Evaluation of Spatial Collagen Morphometry in TNBC versus Non-TNBC Biopsies: A Cross-Sectional SHG/TPEF Imaging and AI-Based Analysis of Tumor and Non-Tumor Regions

Kutbuddin Akbary[1], Li Jiaojiao[1], Ren Yayun[1], Dean Tai[1], Chih-Yang Hsiao[2], Kai-Wen Huang[2]

[1] HistoIndex Pte Ltd, Singapore; [2] Department of Surgery, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

Abstract

BACKGROUND: Triple-Negative Breast Cancer (TNBC) is a heterogeneous and aggressive subtype of breast cancer characterized by the absence of estrogen receptor, progesterone receptor, and HER2 amplification. The tumor microenvironment in breast cancer plays a critical role in disease progression [Acerbi et al. Integr Biol (Camb). 2015 Oct;7(10):1120-34], yet the specific spatial characteristics and morphometric patterns of collagen within different tissue regions remain poorly defined, especially when comparing TNBC and non-TNBC pathologies. Quantitative collagen analysis using second harmonic generation/two-photon excitation fluorescence (SHG/TPEF) imaging and AI-based collagen morphometric evaluation provides an opportunity to characterize fibrosis patterns beyond conventional histopathology. This study aims to delineate and compare collagen morphometric features between tumor and non-tumor regions in TNBC biopsies versus those in non-TNBC biopsies, and to describe region-specific patterns of fibrosis and their similarities and differences.

METHODS: 266 unstained breast cancer biopsy samples acquired from Dept of Surgery, National Taiwan University Hospital were analyzed using SHG/TPEF microscopy and AI analysis. Samples were divided into TNBC (n=21) and non-TNBC (n=245) groups. Collagen morphometric features were identified using a proprietary AI-based analysis using the Genesis®200 scanner (HistoIndex Pte Ltd, Singapore). Four anatomically distinct tissue regions were evaluated: tumor, lobule and duct, stroma-fat, and stroma-fibrosis, with the latter three comprising the non-tumor regions. Normalized Median Relative Differences in feature values were computed to compare collagen parameters between TNBC and non-TNBC groups for each region. Wilcoxon rank-sum test was used to assess statistical significance.

RESULTS: Collagen morphometric analysis revealed region-specific differences between TNBC and non-TNBC biopsies. In tumor regions, collagen parameters were largely comparable between the two groups, with no significant differences detected. In the non-tumor stroma-fibrosis region, some parameters related to collagen fiber distribution showed numerical differences favoring the TNBC group, but these differences did not reach statistical significance. In contrast, the lobule and duct region demonstrated statistically significant differences in multiple collagen morphometric features. Specifically, TNBC biopsies exhibited increased levels of collagen strings (defined as single collagen-connected areas comprising one or more intersecting fibres) and string aggregation features, including those involving various string dimensions such as thick and thin strings. Additionally, several parameters reflecting collagen fiber distribution were significantly elevated in TNBC samples compared to non-TNBC (Figure 1B).

CONCLUSIONS: SHG/TPE-based AI analysis demonstrates that TNBC is associated with a distinct fibrosis profile, particularly characterized by significant alterations in collagen morphometric features within the lobule and duct regions of the non-tumor compartment compared to non-TNBC cases. These region-specific differences in collagen architecture likely reflect underlying desmoplastic remodeling unique to TNBC and may serve as candidate imaging biomarkers for subtype differentiation. Further exploration of these spatial collagen morphometric patterns may enhance understanding of tumor-stroma dynamics and support the development of targeted therapeutic strategies in breast cancer.

Introduction

- TNBC is an aggressive breast cancer subtype lacking ER, PR, and HER2 expression
- TNBC exhibits unique tumor-stroma interactions which can potentially influence disease progression and therapy response
- The extracellular matrix (ECM), particularly collagen organization, plays a key role in cancer cell invasion and mechanical signaling [1]
- Conventional histopathology may not be able to fully capture spatial and morphometric heterogeneity of fibrosis across tissue compartments
- SHG/TPEF microscopy enables stain-free, high-resolution visualization of collagen fibres based on their non-centrosymmetric structure, allowing precise detection of collagen organization, alignment, and density within distinct tissue compartments
- qFibrosis® [2], an AI-driven quantitative analysis framework applied to SHG/TPEF images, measures and quantifies collagen morphometric features (e.g., fiber thickness, aggregation, and orientation) on a continuous numerical scale, providing objective and reproducible assessment of fibrosis heterogeneity
- AIM: To quantify and compare collagen morphometric features in TNBC vs. non-TNBC biopsies using SHG/TPEF imaging and AI-based morphometric analysis, focusing on region-specific fibrosis patterns

Methods and Materials

- **Sample cohort:** 266 unstained breast cancer biopsy samples from NTUH; TNBC (n=21) and non-TNBC (n=245)
- **Imaging platform:** SHG/TPEF microscopy by Genesis®200 (HistoIndex Pte Ltd, Singapore)
- **Tissue zonation:** Four anatomical regions were analyzed, defined based on expert pathologist-guided annotations by AI algorithm (from prior training cohorts):
  a) Tumor region
  b) Non-tumor regions: lobule & duct, stroma-fat, stroma-fibrosis
- **Feature extraction:** AI-based quantification of collagen morphometric parameters (e.g., fiber density, orientation, string metrics)
- **Data Analysis:** Normalized median relative differences for TNBC vs non-TNBC comparison, Statistical test: Wilcoxon rank-sum ($p < 0.05$ considered significant)

Results

- **Comparison between tumor and non-tumor regions:** Collagen parameters similar in TNBC and non-TNBC biopsies (no significant differences)
- **Stroma-fibrosis region:** Numerical trends toward higher collagen distribution heterogeneity in TNBC; not statistically significant
- **Lobule & duct region:**
  a) Significant increase in thickness of collagen strings and string aggregation features in TNBC lobule and duct regions (Figure 1A)
  b) Elevation in multiple collagen distribution parameters (e.g., anisotropy, density variation, Figure 1B & C)
- Indicates enhanced collagen remodeling and altered microstructural alignment in TNBC
- **Overall:** Distinct region-specific collagen profiles in TNBC, most pronounced in non-tumor lobule & duct regions

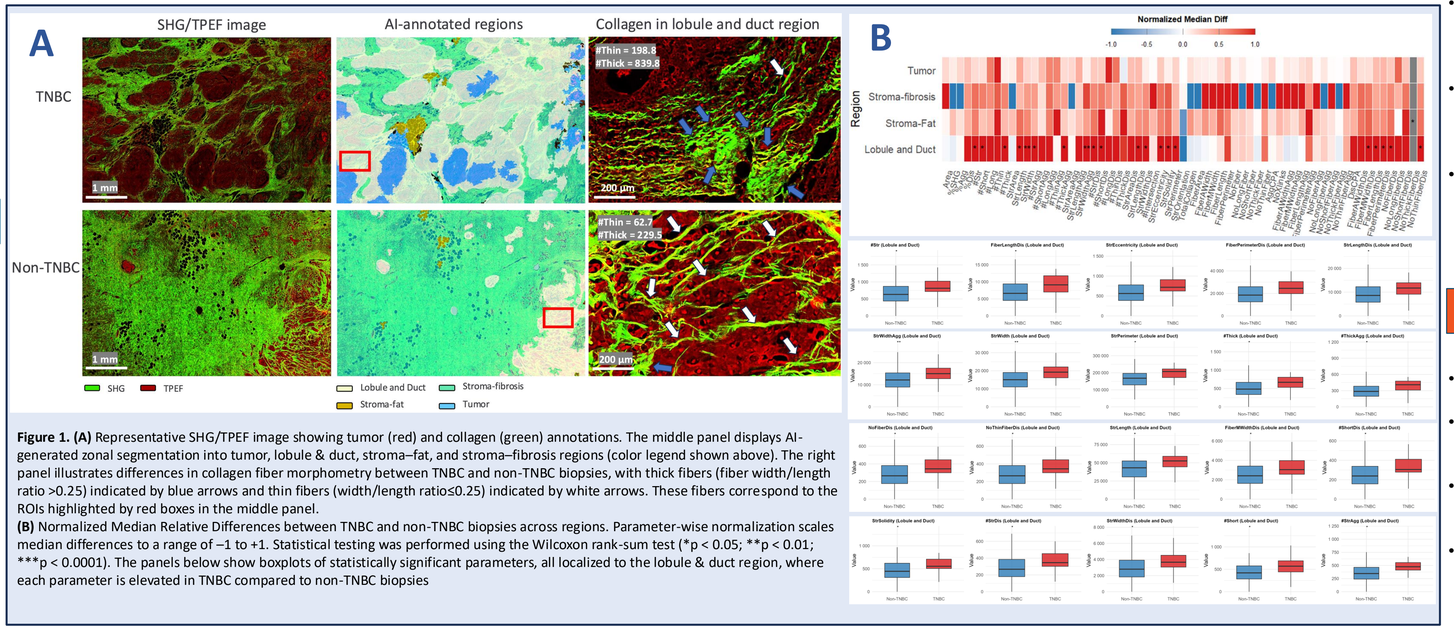


Figure 1. (A) Representative SHG/TPEF image showing tumor (red) and collagen (green) annotations. The middle panel displays AI-generated zonal segmentation into tumor, lobule & duct, stroma–fat, and stroma–fibrosis regions (color legend shown above). The right panel illustrates differences in collagen fiber morphometry between TNBC and non-TNBC biopsies, with thick fibers (fiber width/length ratio >0.25) indicated by blue arrows and thin fibers (width/length ratio≤0.25) indicated by white arrows. These fibers correspond to the ROIs highlighted by red boxes in the middle panel.
(B) Normalized Median Relative Differences between TNBC and non-TNBC biopsies across regions. Parameter-wise normalization scales median differences to a range of –1 to +1. Statistical testing was performed using the Wilcoxon rank-sum test (*p < 0.05; **p < 0.01; ***p < 0.0001). The panels below show boxplots of statistically significant parameters, all localized to the lobule & duct region, where each parameter is elevated in TNBC compared to non-TNBC biopsies

Discussion

- The tumor microenvironment (TME) and ECM are active regulators of breast cancer behavior, influencing cell invasion, migration, and therapy response [3,4]
- The observed region-specific fibrosis differences in this study, particularly in the lobule & duct regions, suggest that TNBC may exhibit ECM remodeling, distinct from the tumor tissue
- Prior studies have shown that collagen alignment and density align with tumor invasiveness and progression [1,5,6]. The increased collagen strings and aggregation features detected in TNBC may represent matrix architectures conducive to invasion/dissemination
- Such stromal collagen morphological patterns in non-tumor regions could also serve as diagnostic aids to conventional pathology in helping to distinguish TNBC vs. non-TNBC phenotypes
- Quantitative collagen parameters derived from qFibrosis® analysis can provide objective, reproducible fibrosis quantification on a continuous scale, facilitating their use as ECM-related imaging biomarkers in breast cancer
- These collagen morphological parameters could also complement emerging ECM- or stroma-targeted drug trials, providing quantitative endpoints to assess treatment response [7]

Conclusions

- TNBC exhibits distinct collagen morphometric alterations localized to the lobule and duct regions
- SHG/TPEF-based AI analysis (qFibrosis®) captures quantitative fibrosis heterogeneity beyond routine pathology
- Region-specific collagen metrics may serve as imaging biomarkers for TNBC subtype differentiation and for monitoring stroma-directed therapeutic responses
- **Future directions:** correlate these collagen morphometric patterns with clinical outcomes to validate prognostic utility

Contact

Dr Kutbuddin Akbary, MD
HistoIndex Pte Ltd, Singapore
Email: akbary.kutbuddin@histoindex.com

References

1. Acerbi I, et al. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. Integr Biol (Camb). 2015 Oct;7(10):1120-34
2. Akbary K, et al. Development of AI Based Fibrosis Detection Algorithm by SHG/TPEF Microscopy for Fully Quantified Liver Fibrosis Assessment in MASH. Liver Int. 2025 Sep;45(9):e70258
3. Pickup MW, et al. The extracellular matrix modulates the hallmarks of cancer. EMBO Reports. 2014;15(12):1243–1253.
4. Levental KR, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell. 2009;139(5):891–906
5. Provenzano PP, et al. Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC Medicine. 2006;4:38
6. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Disease Models & Mechanisms. 2011;4(2):165–178
7. Prakash J, Shaked Y. The Interplay between Extracellular Matrix Remodeling and Cancer Therapeutics. Cancer Discov. 2024 Aug 2;14(8):1375-1388

This presentation is the intellectual property of the author/presenter. Contact them at [akbary.kutbuddin@histoindex.com] for permission to reprint and/or distribute.

© Poster Template by Genigraphics® 1.800.790.4001 www.genigraphics.com