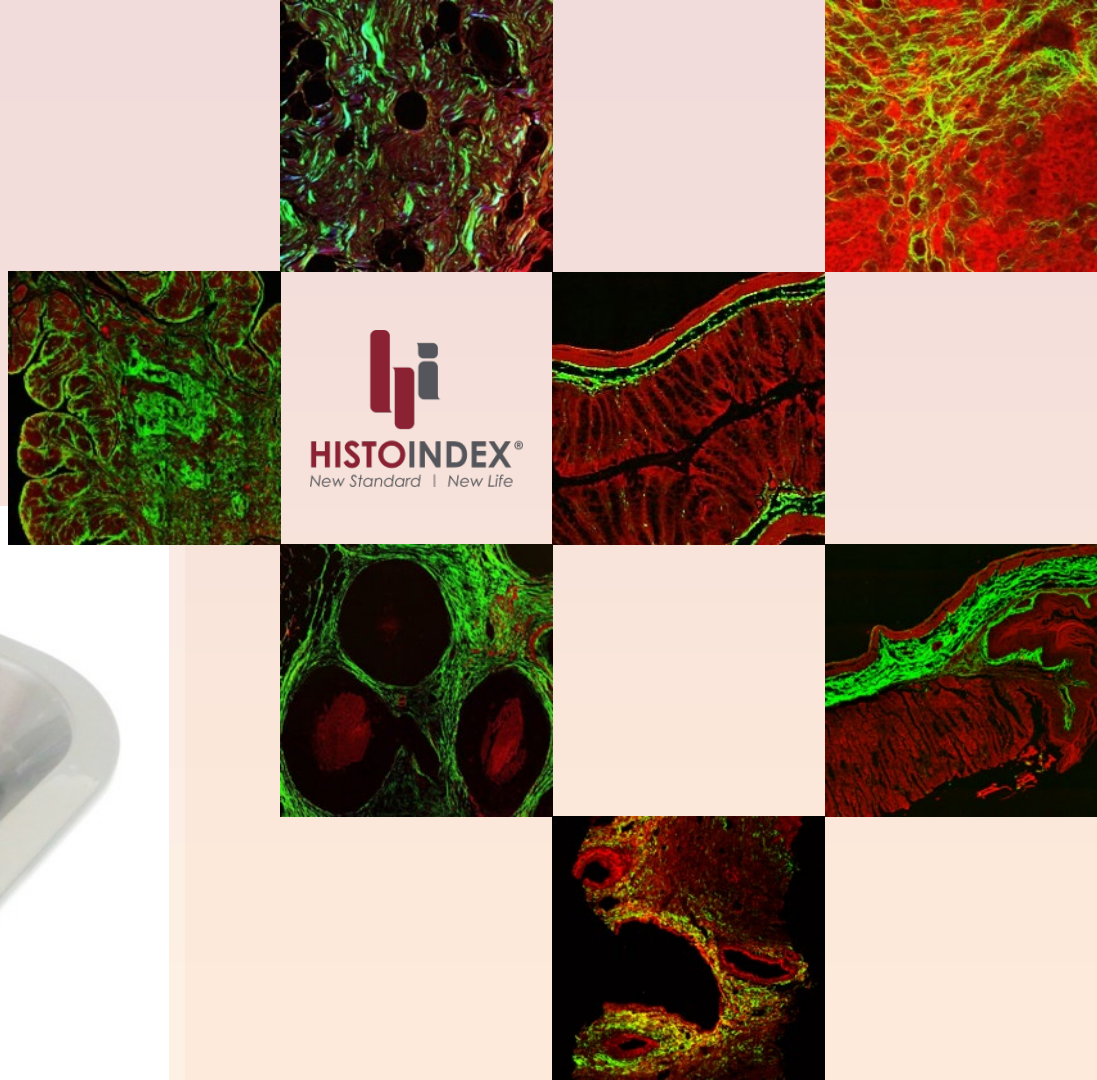
AI-based Digital Pathology in NASH Intervention Efficacy Evaluation



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Challenge of Biopsy #1: Subjective and Semi-quantitative Assessment

*Second Harmonic Generation Microscopy Provides **Accurate Automated** Staging of Liver Fibrosis in Patients with Non-alcoholic Fatty Liver Disease (2018)*

*Chang PE, Goh GBB, Leow WQ, Shen L, Lim KH, et al. **PLOS ONE** 13(6)*

*Improved **Quantitative Assessment** of HBV-associated Liver Fibrosis Using Second-harmonic Generation Microscopy with Feature Selection (2019)*

*Hsiao CY, Teng X, Su TH, Lee PH, et al. **Clin Res Hepatol Gastroenterol***

Challenge of Biopsy #2: Sampling Error

*SHG/TPEF-based Image Technology Improves Liver Fibrosis Assessment of **Minimally Sized Needle Biopsies** (2019)*

*Wang, B., Sun, Y., Zhou, J. et al. **Hepatology International** 13:501–509*

*Quantifying and Monitoring Fibrosis in **Non-Alcoholic Fatty Liver Disease** Using Dual-Photon Microscopy (2019)*

*Wang Y, Wong GL-H, He F-P, Sun J, Chan AW-H, Yang J, Shu SS-T, Liang X, Tse Y, Fan X-T, Hou J, Chan HL-Y, Wong VW-S. **GUT** 2019; 0:1-11.*

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Challenge of Biopsy #3: Discrete NAS Scores & Fibrosis Stages Giving Rise to Inter- & Intra- Observer Variability

Quantification of Hepatic Steatosis *in Chronic Liver Disease Using Novel Automated Method of Second Harmonic Generation and Two-photon Excited Fluorescence (2019)*

George Boon-Bee Goh, Wei Qiang Leow, Shen Liang, Wei Keat Wan, Tony Kiat Hon Lim, Chee Kiat Tan & Pik Eu Chang. ***Scientific Reports*** *2019; 9(2975).*

Dual-Photon Microscopy-Based ***Quantitation of Fibrosis-Related Parameters (q-FP)*** *to Model Disease Progression in Steatohepatitis (2017)*

Yan Wang, Robert Vincent, Jinlian Yang, Amon Asgharpour, Xieer Liang, Michael O. Idowu, Melissa J. Contos, Kalyani Daitya, Mohammed S. Siddiqui, Faridoddin Mirshahi, and Arun J. Sanyal. ***Hepatology*** *2017; 65(6):1891-1903. doi: 10.1002/hep.29090*

Challenge of Biopsy #4: Not Linked to Outcome

Quantitative Assessment of Liver Fibrosis (qFibrosis) ***Reveals Precise Outcomes*** *in Ishak Stable Patients on anti-HBV therapy (2018)*

Yameng Sun, Jialing Zhou, Xiaoning Wu, Yongpeng Chen, Hongxin Piao, Lungen Lu, Huiguo Ding, Yuemin Nan, Wei Jiang, Tailing Wang, Hui Liu, Xiaojuan Ou, Aileen Wee, Neil D. Theise, Jidong Jia, Hong You. ***Scientific Reports*** *2018; 14;8(1).*

Advanced Septa Size Quantitation Determines the Evaluation of Histological ***Fibrosis Outcome*** *in Chronic Hepatitis B Patients (2018)*

Bingqiong Wang, Yameng Sun, Jialing Zhou, Xiaoning Wu, Shuyan Chen, Shanshan Wu, Hui Liu, Tailing Wang, Xiaojuan Ou, Jidong Jia & Hong You. ***Modern Pathology*** *2018; 31(10):1567-1577.*

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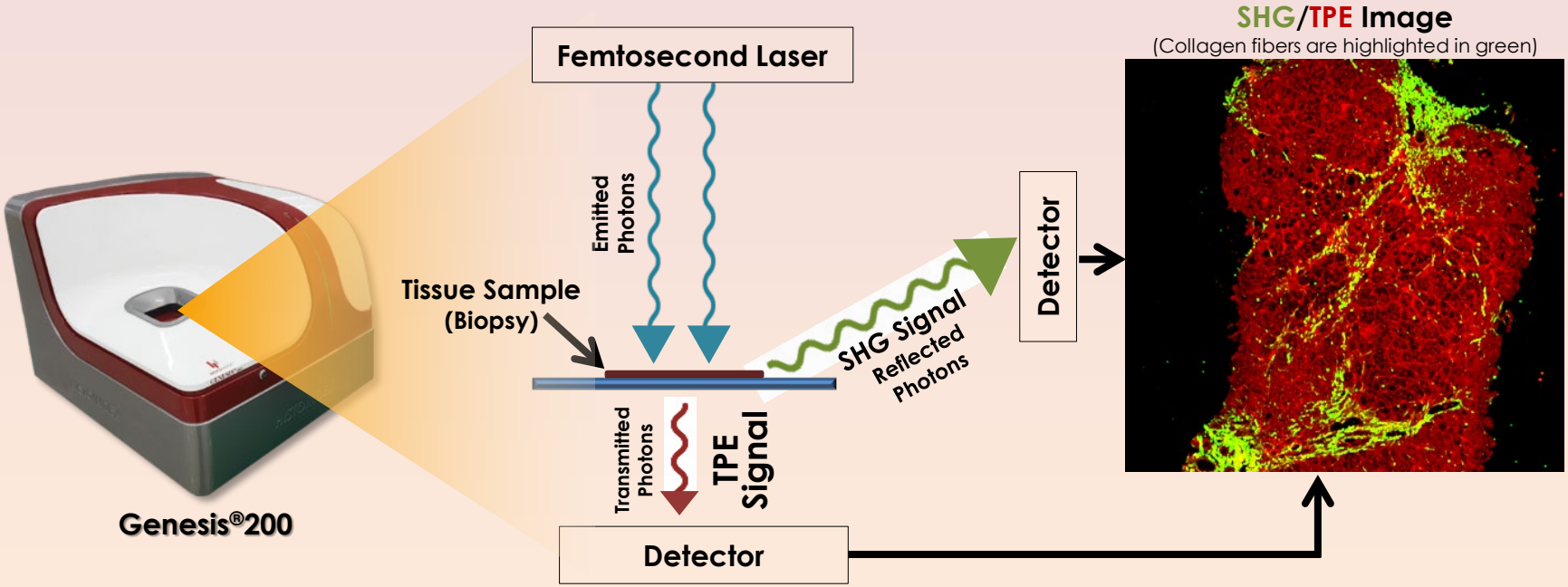
Stain-free Imaging Technology with Digital Pathology Reveals Fine Details for the FIRST TIME



Reference: Sun W, Chang S, Tai D C, et al. Nonlinear Optical Microscopy: Use of Second Harmonic Generation and Two-photon Microscopy for Automated Quantitative Liver Fibrosis Studies[J]. Journal of Biomedical Optics, 2008, 13(6):7-0.

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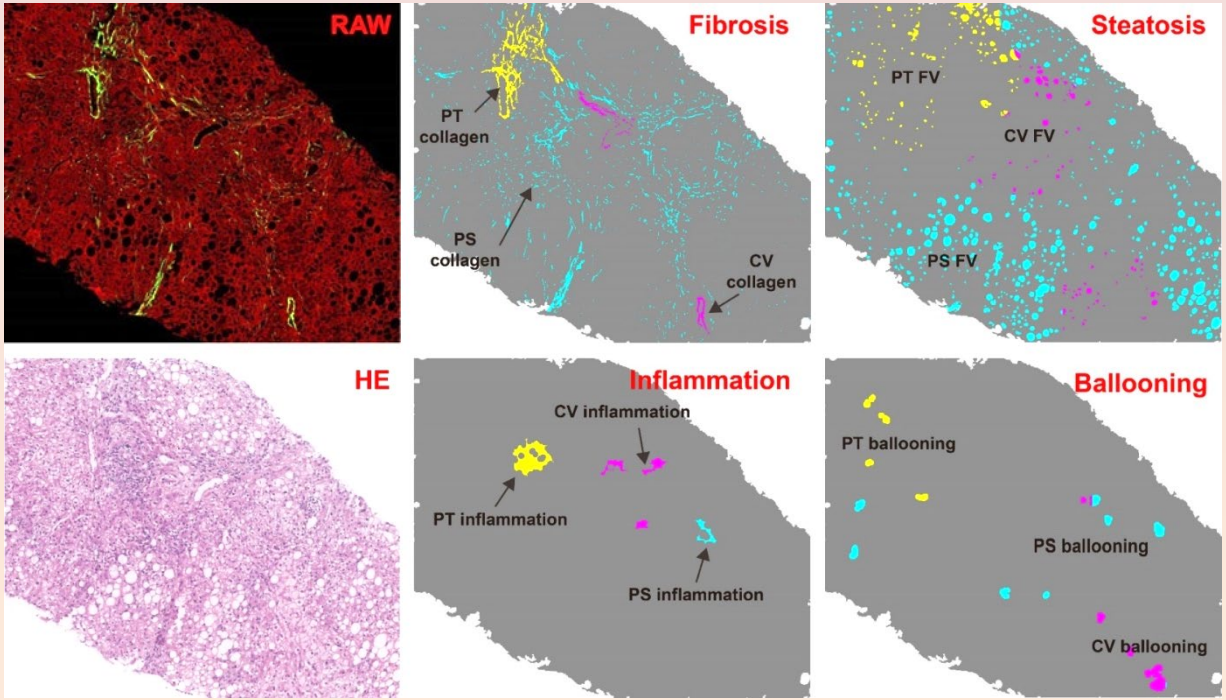
Platform for Mechanism of Action Analysis: Fibrosis, Inflammation, Ballooning and Steatosis on the Same Slide



PT – Portal tract CV – Central Vein PS – Perisinusoidal FV – Fat vacuole

***qFIBS**: A Novel Automated Technique for **Quantitative Evaluation of Fibrosis, Inflammation, Ballooning, and Steatosis** in Patients with Non-alcoholic Steatohepatitis (2020)*
*Liu F, Boon-BeeGoh G, Tiniakos D, Wee A, Leow W, Zhao JM, Rao HY, Wang X, Wang Q, Wan WK, Lim KH, Romero-Gomez M, Petta S, Bugianesi E, Tan CK, Harrison SA, Anstee QM, Chang PJ, Wei L. **Hepatology 71(6)**, p1953-1966, June 2020.*

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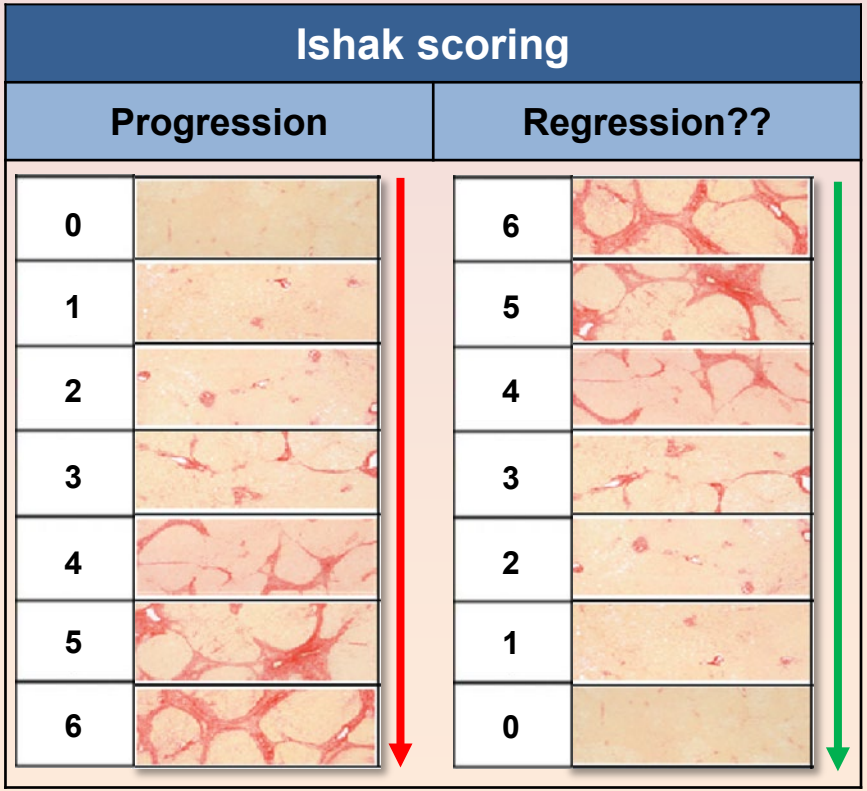
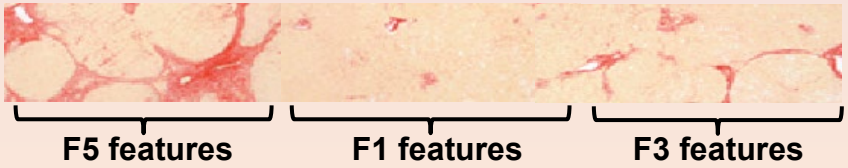
Challenge #1: Subjective and Semi-Quantitative Assessment



Ishak et al. J Hepatol. 1995

But this is what we see in post-intervention biopsies



Not explained in the system
→ Subjective interpretation on semi-quantitative system

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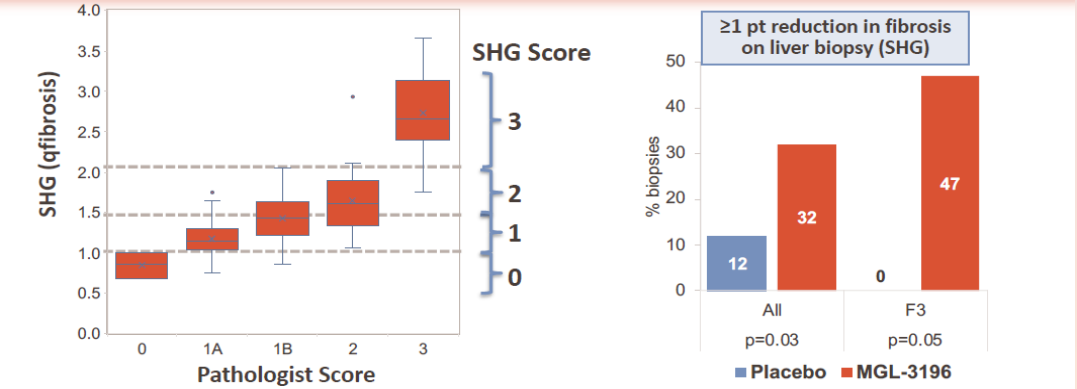
qFibrosis quantifies all features described in NASH-CRN system simultaneously and consistently

Week 36: Change in Fibrosis Score on Liver Biopsy



- Second Harmonic Generation (SHG) microscopy provides automated fully quantitative assessment of fibrosis on liver biopsy slides based on unique architectural features of collagen
- SHG score was generated and aligned with the pathologist baseline score (baseline, r=0.76), (*left panel*), blinded to treatment code
- Using SHG, MGL-3196 treated compared with placebo showed a statistically significant ≥1-pt reduction in fibrosis score at Week 36. Based on pathology score, fibrosis was reduced by ≥ 1 point in 29% of MGL-3196 treated patients vs. 23% in placebo

https://doi.org/10.1371/journal.pone.0199166
Week 36 pathology scores and treatment code were not provided to SHG readers.

13

Madrigal Pharmaceuticals

Phase 2 Results for Madrigal's MGL-3196 in Non-Alcoholic Steatohepatitis (NASH) Presented during Presidential Plenary Clinical Session of The AASLD Liver Meeting®

Data Source: Madrigal Pharmaceuticals

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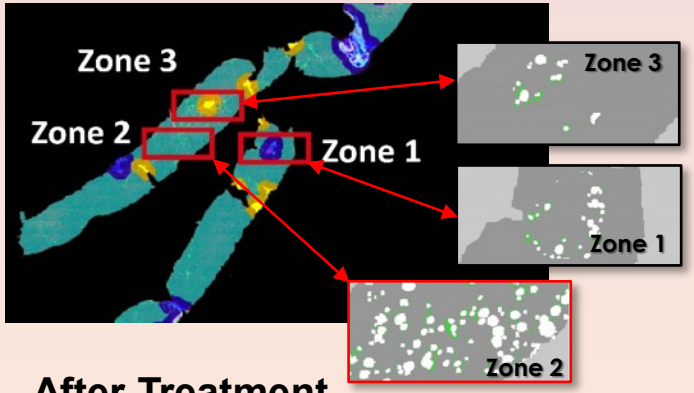
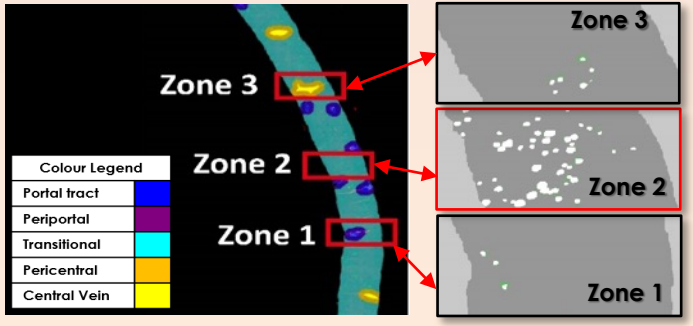
Visualization and Quantification for Mechanism of Action

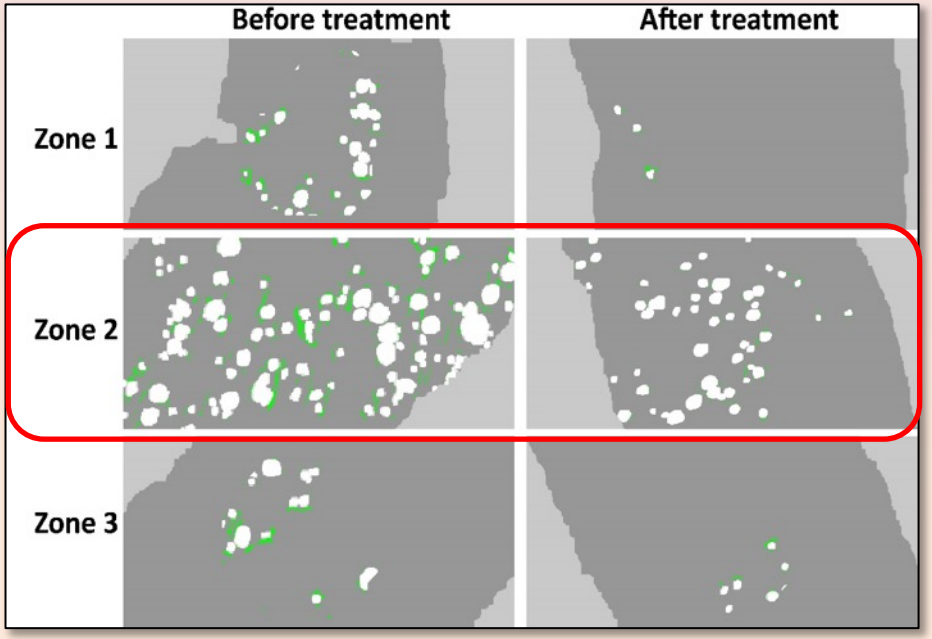
Co-localisation of qFibrosis and qSteatosis

Before Treatment



After Treatment





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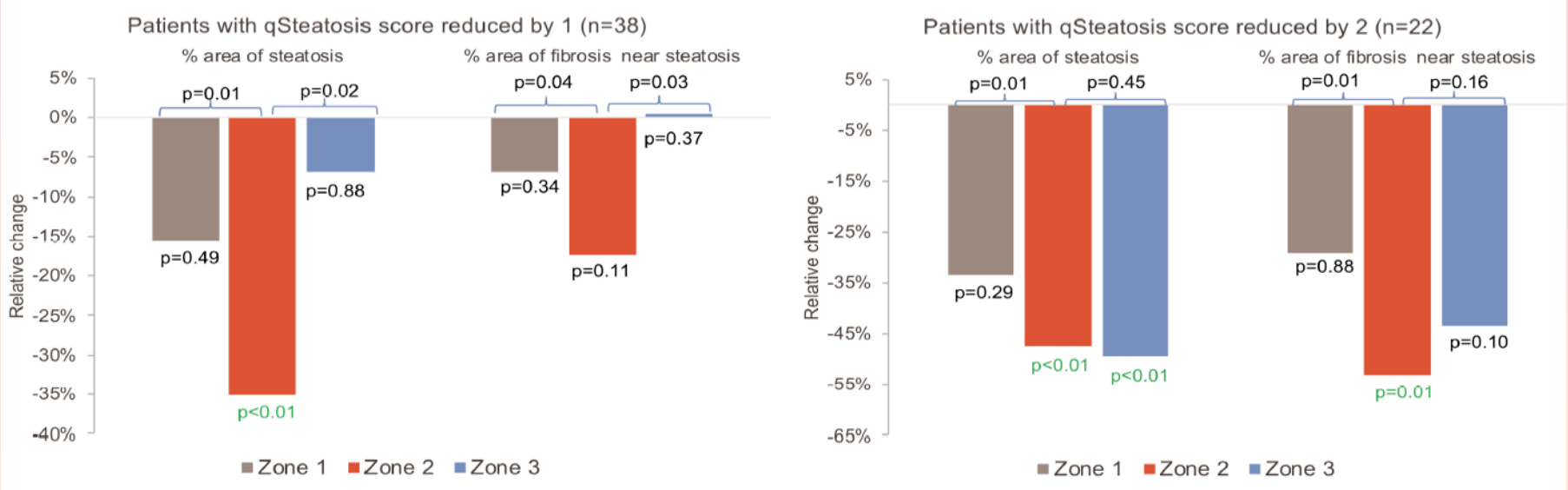
Visualization and Quantification for Mechanism of Action

Results from Resmetirom (MGL-3196)



The ability to directly co-localize Fibrosis with Steatosis on the same slide reveals MOA and is vital to assess and quantify drug efficacy.

Data Source: AASLD 2019, "Steatosis and Fibrosis Measured as Continuous Variables on Paired, Serial Liver Biopsies in the Resmetirom (MGL-3196) 36-Week Phase 2 NASH Study", Stephen Harrison et. al.

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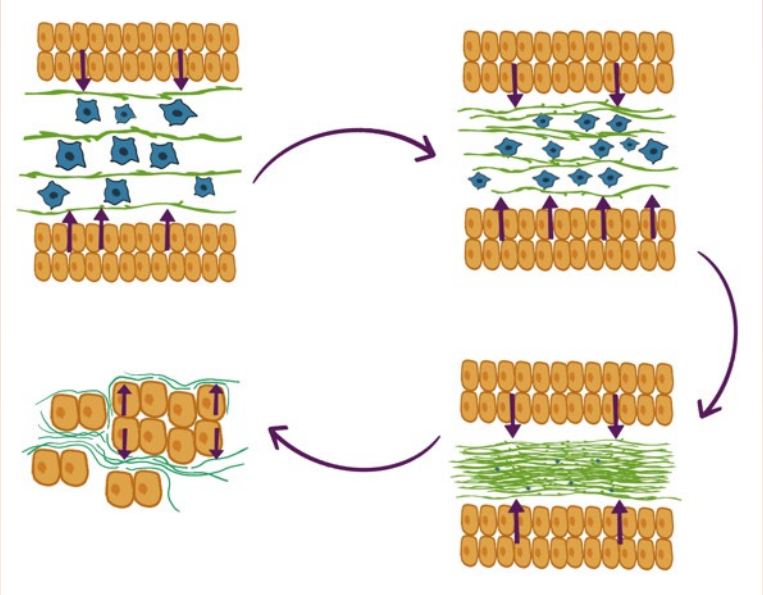
Distinguish Progression/Regression within Same Stages

Progressive Septa
Fine collagen fiber runs between inflammatory cells, in parallel formation

Broken Septa
Septa been infiltrated by newly regenerated hepatocytes, and collagen fiber runs around hepatocytes in circular formation

NASH-CRN F1/2 **NASH-CRN F3/4**



Established Septa
Fine collagen fiber collapsed into denser bundles with less inflammatory cells in between

Regressive Septa
Septa been squeezed into thin and dense strings by newly regenerated hepatocytes from both sides

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More Data to come in AASLD

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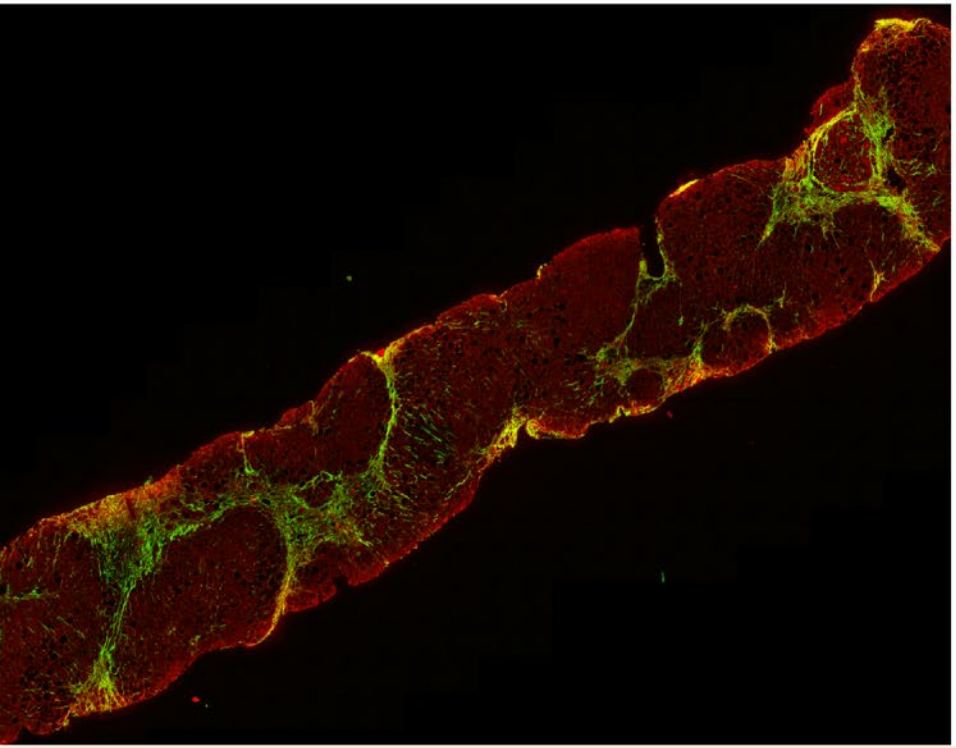
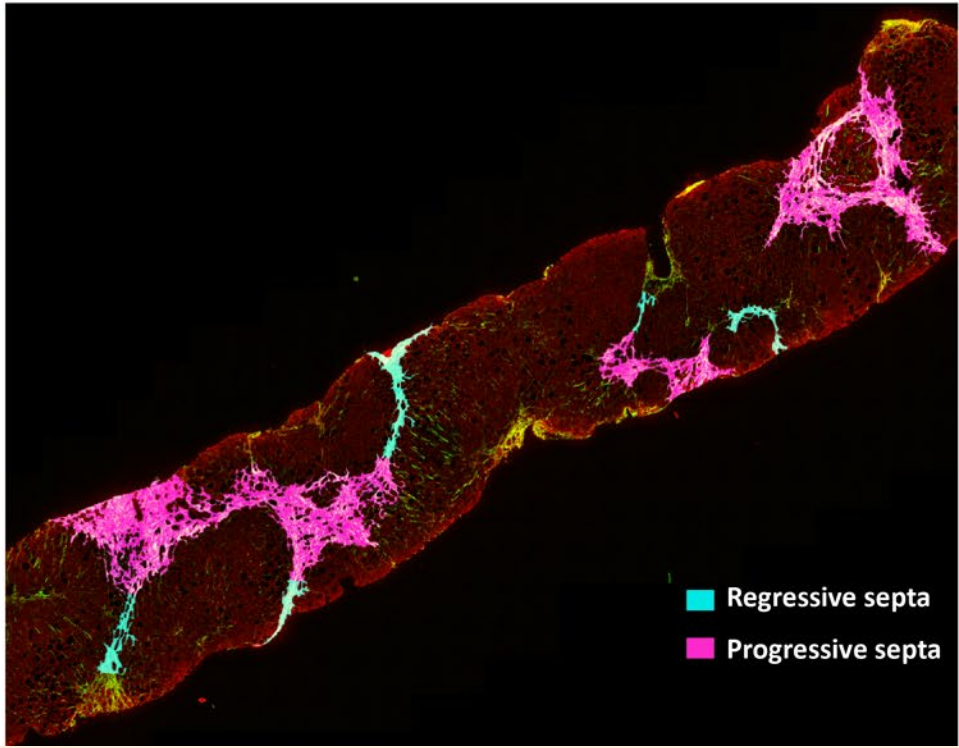
SHG/TPEF image



Septa region



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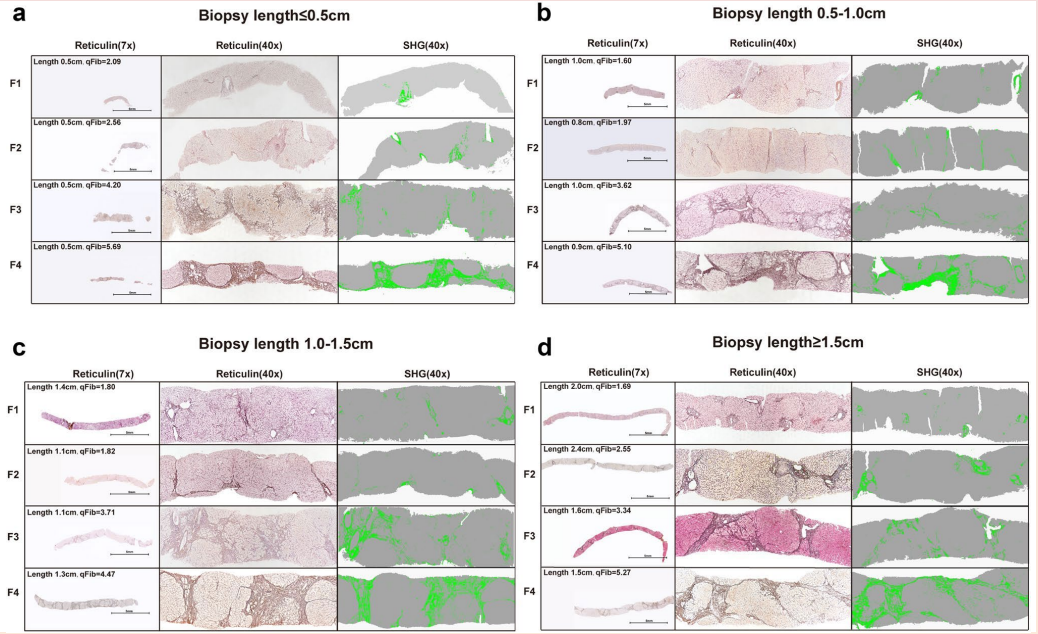
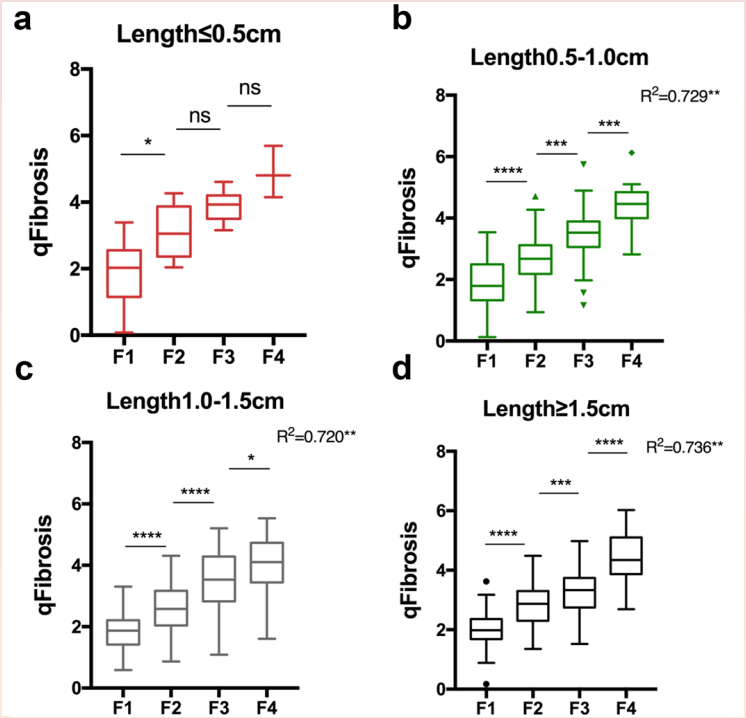
Challenge #2: Sampling Error





These minute alterations are likely to be more diffuse than major architectural changes, such as septa, and thus relatively free of sampling error.

Wanless. Clin Res Hepa & Gastro 2019

Wang et al, Hepatology International 2019

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Challenge #3: Inter- & Intra- Observer Variability

Wang et al, Hepatology 2017

Table 2. AUROCs of SHG B-index for prediction of Brunt fibrosis.

NAFLD Fibrosis Group	Brunt fibrosis stage	AUROC	*P*	95% CI	SHG B-index cut-off value
Mild fibrosis	0 vs 1/2/3/4	0.853	<0.001	0.774–0.933	1.18
Significant fibrosis	0/1 vs 2/3/4	0.967	<0.001	0.933–1.000	1.33
Bridging fibrosis	0/1/2 vs 3/4	0.985	<0.001	0.966–1.000	1.76
Cirrhosis	0/1/2/3 vs 4	0.941	<0.001	0.892–0.990	2.76

Chang et al, PLoS ONE 2018

Highly reproducible qFibrosis assessment

- In single parameter
- In combined indices

Also in qInflammation, qBallooning, and qSteatosis

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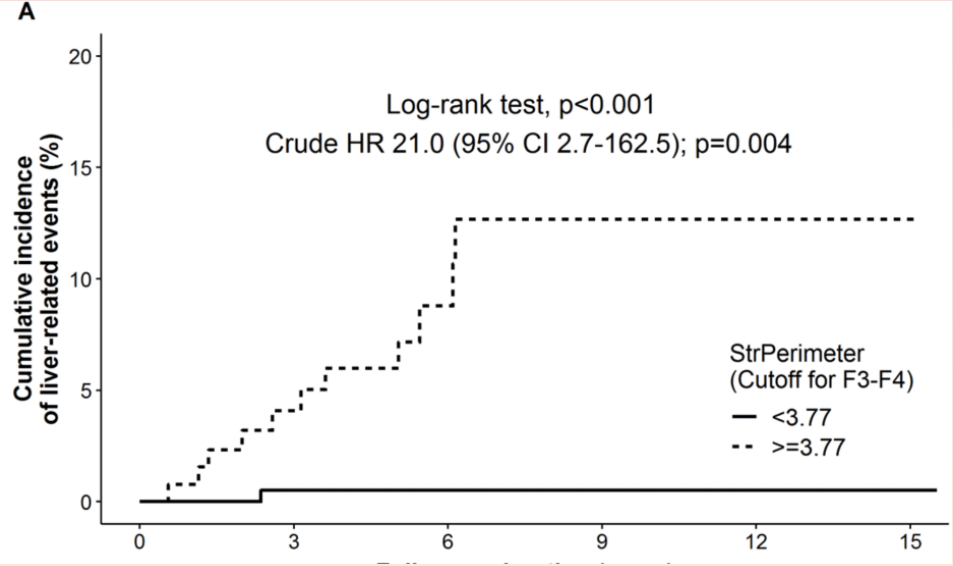
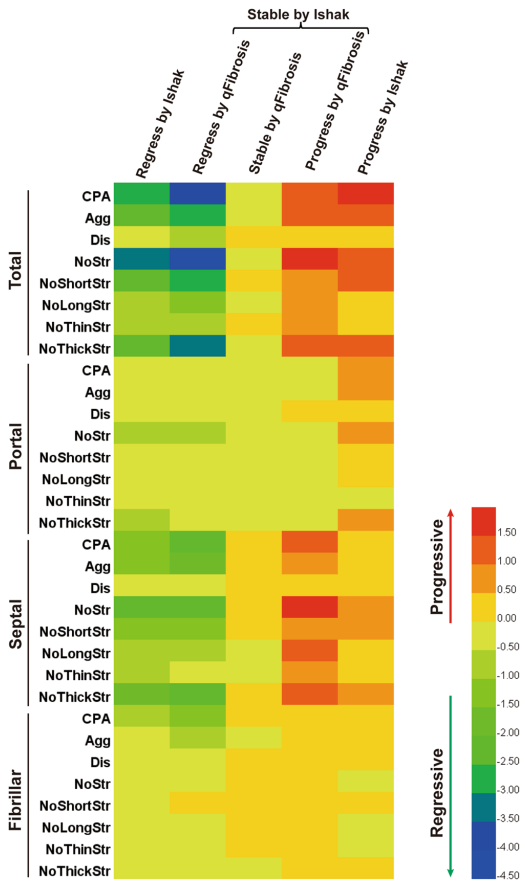
Challenge #4: Link to Outcome



Wang et al, GUT 2019

Validated in NASH and viral hepatitis patients



Sun et al, Sci Report 2018

Figure 5. Changes of multi-morphological collagen features pre- and post-treatment by Heatmap analysis. The color scale represents the changes of collagen features ((post-pre)/pretreatment). Red color indicates fibrosis progression, whereas green or blue color indicates regression. Changes of collagen features were comparable between "*Regression by Ishak*" and "*Regression by qFibrosis*" groups. Most of these features hardly changed in "*Stable by qFibrosis*" group, but increased in "*Progression by qFibrosis*" and "*Progression by Ishak*" groups. CPA, Collagen Percentage Area; Agg, Aggregated Collagen Percentage Area; Dis, Distributed Collagen Percentage Area; NoStr, Number of Strings; NoShortStr, Number of Short Strings; NoLongStr, Number of Long Strings; NoThinStr, Number of Thin Strings; NoThickStr, Number of Thick Strings.

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Current Progress in Validation and Regulation Approval

NASH Clinical Trials

- **40% of all NASH Ph II/III clinical trials use HistoIndex's SHG and AI-based SHG digital pathology platform**

90% Pharma and Biopharmas; 10% Academic/Research Institutions

NASH Preclinical and Clinical Studies

- **Working closely with 30 sponsors across industries on Preclinical projects.**

50% Pharma & Biotech; 50% from CRO and Academic/Research Institutes

FDA Application Process

- **IVD under FDA CDRH (conducted pre-submission meeting with FDA)**
- **BQP under FDA CDER (planning phase)**

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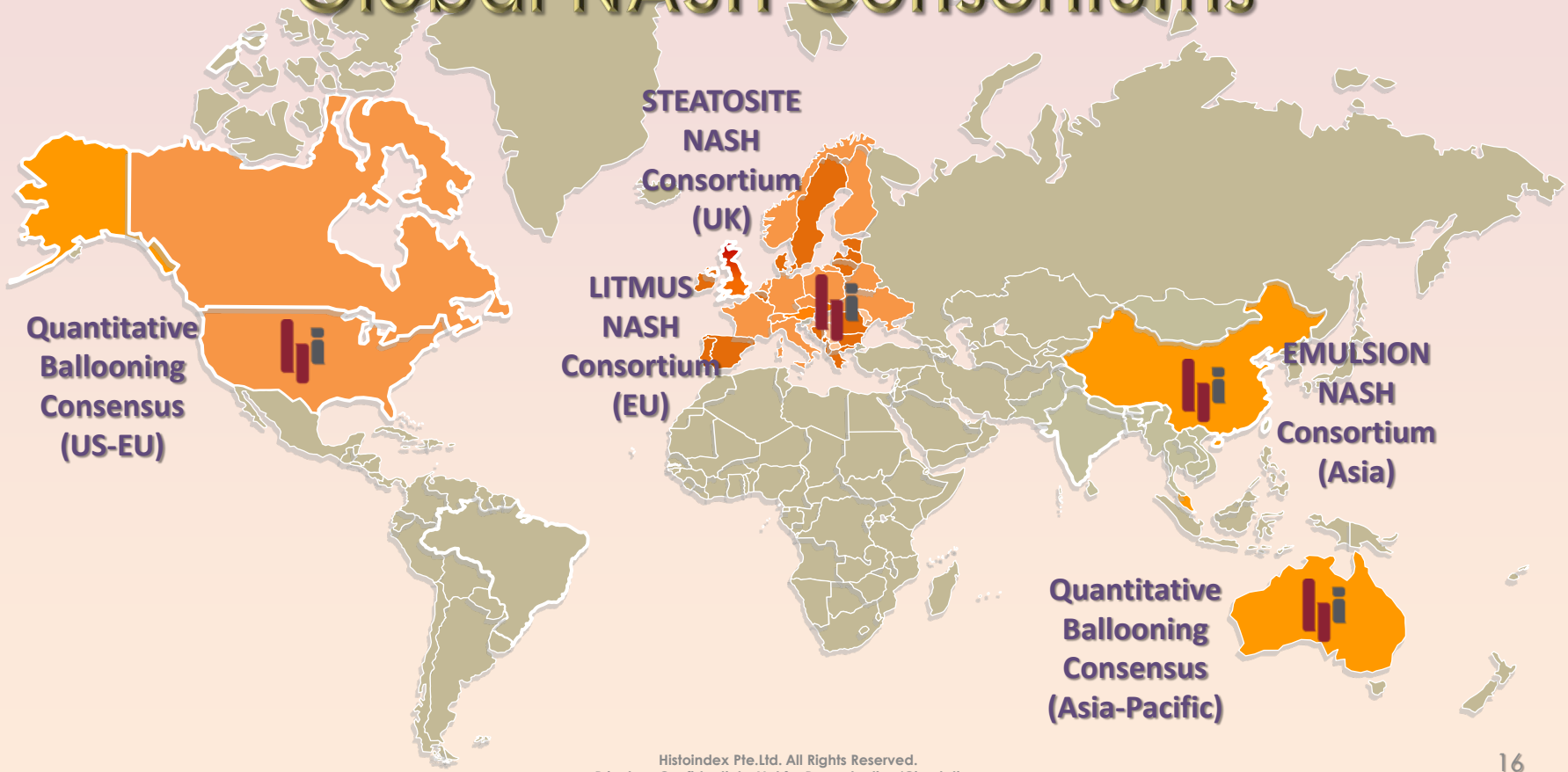
HistoIndex's Involvement in Global NASH Consortiums



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