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Digital Pathology Approach to Mapping Collagen VI Tumor Spatial Arrangement in COVID-19 Tissue Slides Using ImageJ

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INTRODUCTION & AIM

Background

Collagen VI is a structural ECM protein that regulates cell adhesion, migration, and signaling, contributing to tumour invasion and growth. In Hodgkin lymphoma, increased collagen cross-linking mediated by lysyl oxidase elevates ECM stiffness and promotes tumour invasion. Traditional histopathology and pixel-intensity metrics fail to capture the spatial organisation and topological complexity of collagen networks, limiting quantitative assessment of tumour-associated stromal remodelling.

Gap

Conventional area-based collagen metrics do not reflect the architectural complexity governing tumour behaviour, invasion potential, and disease severity.

Aim

To develop and validate a reproducible ImageJ (Fiji)-based digital pathology pipeline for quantitative assessment of collagen VI cellular spatial arrangements in cancer-related tissue, using Hodgkin lymphoma as the primary disease context, validated on an independent COVID-19 lung tissue cohort (n = 62 slides).

METHODS

01 Image Preprocessing
Colour deconvolution (H-DAB), 8-bit conversion, inversion. Workflow developed on Hodgkin lymphoma tissue; subsequently applied to COVID-19 whole-slide images at native resolution.

02 Collagen Quantification
Otsu thresholding with manual optimisation; binary mask generation. Collagen fractional area (% DAB-positive pixels) and perimeter-to-area (P/A) ratio extracted as fibre fragmentation proxy.

03 Distance Mapping
Collagen distance map generated; tumour cells converted to ROIs via Analyse Particles plugin (size: 20 to infinity px). Centroid-to-collagen distances measured in microns post-scale calibration.

04 Skeletonization and BCI
Collagen fibres reduced to 1-pixel-wide skeletons. Analyse Skeleton (2D/3D) plugin extracted branches, junctions, triple and quadruple points.
BCI = (Junctions + 2 x Triple + 3 x Quadruple) / Branches

05 Statistical Analysis
Spearman rank correlation (ρ) for structural-histological associations. Bland-Altman analysis for inter-analyser reproducibility across two independent analysts. All analyses in Microsoft Excel.

STUDY COHORT

21	62	3	13
Patients	Slides Analysed	Cohorts	Metrics Extracted

CONCLUSIONS

Key Findings

ImageJ-based skeleton analysis provides a robust, reproducible framework for quantifying collagen VI spatial architecture and ECM remodelling in cancer-related tissue.

BCI, branch density, and junction density exhibit biologically plausible associations with collagen deposition, with stronger associations in COVID-19 diseased tissue than normal controls.

Bland-Altman analysis confirmed strong inter-analyser reproducibility for collagen area (mean diff −0.36) and BCI (mean diff −0.03), establishing methodological robustness for multi-analyst studies.

Future Work

Extension to full Hodgkin lymphoma cohort (n = 300) for tumour microenvironment characterisation and therapeutic target identification; integration with clinical staging data; machine learning automation of thresholding.

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“Multi-omic spatial profiling reveals the unique SARS-CoV-2 lung microenvironment and collagen VI as a predictive biomarker in severe COVID-19”: Eanna Fennel, Graham S. Taylor, Clara I. Leahy, Aisling M. Ross, Gary Reynolds, Tracey Perry, Esther Youd, Jacob Skidmore, Radwan Ramzi Radwan Darwish, Kelly J. Hunter, Benjamin E. Willcox, Philip Jermann, Chowdhury Arif Jahangir, Arman Rahman, William M. Gallagher, Nadezhda Nikulina, Bassem Ben Cheikh, Oliver Braubach, Aaron T. Mayer, Lawrence S. Young, Dimitris Grammatopoulos, Sian Faustini, Alex Richter, Alexander C. Dowell, Tonny Venith, Onn S. Thein, Dhruv Parekh, Kylie B.R. Belchamber, David R. Thickett, Aaron Scott, Richard Attanoos, Lucia Mundo, Stefano Lazzi, Lorenzo Leonicini, Gareth Leopold, Neil Steven, Jannie Marie Bulow Sand, Morten A. Karsdal, Diana Julie Leeming, Stefan Dojcinov, Aedin Culhane, Paul G. Murray, and Matthew R. Pugh

RESULTS & DISCUSSION

Spearman Correlation: Structural Metrics vs Collagen Fractional Area

Cohort	BCI (ρ)	Branch Density (ρ)	Junction Density (ρ)
Normal lung	−0.07	0.50	0.63
UK COVID-19	0.28	0.64	0.65
Italian COVID-19	0.76	0.40	0.46

Reproducibility (Bland-Altman): Collagen area: mean diff −0.36; LoA −6.48 to 5.77. BCI: mean diff −0.03; LoA −0.24 to 0.19. Distance mapping: mean diff 32.43 (limited reproducibility; suboptimal dichromatic staining).

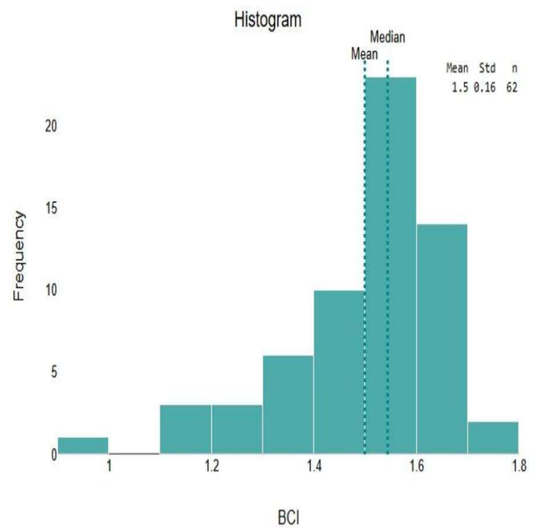

Fig 1. BCI distribution across all lung tissue slides (mean 1.50, SD 0.16, n = 62). Left-skewed distribution reflects higher architectural complexity in diseased vs normal tissue.

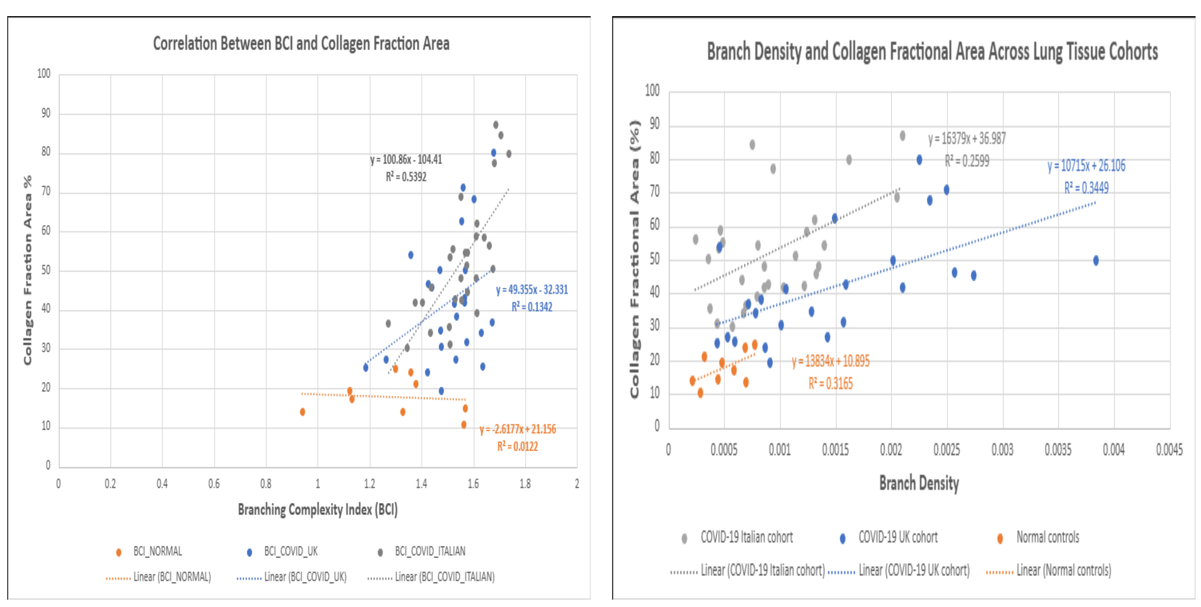

Fig 2. Left: BCI vs collagen fractional area (normal ρ = −0.07; UK COVID ρ = 0.28; Italian COVID ρ = 0.76). Right: Branch density vs collagen fractional area across cohorts.

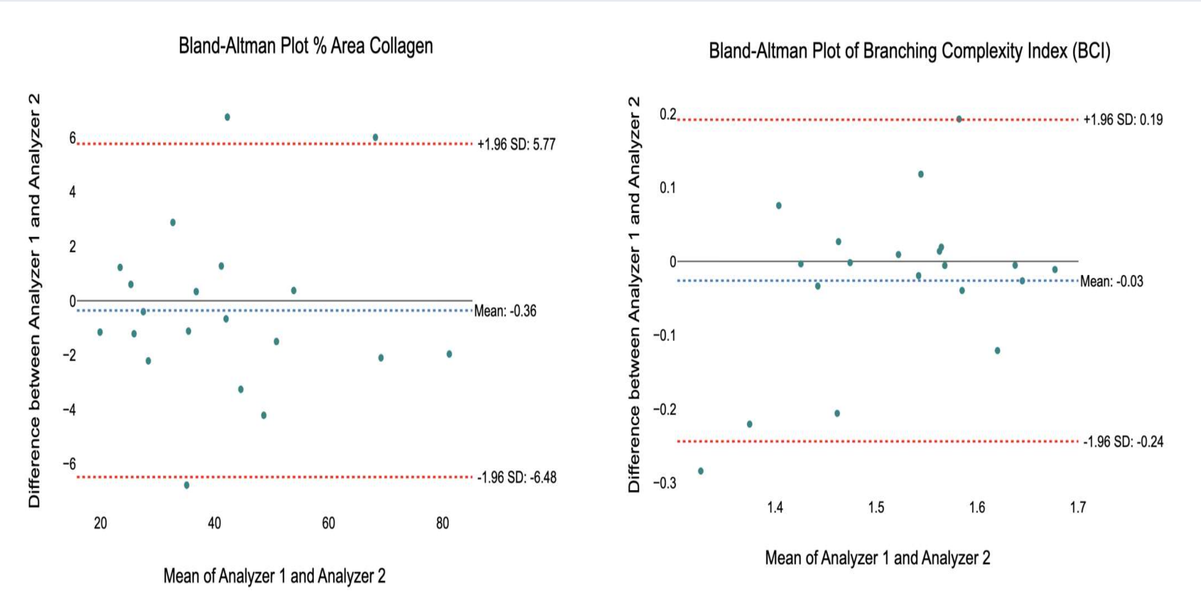

Fig 3. Bland-Altman plots for collagen area (mean diff −0.36; LoA −6.48 to 5.77) and BCI (mean diff −0.03; LoA −0.24 to 0.19), confirming strong inter-analyser reproducibility.

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