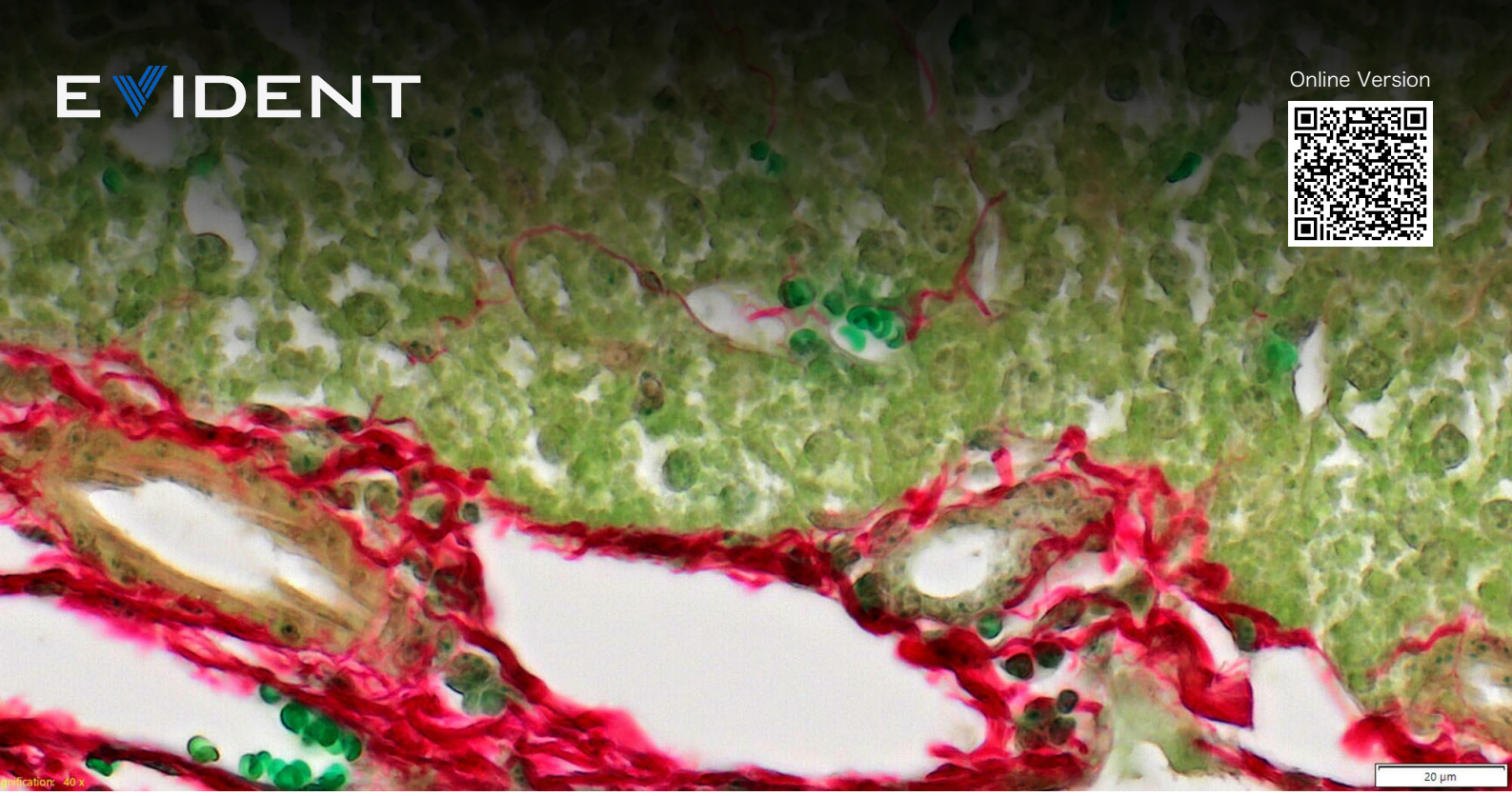


Online Version

Application Notes

Oct 03 2023

Identifying Cirrhosis in Liver Tissue Using a Research Slide Scanner

Introduction

Cirrhosis, also known as liver cirrhosis or hepatic cirrhosis, is a condition where the liver improperly functions due to long-term damage. This damage is characterized by the replacement of normal liver tissue by scar tissue. Typically, the disease develops slowly over months or years. Early on, there are often no symptoms.

Cirrhosis is commonly caused by alcohol, hepatitis B, hepatitis C, and nonalcoholic fatty liver disease.

A diagnostic factor is the amount of collagen fibers inside the liver tissue. Special staining methods can be used to highlight these fibers for image analysis.

Experiment

Mouse liver biopsies were taken and immobilized on a glass slide. Next, the tissue was stained with a Picro-Sirius-Red & Fast-Green stain to highlight fibrosis tissue.

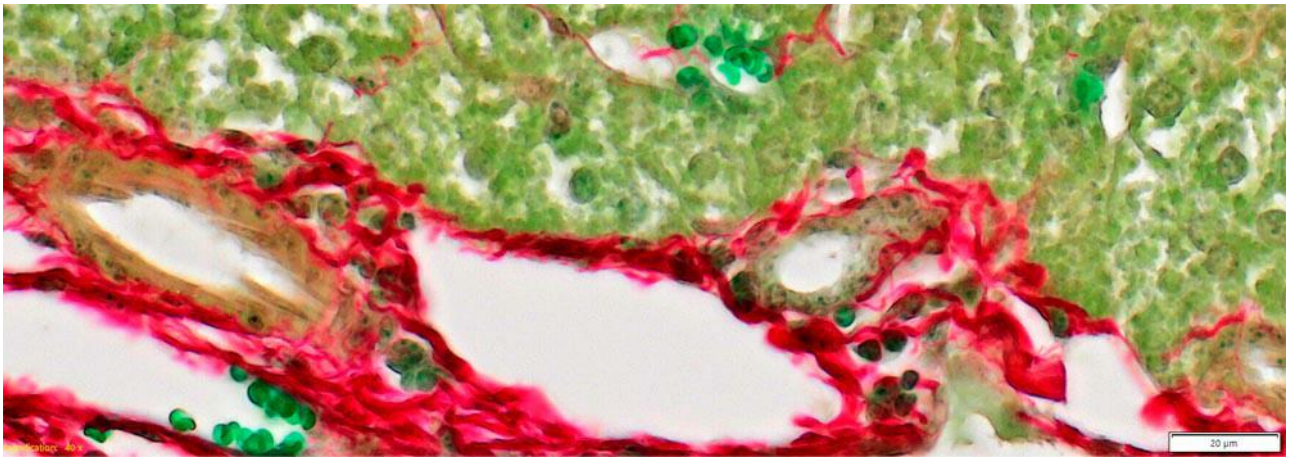
The Picro-Sirius-Red & Fast-Green stain is a variant of the Pico Sirius Red stain where a counterstain of the surrounding tissue with Fast Green is performed to better highlight the red stained collagen fibers.

In histology, Sirius Red staining is used in various domains of diagnostics to observe fibrosis levels in many cases of inflammation induced by cancer, vascular, or metabolic pathologies.

Using brightfield microscopy, the following can be observed:

- The nuclei in yellow
- The cytoplasm in yellow
- Collagen fibers in red
- Muscular fibers in yellow
- Red blood cells in yellow

The stained slides were imaged with an Olympus SLIDEVIEW™ VS200 research slide scanner at 40x magnification using a 40x UPlanXApo objective lens with a 0.95 NA to get the best possible resolution (0.17 μm/pixel).



Mouse liver tissue. Thickness: 4μm, staining: Picro-Sirius-Red & Fast-Green (Labor: Medizinische Universität Wien, Innere Medizin III, HEPEX Labor für Portale Hypertension und Fibrose bei Lebererkrankungen, Austria)

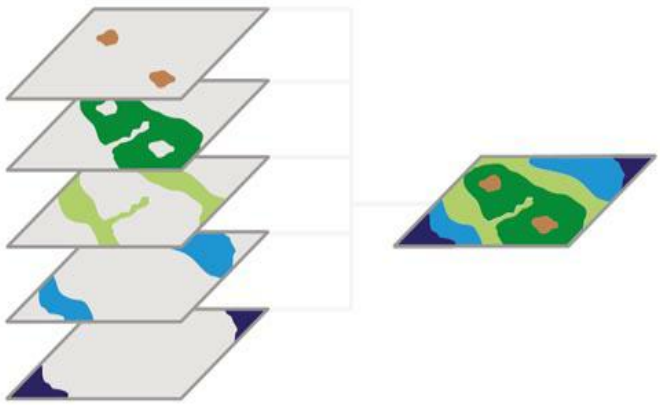
However, the 40x UPlanXApo objective lens has a depth of focus of only 0.82 μm. As liver tissue sections are thin (around 4 μm), applying the extended focal imaging (EFI) algorithm also improves the image quality in terms of focus.

EFI enables you to acquire images that have practically unlimited depth of focus. For this purpose,

the software calculates a composite image that is sharp in all areas from many differently-focused images.

The image acquisition process first acquires a Z-stack, then computes an EFI image from the Z-stack.

The illustration below shows several frames that were acquired at different Z-positions. In each of these frames, there are only a few image segments that are sharply focused. These segments are shown in color, and the sharply focused image segments will be assembled into the EFI image.

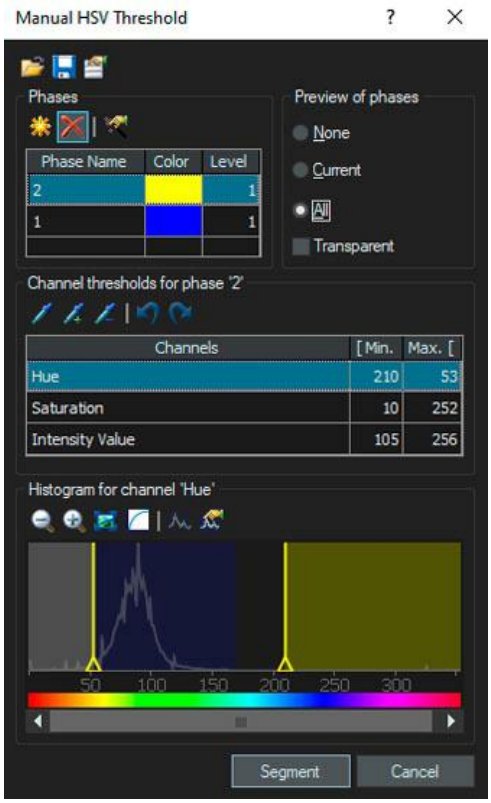


To detect the fibrosis (collagen fibers) tissue stained in red, we used the VS200-Detect solution combined with the VS200-ASW desktop software.

In order to turn a colored image, such as the one captured by the VS200, into a binary image, to detect fibers or background, we need to threshold the image using the hue, saturation, and value of each pixel.

HSV allows you to not only filter based on the colors of the pixels, but also by the intensity of color and the brightness.

A Manual HSV threshold was applied for the two phases: yellow for fibrosis tissue and blue for all other tissue.



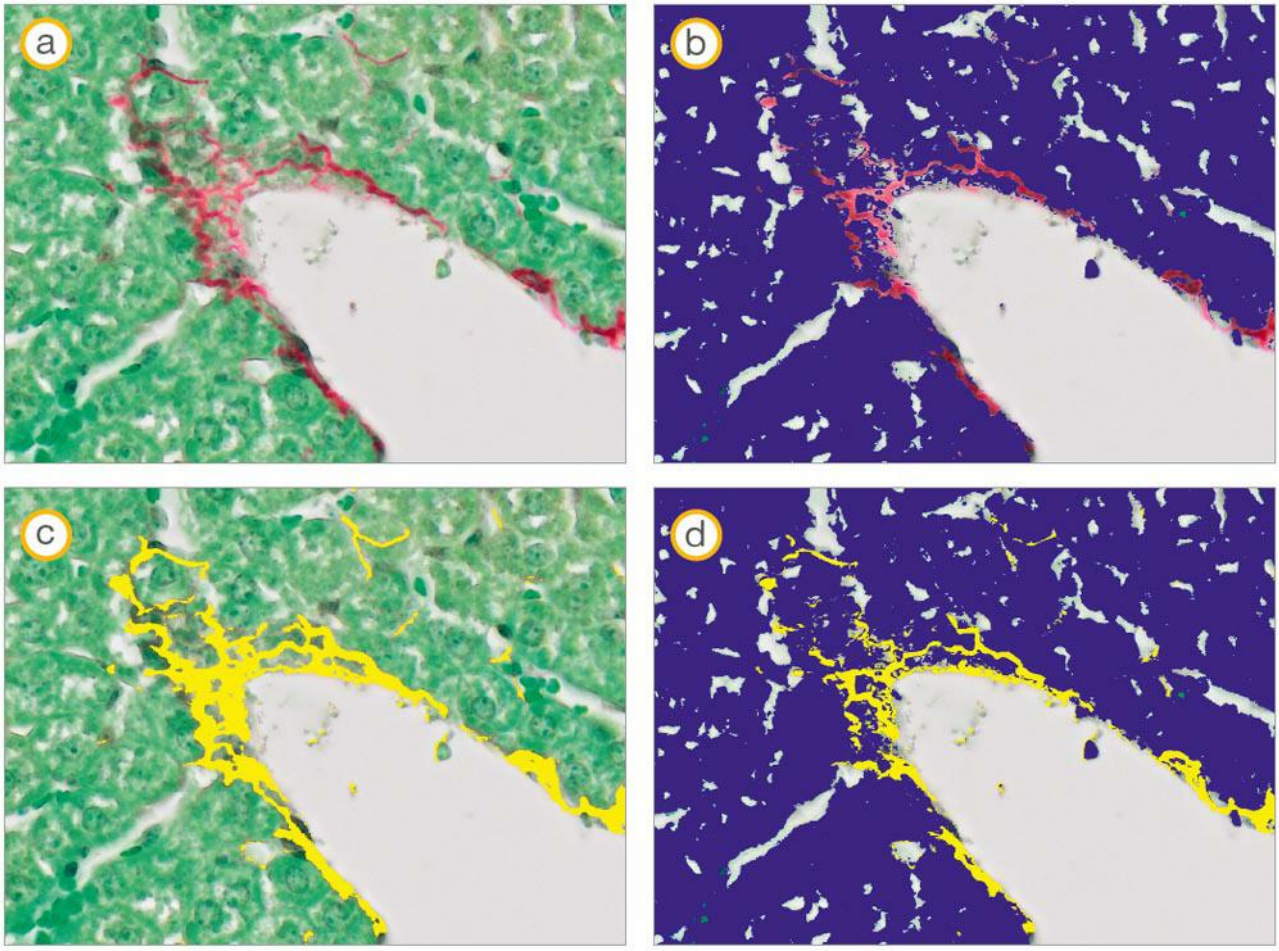
Manual HSV threshold

HSV stands for:

Hue: Measures the color of the pixel.

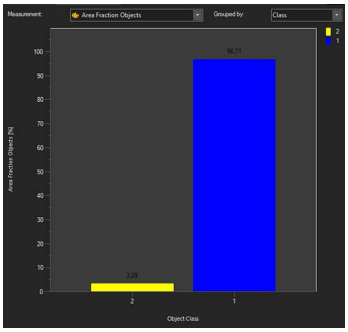
Saturation: Measures the intensity of color of the pixel.

Value: Measures the brighness of the pixel

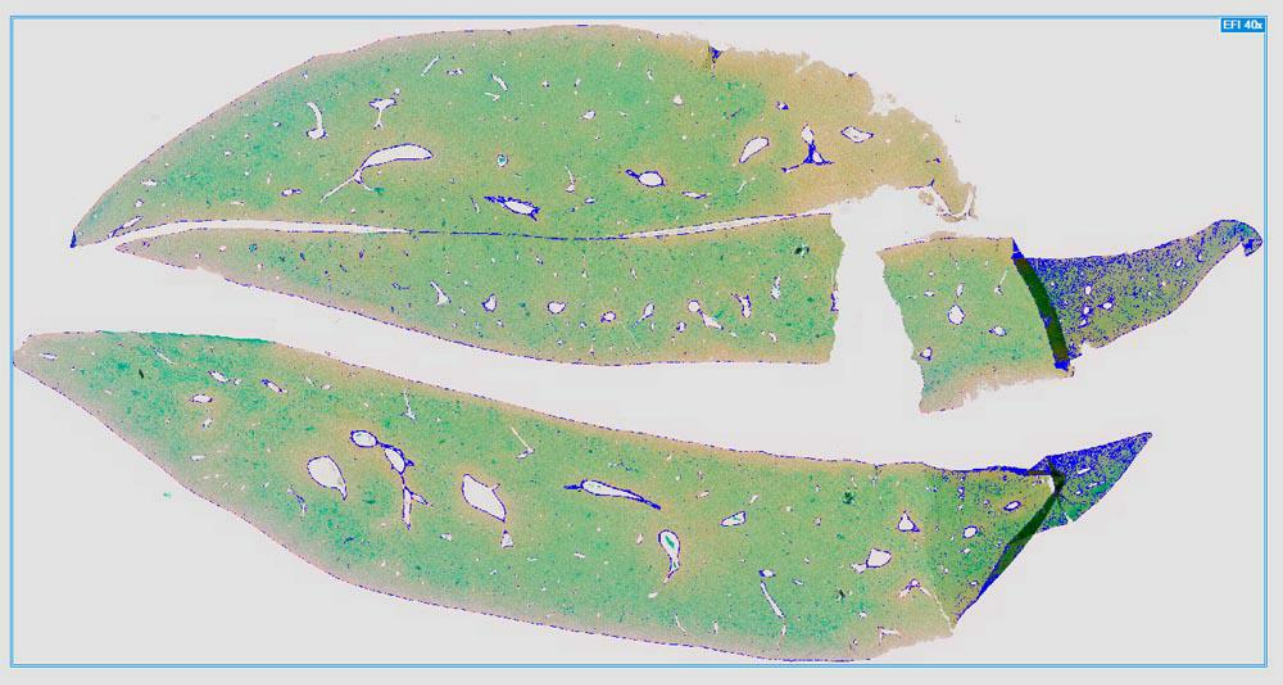


Mouse liver tissue: (a) source image, (b) manual threshold setting (blue) for the green stained tissue, (c) manual threshold setting (yellow) for the red stained fibers and (d) combined threshold of the two phases

Next, the Count and Measure on layer procedure showed the following result: 3.29% (yellow column) of the complete mouse liver biopsy was stained positive for fibrosis tissue.



The results of the Count and Measure on layer procedure: 3.29% (yellow column) of the complete mouse liver biopsy was stained positive for fibrosis tissue



The analysis can be performed on the whole mouse liver section

Related Product

Research Slide Scanner

VS200

- Outstanding image quality for whole slide imaging, from 2X to 100X, including oil scanning
- Accommodates multiple slide sizes and observation methods
- VS-SILA optical sectioning device enables you to obtain high-contrast images free from blur across the entire slide
- Simplified and powerful workflow, from brightfield to fluorescence multiplexing
- Uniquely configurable software and hardware, from manual brightfield scanning to AI recognition and fully automated scanning

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