Abstract #3370

Quantitative multimodal anisotropy imaging enables automated fibrosis assessment of H&E-stained tissue

STUDY BACKGROUND

Non-alcoholic steatohepatitis (NASH) disease severity is graded by Clinical Research Network (CRN) histologic scoring using two differently stained tissue sections per tissue block (1). To visualize and grade steatosis, inflammation, and ballooning, one tissue section must be stained with hematoxylin and eosin (H&E) and, to stage fibrosis, the other tissue section must be stained with Masson's Trichrome (MT). The necessity for two differently-stained tissue samples to assess each patient at each time point is cumbersome, requires more tissue and resources than a single section, and may introduce interpretability challenges due to variability in staining and intra-biopsy sample heterogeneity (2,3)

Here, we report the development of quantitative multimodal anisotropy imaging (QMAI) which can highlight structured substances like collagen in tissue and apply this to visualize fibrosis in H&E-stained liver tissue sections from patients with NASH. QMAI performance was evaluated by comparison to MT-stain color-based fibrosis quantification.

METHODS

Hardware: An Olympus BX63 microscope was modified to enable multispectral, polarization, and quantitative phase imaging, in combination facilitating Quantitative Multimodal Anisotropy Imaging (QMAI; **Figure 1**).

Figure 1. Overview of Multiple Imaging Modalities Required for QMAI

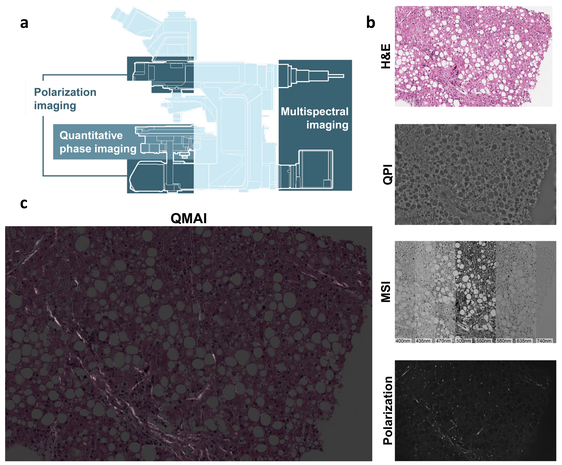


Diagram of QMAI setup highlighting modified microscope modules (**a**); Example system images (**b**) used to generate a QMAI fibrosis overlay (**c**).

Imaging: Tissue from 24 NASH liver core biopsies (CRN fibrosis stages 0-4) was sectioned and stained with hematoxylin and eosin (H&E)- and trichrome (MT; N=48 slides) at PathAI Diagnostics (Memphis, TN). H&E slides were imaged using the QMAI microscope. MT slides were imaged using a Leica Aperio AT2 scanner.

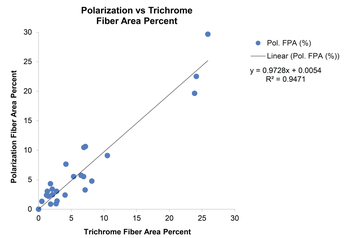
METHODS

Fibrosis Quantification and QMAI Validation: All WSIs were screened for quality by a board-certified expert NASH pathologist. Regions of tissue artifact were excluded from analysis for each WSI and the associated QMAI H&E image. A pixel-intensity threshold was applied to QMAI images of H&E sections to locate fibrotic regions and compute fibrosis proportionate area (FPA). For MT-stained WSIs, color-based segmentation was employed to obtain a binarized fibrosis heatmap from which FPA was quantified. For all tissue sections, a background/foreground algorithm generated the denominator for FPA measurements. Agreement between QMAI H&E- and MT-derived FPA was evaluated via Lin's Concordance.

RESULTS

FPA computed from MT images vs. via QMAI of H&E images were strongly concordant in analysis of paired images from the same biopsy (Lin's CCC = 0.950, 95% CI: [0.889, 0.978]; **Figure 2**).

Figure 2. Comparison of FPA Derived from QMAI and MT Imaging Methods

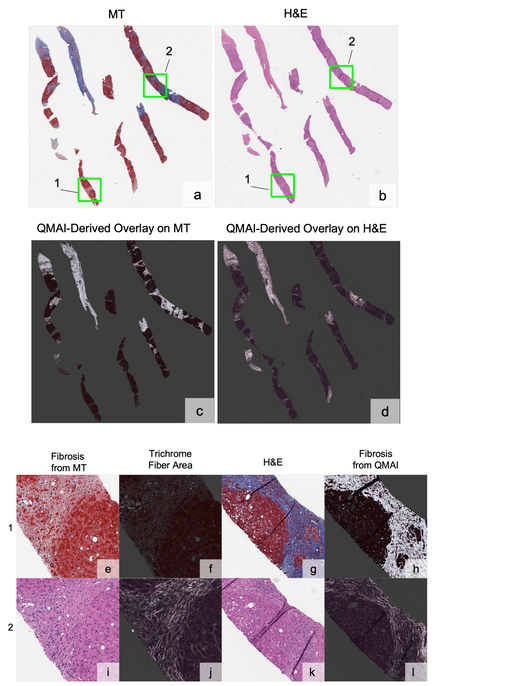


Visual examination of fibrosis overlays from QMAI of H&E slides and blue/red contrast of MT stain shows broad qualitative concordance between regions of fibrosis detected in paired H&E/MT sections (**Fig. 3c-d**).

Examination of regions where there is apparent visual discordance (**Fig. 3e-f**) suggests that discrepancies between QMAI-derived FPA and color-based segmentation of MT may be attributable to inter- and intra- slide MT stain variability, resulting in over-/under-call of fibrosis.

RESULTS

Figure 3. Comparison of collagen fiber detection using MT- and QMAI-based imaging



Examples of collagen fiber detection using MT blue-detection vs. QMAI. Biopsy sections were stained with MT (**a**) or H&E (**b**), and visualized alongside corresponding blue-detection (**c**) and QMAI (**d**) fibrosis overlays. Two regions of interest (1 and 2) from these samples are shown at high magnification in **e-l**.

CONCLUSIONS

Quantitative multimodal anisotropy imaging enables fibrosis quantification from H&E-stained NASH biopsy samples that is consistent with conventional fibrosis quantification from MT-stained tissue. By eliminating the need for MT-stained slides, automated fibrosis quantification and staging from H&E sections may simplify and reduce the cost of NASH studies as well as lessen the impact of MT stain-variability and intra-sample heterogeneity on subject screening and endpoint assessment. Additionally, detecting fibrosis from H&E sections may enable improved interrogation of spatial relationships between fibrosis and various NASH features commonly assessed on H&E (e.g., steatosis, ballooning, and/or inflammation).

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