RESULTS: Liver collagen content was determined in a stepwise process to determine area of PSR-positive and total tissue area. From these area measurements, percent PSR-positive area was determined. In order to accurately compare control to late stage model fibrosis, collagen on PSR-stained tissue was quantified and total cross-section liver area was measured. The results were used to evaluate therapeutic intervention in the development of NASH.

Figure 1: Image analysis approach to identify PSR-positive staining in fibrotic liver tissue sections. The PSR-stained tissue image was analyzed by surrounding and excluding hepatic nuclei, the outer collagen matrix is identified (b), color deconvolution is applied isolating the positive staining of interest (c), and the area of positive staining is determined as well as the total liver area (d).

Figure 2: Image analysis approach to identify PSR-positive staining in fibrotic liver tissue sections. The PSR-stained tissue image was analyzed by surrounding and excluding hepatic nuclei, the outer collagen matrix is identified (b), color deconvolution is applied isolating the positive staining of interest (c), and the area of positive staining is determined as well as the total liver area (d).

Figure 3: Quantitative tissue image analysis is effective in determining percent fibrosis using collagen as the marker of fibrosis. Over 300 tissue sections from multiple animal models have been evaluated with 34 methodology. Results allow discrimination of induction and therapy-related reductions in collagen amounts at less than 5% increments. Studies have demonstrated that even at low incremental changes, therapeutic intervention may be effective. Small incremental reductions in collagen deposition may be subjectively observed by trained pathologists, but cannot be accurately measured without computer-aided assistance. This tMk model of fibrosis is being applied to different liver fibrosis-inducing models and in different species. This same approach has also been applied to measure fibrosis in cardiac and renal tissues and is being investigated for use in pulmonary fibrosis animal models.

Figure 4: Examples of quantitative area determination of PSR-staining. StainMap’s Vessel-stained approach distinctly detects and measures PSR positivity, and total area determination is unaffected by hemorrhage or vascular components.

Figure 5: Vacuolated hepatocytes may contribute to local total cross-sectional area measurements. Liver space due to vascular structures, bile ducts and vacuolated cytoplasm are recognized and categorized as non-tissue and are not included in tissue area measurements. "Filling" the vacuolated cytoplasm-necrosis area measurements. Optimizing the algorithm to include the vacuolated regions in total area measurements results in more accurate area measurements.

CONCLUSIONS: Quantitative tissue image analysis is effective in determining percent fibrosis using collagen as the marker of fibrosis. Over 300 tissue sections from multiple animal models have been evaluated with 34 methodology. Results allow discrimination of induction and therapy-related reductions in collagen amounts at less than 5% increments. Studies have demonstrated that even at low incremental changes, therapeutic intervention may be effective. Small incremental reductions in collagen deposition may be subjectively observed by trained pathologists, but cannot be accurately measured without computer-aided assistance. This tMk model of fibrosis is being applied to different liver fibrosis-inducing models and in different species. This same approach has also been applied to measure fibrosis in cardiac and renal tissues and is being investigated for use in pulmonary fibrosis animal models.

Nonalcoholic steatohepatitis (NASH) is a common, often "silent" chronic liver disease. While resembling alcoholic liver disease, it occurs in people who drink little or no alcohol. The lesions most commonly accepted for NASH include steatosis, hepatocyte ballooning degeneration, mild diffuse lobular mixed acute and chronic inflammation, and periportal, perisinusoidal collagen deposition. Progression of fibrosis may result in bridging septa and cirrhosis, ultimately leading to liver failure. There are no specific therapies for NASH. Current treatment focuses on controlling associated medical conditions, such as diabetes and obesity, and on monitoring for progression. Emerging antifibrotic therapies are aimed at inhibiting the accumulation of fibrogenic cells and/or preventing the deposition of extracellular matrix proteins. Development of a reproducible murine model recapitulating the progressive nature of NASH with accurate and reproducible detection and analysis of fibrosis progression would be a useful tool for studying the natural history, molecular mechanisms and biology of NASH.

Animal models mimicking NASH in adult tissues. A major obstacle in their utilization is the identification and quantification of induced fibrosis and its amelioration due to therapy. Previous attempts to quantitate such models have reproduced the metabolic and inflammatory aspects of NASH without the development of progressive liver fibrosis. A diet-induced murine model has resulted in a liver with many clinical features of NASH including hepatic fibrosis. However, fibrosis generally occurs at a low area percentage in tissues, and manual subjective evaluation cannot accurately discard discrepancies at low levels. Quantitative tissue image analysis (QIA) solutions have been developed to accurately and consistently determine percentages of induced fibrosis in various experimental animal models. This data in combination with histomorphologic and molecular data allows a greater utilization of these animals in antifibrotic drug development.

METHODS: Mice from male mice fed a NASH-like inducing diet were collected and analyzed by IVA using StainMap, a proprietary Flagship algorithm. Liver sections were stained with H&E, Picricirus Red (PSR) and Masson’s Trichrome (MTR) to visually determine increased collagen levels associated with induced fibrosis. Histology was assessed according to the Brunt staging system (1). Quantitative image analysis for percent collagen was performed on liver sections stained with Masson’s Trichrome stain (MTR) or PSR. While both stains provided acceptable measurements using StainMap, PSR was chosen as the stain of choice for IVA, based on ease of visualization of PSR-positive collagen deposition and correlation with image analysis markup images. MTR staining resulted in increased false positive regions with the liver sections.

RESULTS: Liver collagen content was determined in a stepwise process to determine area of PSR-positive and total tissue area. From these area measurements, percent PSR-positive area was determined. In order to accurately compare control to late stage model fibrosis, collagen on PSR-stained tissue was quantified and total cross-section liver area was measured. The results were used to evaluate therapeutic intervention in the development of NASH.

Progression of disease presents varying histomorphologic appearances to the liver with corresponding challenges to image analysis solutions. Optimized IVA algorithms were applied against liver sections to determine total tissue area and area of collagen deposition. Results allow discrimination of induction and therapy-related reductions in collagen amounts at less than 5% increments.