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**TITLE**: Automated 3D reconstruction of the normal and cirrhotic rat hepatic microcirculation using confocal microscopy: a feasibility study

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**Abstract max: 300 words.**

**AIM**
Liver cirrhosis is a chronic disease of the liver, comprising a wide spectrum of pathological characteristics affecting the hepatic architecture and function. To date, little is known about the hemodynamic consequences caused by cirrhosis, especially at the microscopic level. In order to analyze the adaptive morphology and perform computational flow simulations, 3D reconstructions of the hepatic microcirculation are essential. In this work, we show that the combination of immunohistochemistry and confocal laser microscopy enables acquiring detailed 3D geometrical data of the liver microarchitecture.

**METHODS**
After whole animal perfusion fixation with 4% paraformaldehyde, normal and cirrhotic livers were resected from male Wistar rats. Subsequently, immunohistochemistry was applied to 200 µm thick slices by staining the endothelial cells with the monoclonal antibody RECA (Rat Endothelial Cell Antigen) and the fluorescent cyanine dye Cy3. 2D image stacks were recorded with a confocal microscope at magnifications of 40x. Afterwards, the resulting datasets were automatically processed and segmented with an in-house developed software using ITK and Qt libraries (see Figure 1) in order to visualize the liver sinusoids in 3D.

**RESULTS**
The results indicate that automatic reconstruction of the hepatic vascular network is feasible for normal and cirrhotic livers (see Figure 1). Currently, the visualization depth is limited to 40 - 50 µm for rat livers. Several techniques (stitching, registration, bidirectional imaging etc.) are being explored to increase the imaging depth.

**CONCLUSION**
The aforementioned technique provides a useful tool to reconstruct the 3D architecture of the hepatic microcirculation, which may lead to new insights in the adaptive morphology of liver cirrhosis. In addition, immunohistochemistry of liver slices is not restricted to the vascular network, but can be
extended to the simultaneous staining of the biliary network as this ramifying tree and its respective functions are also affected by cirrhosis.

Figure 1: (a) Raw dataset of a RECA-stained cirrhotic rat liver slice, obtained with confocal microscopy. (b) Automatically processed and segmented dataset indicating the vascular network. (c) Anatomically correct 3D reconstruction of the cirrhotic microcirculation of a rat liver.