

Standardized quantification on liver fibrosis using second harmonic generation and two-photon microscopy

Tai DCS¹, Tan N^{2,4}, Kang CH¹, Chen CL³, Chia SM⁴, Xiao GF⁴, Sun WX¹, and Yu H^{1,4}

¹Institute of Bioengineering and Nanotechnology, A*STAR, The Nanos, Singapore

²Department of Paediatrics, KK Women's and Children's Hospital, Singapore

³Department of Pathology, National University Hospital, Singapore

⁴Department of Physiology, Yong Loo Lin School of Medicine, Singapore

E-mail: dtai@ibn.a-star.edu.sg

KEY WORDS: Liver fibrosis, Collagen, Second harmonic generation, Two-photon microscopy

1. BACKGROUND

Determining the extent of liver fibrosis has clinically been difficult due to the lack of a simple, objective method that can accurately quantify the amount of collagen in the diseased tissue. Second harmonic generation (SHG) microscopy has been shown to produce bright and robust signals from non-centrosymmetric fibrillar collagen. We designed a SHG system that can objectively quantify liver fibrosis in an animal model in an efficient, standardized and reproducible manner.

2. METHOD

SHG and two-photon microscopies were performed on livers harvested from bile duct ligated Wistar rats using a confocal microscope with a mode-locked Ti:Sapphire laser. Images acquired were later analyzed with a custom-developed algorithm, producing a collagen index (Fibro-C-index).

3. RESULTS

A linear correlation between Fibro-C-index and the amount of tissue type I collagen was obtained. Fibro-C-index results agree with pathologist scoring closely, which validates our quantification approach. Furthermore, using the Fibro-C-index, early fibrosis (score 1), undifferentiable by light microscopy, showed up to 400% difference in levels of collagen.

4. CONCLUSION

Fibro-C-index provides sensitive measurement for liver fibrosis and accurately reflects the progression of liver fibrosis, especially in early stages. Fibrosis development is easily quantified using a stain-free technique and a fully-automated algorithm, and is shown to be a standardized index system.