# New Standards in Fibrosis Quantitation: The Value of Label-Free Second Harmonic Generation Imaging


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## Background

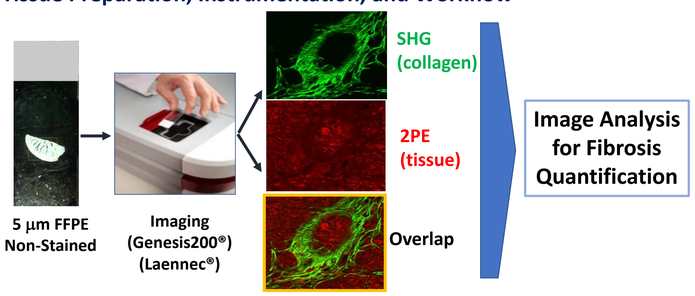
Fibrosis is associated with excessive accumulation of extracellular matrix in response to persistent injury, inflammation, and abnormal wound healing. The current standard to assess fibrosis are conventional staining and histopathological criteria scoring. This assessment has limitation as it uses a narrow range scoring (when scoring exists), it is prone to observer variations, and requires multiple histopathology workflows and stains.

- Here, we use second harmonic generation (SHG) together with two-photon excitation fluorescence (2PE) imaging and computerized image analysis algorithms to provide a novel, sensitive, and efficient method for collagen quantitation. We extract information on the collagen fibers in various animal models to assess fibrosis as it advances or regress in response to therapeutic compounds.
- We present animal models including NASH livers, IPF lungs and UUO kidneys treated with or without reference anti-fibrotic drugs including Nintedanib and Pirfenidone.
- In addition to basic quantifiable metrics including total collagen, we offer novel analysis capabilities that conventional method are not able to provide such as tissue regional segmentation (for livers, lung, kidney), collagen network structure, collagen fiber density, etc. These metrics maybe the key to better fibrosis scoring and staging.
- 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.


*We thank GenScript and Intercept Pharmaceuticals for providing the conventional staining images and respective graphs. We thank HistoIndex and A*STAR (Singapore) for their assistance in the image analysis of the IPF rodent model images.


## METHOD

### Tissue Preparation, Instrumentation, and Workflow




- 5-200µM FFPE or Frozen sections
- Stain-Free and label-free imaging
- Fully quantitation of collagen fibers
- High Resolution (0.39um @ 20X)
- Non-destructive (tissue re-usable)
- 2-Photon Excitation (2PE): auto fluorescence to delineate tissue morphology and injury
- Second Harmonic Generation (SHG): Collagens I and III
- 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.

### IPF Lung Model

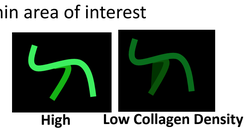
C57BL/6 mice were subjected to intratracheal instillation of bleomycin for induction of lung epithelial injury, inflammation, and fibrosis. Pirfenidone or Nintedanib was given 7days after start of bleomycin (21days study).

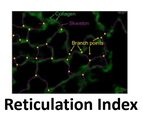
### UUO Kidney Model

SD rats were subject to surgical ligation of ureter of left kidney, while leaving the right contralateral kidney as control. This induces progressive epithelial injury, tubulointerstitial inflammation and fibrosis of the left kidney. Pirfenidone is given 7 days after start of ureter unilateral obstruction (UUO) (28 days study).

| Models | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| NASH Livers | Chow | NASH | NASH + OCA | NASH + Drug A |
| IFP Lungs | Saline | Bleomycin (BLM) | BLM + Nintedanib (NTN) | BLM+ Pirfenidone (PFD) |
| UUO Kidney | Sham | UUO | UUO+PFD | |

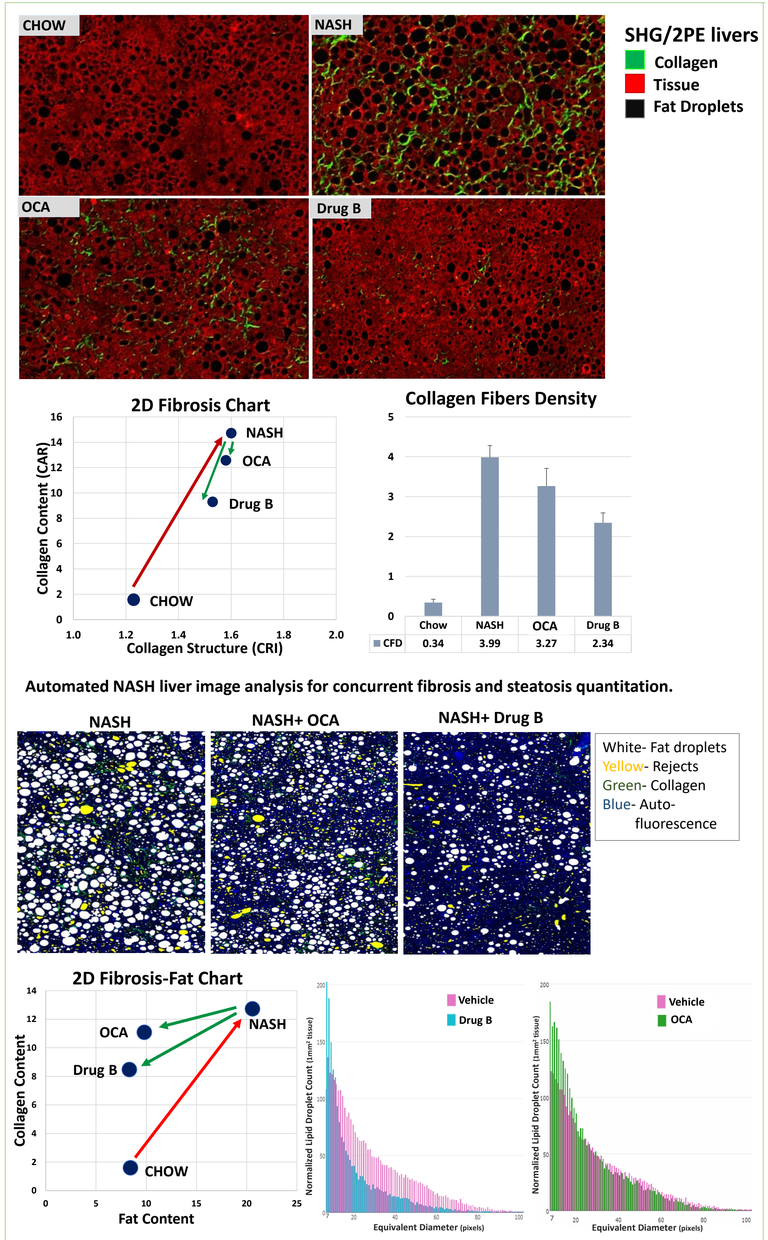
- Collagen Area Ratio (CAR): % collagen area within area of interest
- Collagen Reticulation Index (CRI): measures complexity of the collagen fiber network
- Collagen Fiber Density (CFD): collagen density within fiber based on pixel intensity
- Fat area Ratio (FAR): % fat area within area of interest




## RESULT

**Figure 1. Modified ALIOS diet-feed mice induces hepatic fibrosis and steatosis treated with or without obeticholic acid (OCA) or Drug B.**




**Automated NASH liver image analysis for concurrent fibrosis and steatosis quantitation.**







**Figure 2. Modified ALIOS diet-feed mice induces steatosis and fibrosis. Conventional staining for comparison. Collagen Ia1(brown)**

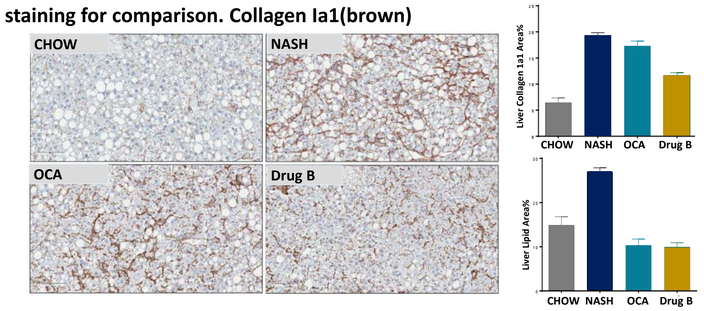



**Figure 4. Bleomycin induced lung fibrosis and treated with or without Nintedanib or Pirfenidone**

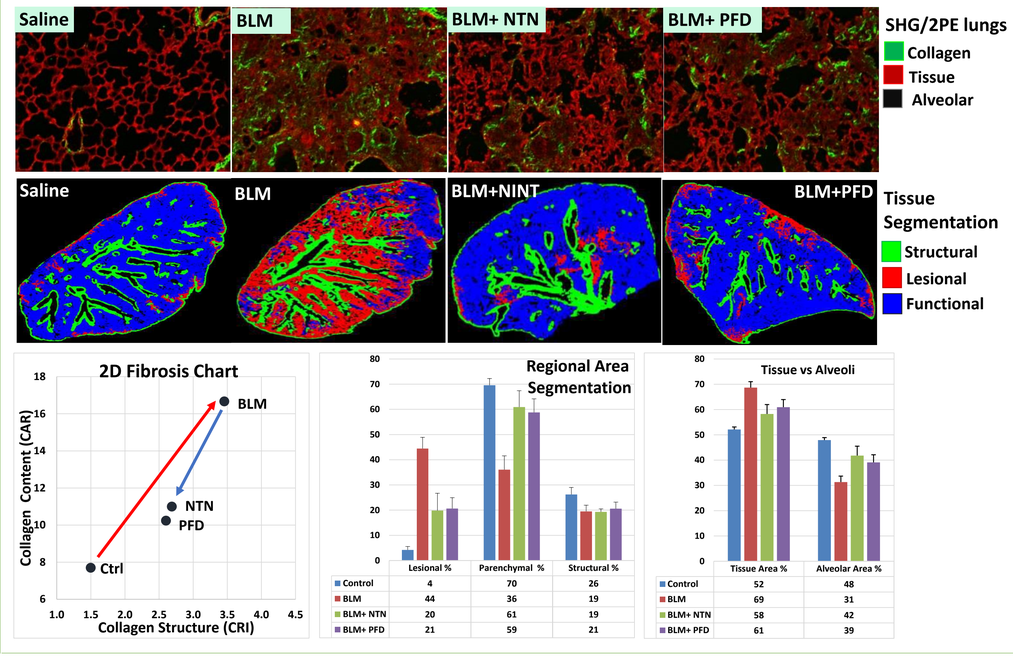
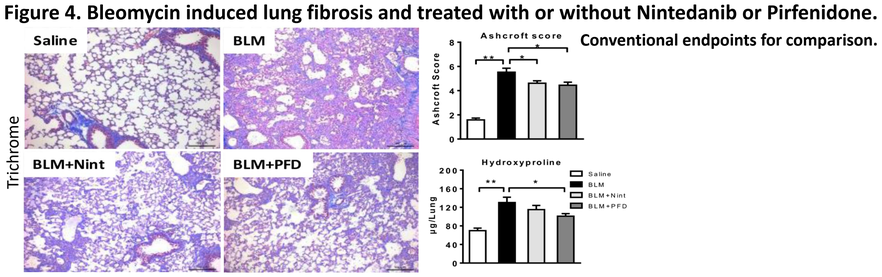



**Figure 4. Bleomycin induced lung fibrosis and treated with or without Nintedanib or Pirfenidone.**




Conventional endpoints for comparison.

**Figure 5. UUO induced kidney fibrosis in rat treated with or without Pirfenidone**

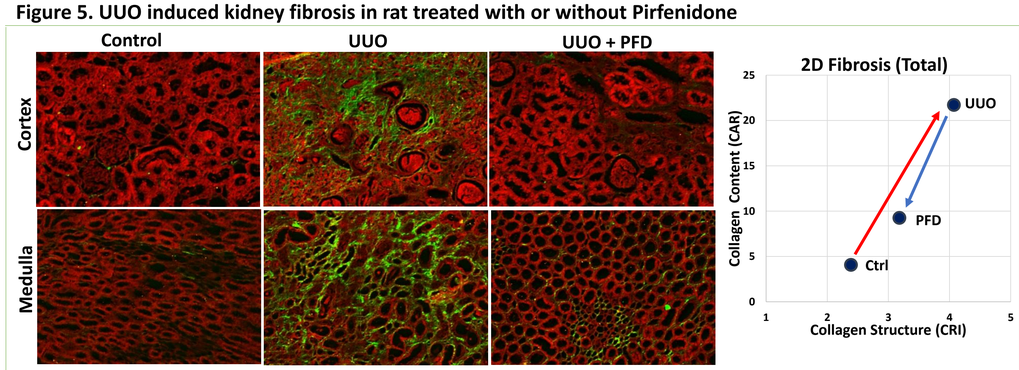
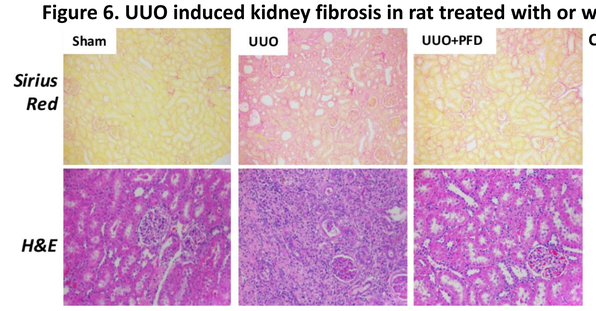



**Figure 6. UUO induced kidney fibrosis in rat treated with or without Pirfenidone.**




Conventional endpoints for comparison.