Concurrent Semi-Automated Quantification of Hepatic Fibrosis and Steatosis using Label-Free Second Harmonic Generation Imaging in Animal Models

Li Chen[1], Joel Schwartz[2], Mathieu Petitjean[1]

[1] Genesis Imaging Services, PharmaNest, Princeton, NJ, USA

[2] Schwozny Analytics, Amesbury, MA, USA

Genesis Imaging Services

ABSTRACT

Background

Nonalcoholic fatty liver disease (NAFLD) is a prevalent liver disease that can progress from a simple fatty liver (hepatic steatosis) to a more advanced stage nonalcoholic steatohepatitis (NASH) with fibrosis. The current standard to assess liver fibrosis and steatosis are conventional staining and histopathological criteria scoring. This assessment has limitation as it uses a narrow range scoring, it is prone to observer variations, and requires multiple histopathology workflows and stains. Here we use second harmonic generation (SHG) and two-photon excitation fluorescence (2PE) imaging, combined with machine-learning image analysis algorithms, to provide a novel, sensitive, and efficient method for concurrent quantification of fibrosis (SHG) and fat droplets (2PE) from a single stained-free histological tissue section.

Method

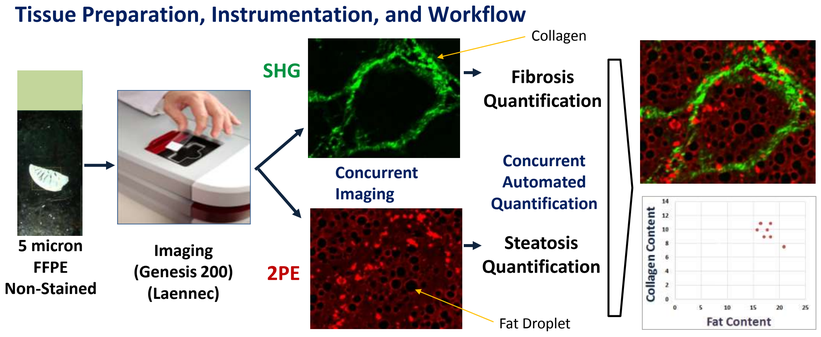
In this study, leptin- deficient mice (lep $^{0b/0b}$) are feed with normal chow or high trans-fat diet with or without therapeutic drug treatment. A modified ALIOS diet is used to induce NASH. FFPE liver histological section (5μm) slides were imaged (without staining nor labels) using Genesis 200®, a nonlinear optical imaging system, which combines SHG (780nm in transmission) to produce collagen specific images (collagens I and III) and 2PE (550+/-88nm) to delineate tissue morphology. Because fat do not produce intrinsic fluorescence and due to its morphology and size, fat droplets shown as circular dark objects can be segregated from tissue and quantitated. It can also be distinguish from vasculature due to the absence of hemoglobin and surrounding collagen. We report results on metrics for fibrosis including collagen content, collagen reticulation index, and collagen fiber density, and for steatosis including fat content, droplet size, and morphometrics.

Results and Conclusion

The study result revealed that modified ALIOS diet induces NASH and fibrosis in leptin-deficient mice and this condition is ameliorated with bile acid receptor agonist therapeutic drugs. SHG/ 2PE imaging is a novel stained-free imaging technique combined with a robust image analysis system to provide an automated and fully quantitative extraction of information for liver fibrosis and steatosis. This sensitive technology can help trace the development, progression, and reversal of NAFLD from simple steatosis to NASH with fibrosis.

METHOD

Tissue Preparation, Instrumentation, and Workflow



- 5-200uM FFPE or Frozen sections
- Stain-Free and Label- Free imaging
- Fully Quantitative collagen & fat
- High Resolution (0.4 uM @ 20x)
- Non-destructive (tissue can be save for further study)
- Two-Photon Excitation (2PE): auto fluorescence for tissue morphology depict cellular structure and injury
- Second Harmonic Generation (SHG): specific for collagens I and III
- Machine- learning Image Analysis Software optimized and validated with pathologists

Amylin Liver NASH Model

Leptin-deficient (lep $^{0b/0b}$) mice were allowed ad libitum access to normal chow (low-fat diet w no fructose nor cholesterol) or to modified ALIOS diet (high trans fat (40%), fructose (22%), cholesterol (2%) in food pellets) with or without therapeutic drugs.

Group 1	Group 2	Group 3	Group 4
Chow	NASH	NASH + *Drug A	NASH + *Drug B

*Drugs A and B are investigational drugs (OCA and INT-767, respectively) provided by Intercept Pharmaceutical which are currently in clinical trials to treat patients with NASH and liver fibrosis. OCA (bile acid) is a FXR agonist. INT-767 is a dual FXR and TGR5 (bile acid receptors) agonist.

Analysis Parameters

1. Histological comparison of SHG/2PE images to conventional staining images

2. Fibrosis

-Collagen Content: collagen area%
-Collagen Reticulation Index: measures collagen fiber network
-Collagen Fiber Density: collagen density within fiber
(intensity directly proportional to amount collagen within fiber)

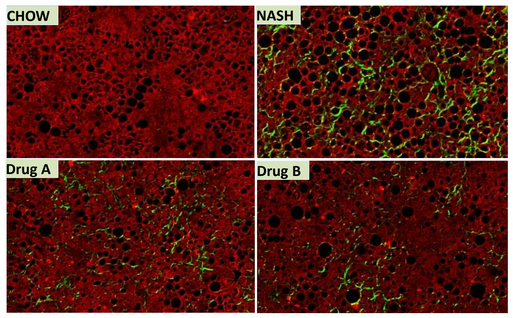
3. Steatosis

-Fat Content: fat area%
-Fat Droplet Size: average area per droplet (pixel)
-Fat Droplet morphometrics

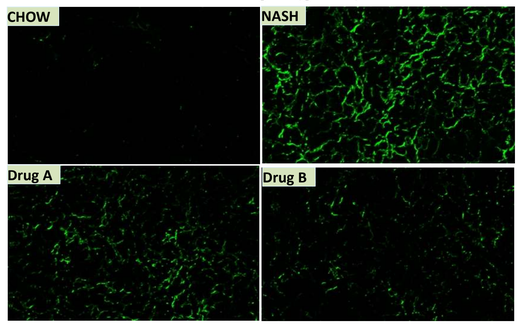
RESULT

Figure 1. Modified ALIOS diet-feed mice induces hepatic fibrosis and steatosis in histological sections.

A. SHG/ 2PE livers. Collagen (green), Tissue (red), fat droplets (black)



B. SHG Livers. Perisinusoidal collagen (green)



C. Quantitation of collagen, collagen structure network, and collagen fiber density.

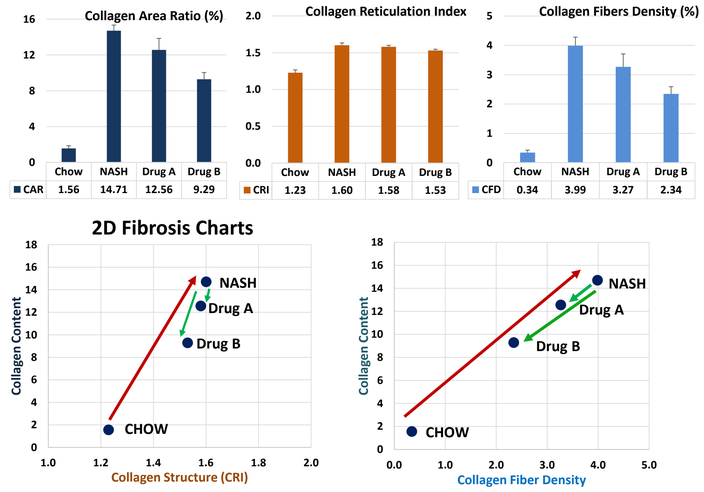
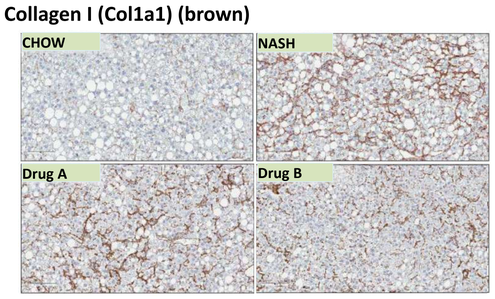
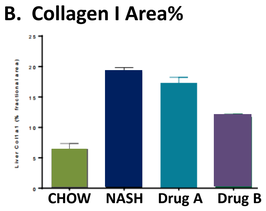


Figure 2. @ Modified ALIOS diet-feed mice induces steatosis and fibrosis in histological sections. Conventional staining for comparison.

A. Collagen I (Col1a1) (brown)



B. Collagen I Area%



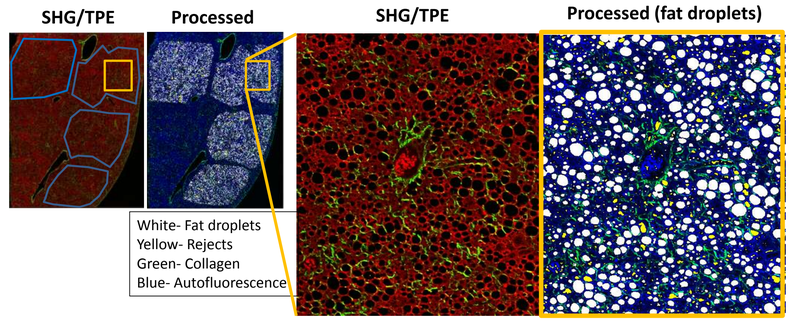
C. Histopathological Scoring (group average)

	Steatosis	Inflammation	Fibrosis	NAS
Chow	1.3	0	0	1.3
NASH	3.0	2.5	2.8	6
Drug A	1.5	2.3	1.8	4.5
Drug B	1.5	2.5	1.3	4

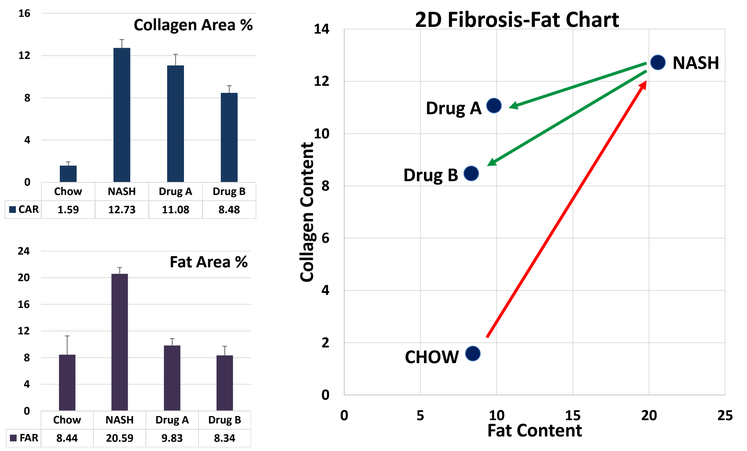
@ Data from conventional method is provided by Intercept

Figure 3. Automated image analysis for concurrent fibrosis and steatosis quantitation

A. Selection of ROI



B. Simultaneous quantitation of Collagen and Fat Area%



C. Fat Morphometric

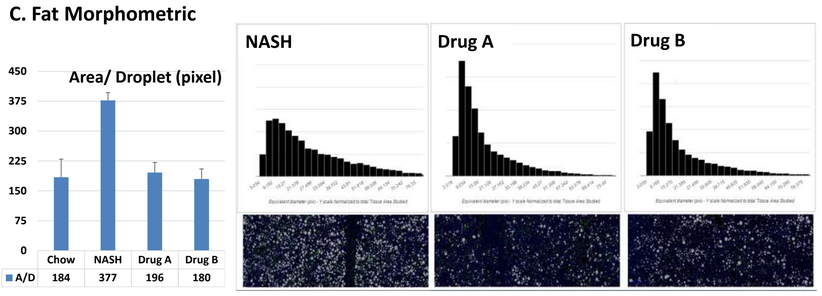
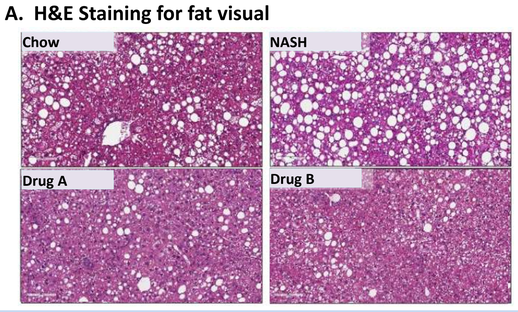
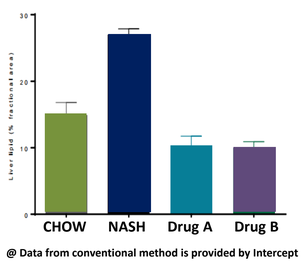


Figure 4. @Conventional histological staining for comparison.

A. H&E Staining for fat visual



B. Fat Area%



@ Data from conventional method is provided by Intercept

CONCLUSION

- SHG/2PE technology is a sensitive stain-free novel imaging technique combined with automated concurrent quantitation of fibrosis and steatosis. This provides a tool to assess across various stages of NAFLD from simple hepatic steatosis to fibrosing NASH.
- In addition to basic metrics such as collagen and fat measurements, we offer unique metrics including collagen fiber density and collagen structure network, and fat droplet morphometric for a more in depth understanding of both hepatic fibrosis and steatosis development.
- Due to the nature of the technology, this stain-free SHG/2PE imaging and automated image analysis allow for a reliable and quick turnaround time for data results.