

QUANTIFICATION OF COLLAGEN IN SECOND HARMONIC IMAGING OF LIVER FIBROSIS

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The excessive accumulation of extracellular matrix proteins such as collagen is a symptom of liver fibrosis in most chronic hepatic diseases which inevitably leads to liver failure [1]. The relationship of the degree of liver fibrosis and the amount of collagen in the liver slices is therefore an important indicator in determining the phase of liver fibrosis. At present, biopsy were undertaken and scoring of collagen has been subjective to the experience of the patho-physiologist in determining the extent of liver fibrosis as collagen was counted through a staining technique under a light microscope based primarily on a qualitative description of the morphological features of the specimen [2].

In our study, we have developed a fully automated algorithm for quantitative evaluation of collagen distribution. In particular, we have made use of both second harmonic generation (SHG) and two-photon excited fluorescence (TPEF) images to identify the collagen as oppose to using SHG results only. TPEF images contain information such as cell types and cell morphology. With prior knowledge about the location of collagen in some particular cell types, such as bile duct cells or around the vessel wall, we added weight to these regions in the SHG signal. Following the weight adjustment, we used automated segmentation algorithm to identify collagen. In contrast to existing algorithms, this program seeks to quantify not only the aggregated collagen around the portal regions and distributed collagen but collagen in the bile canaliculi as well and will provide us with a collagen index (Fibro-C-index) of the diseased tissues. The enhancement is critical in that the collagen present in the bile canaliculi, though minute in intensity are numerous in occurrence, would have been disregarded after thresholding and reflect a smaller contribution to the overall amount of collagen, hence, a false representation of the extent of fibrosis. Results have shown a significant improvement in collagen identification, and hence, a more accurate quantitative estimate of collagen progression in diseased livers. We have thus shown that the Fibro-C-index provides an accurate, sensitive and linearly correlated measure of fibrosis development which would be important to clinicians in their diagnoses of liver failing patients.

[1] R. Bataller and D. A. Brenner, "Liver Fibrosis" *The Journal of Clinical Investigation* **115**, 209 (2005)

[2] Z. D. Goodman, "Grading and staging systems for inflammation and fibrosis in chronic liver diseases" *Journal of Hepatology* **47**, 598 (2007)