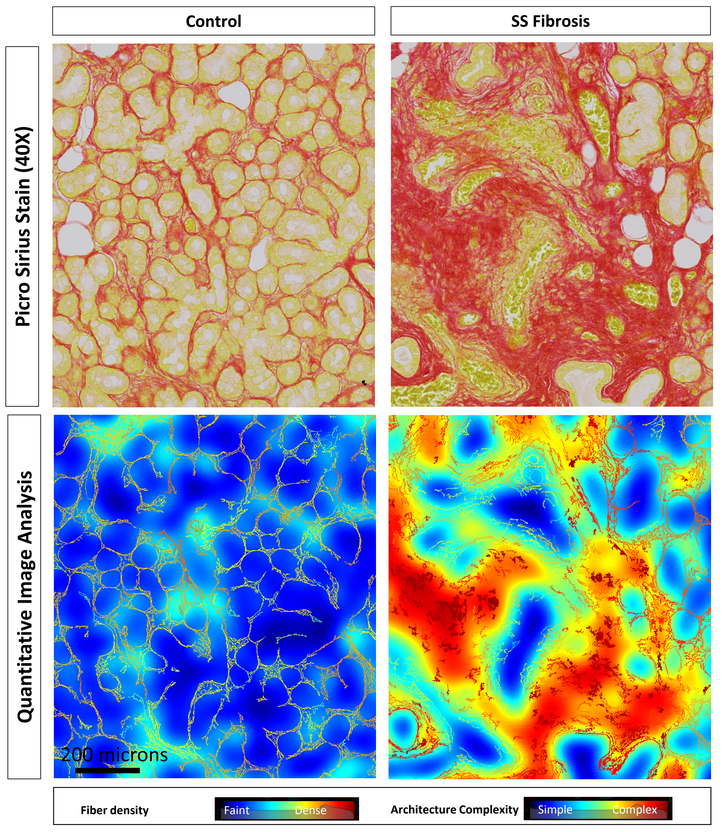
- 12 verified healthy controls (n=55 tissues)
- 7 patients meeting the 2016 ACR/EULAR criteria for SS (n=38)
- 5 patients with non-SS sicca (n=28 tissues).



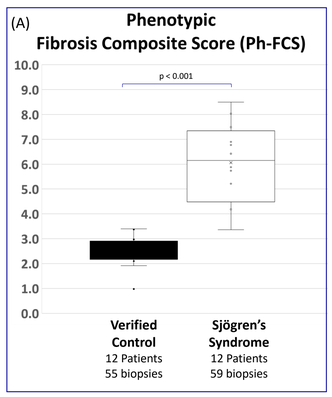

Histological sections were stained with Picro Sirus Red and imaged at 40X (0.086 µm/p) with a Zeiss Axioscan 7 scanner running Zen 3.5 software.

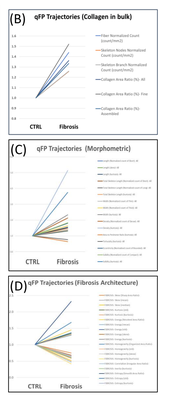
Using quantitative image analysis, the fibrosis phenotype was described for its collagen features (12 traits), the morphometric traits of the collagen fibers (12), and fibrosis architecture traits (7). Each trait was quantified with 7 quantitative parameters (qFTs) to account for severity, distortion, 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 SS groups. Such principal qFT are assembled into a normalized Phenotypic Fibrosis Composite Score (Ph-FCS) and displayed in the form of a heat chart (figure).

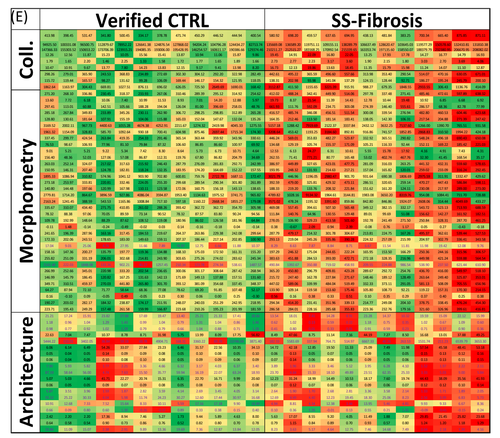
## 4 Representative Images




## Results



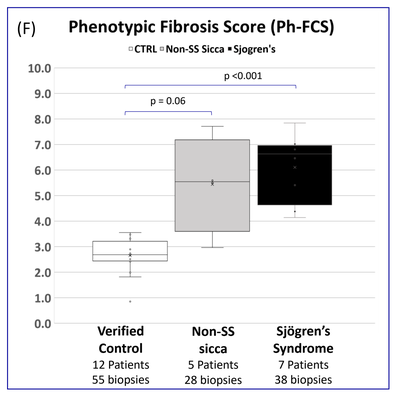



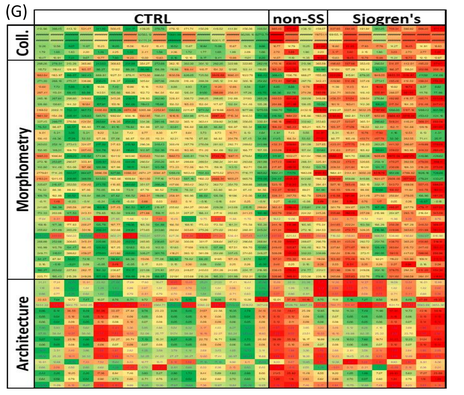




The Ph-FCS (Fig. A) classifies the control patient group from the group of patients with Sjögren's syndrome with an exceptional performance (X2.15-fold change of group means, $p>0.001$) and scores the severity of fibrosis in a continuous way with a large dynamic range (PhFCS range from 4.1 to 7.8 in the SS group). The phenotypic Heat Chart (Fig. E) and principal qFT trajectories (Fig. B-C-D) illustrate the related changes in the three complementary phenotypic layers for bulk collagen, Fibers Morphometry and Fibrosis Architecture)

The same Ph-FCS score cannot distinguish non-SS sicca patients from Sjogren's patients (Fig. F).

The Phenotypic charts and Principal qFTs offer a wealth of information that can be used to study sub phenotypes of fibrosis.




## 5 Conclusions

An automated scoring system of the fibrosis histological phenotype has been developed. This scoring system can differentiate both Sjogren's and non-SS sicca minor salivary glands from healthy control minor salivary glands. The scoring will be useful for investigating patient heterogeneity and fibrotic pathology in Sjogren's and perhaps also some individuals with non-SS sicca.

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