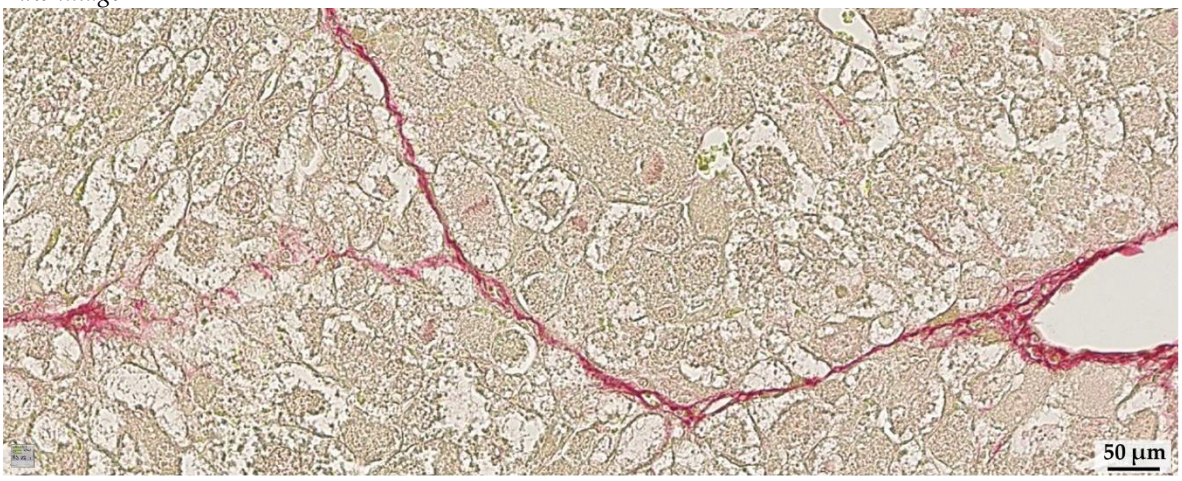
biomolecules

MDPI

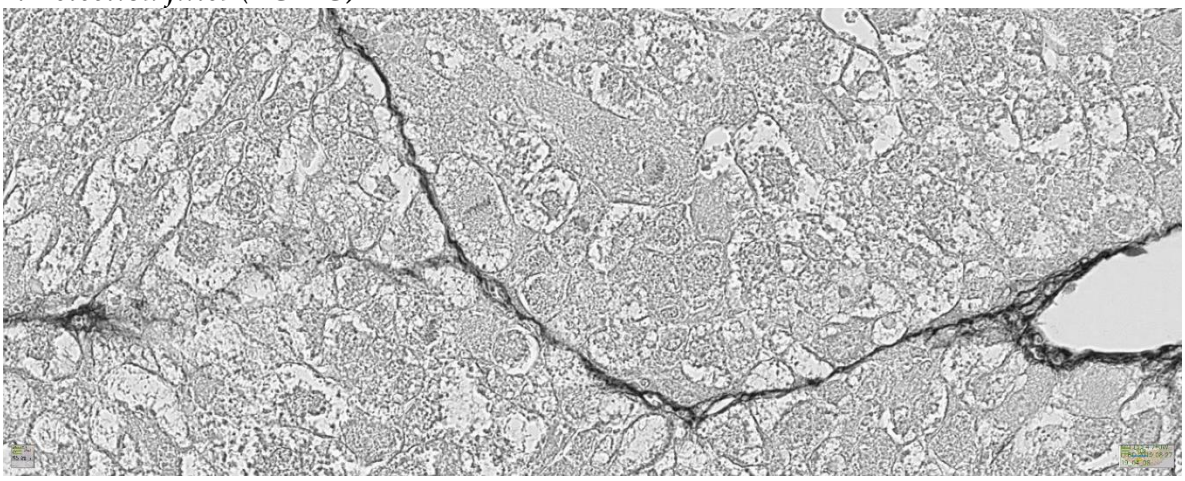
Supplementary Material 2: Detection filters and threshold determination for the RGB-based or the PSR-optimized methods. An illustration is given in the liver.

Raw image



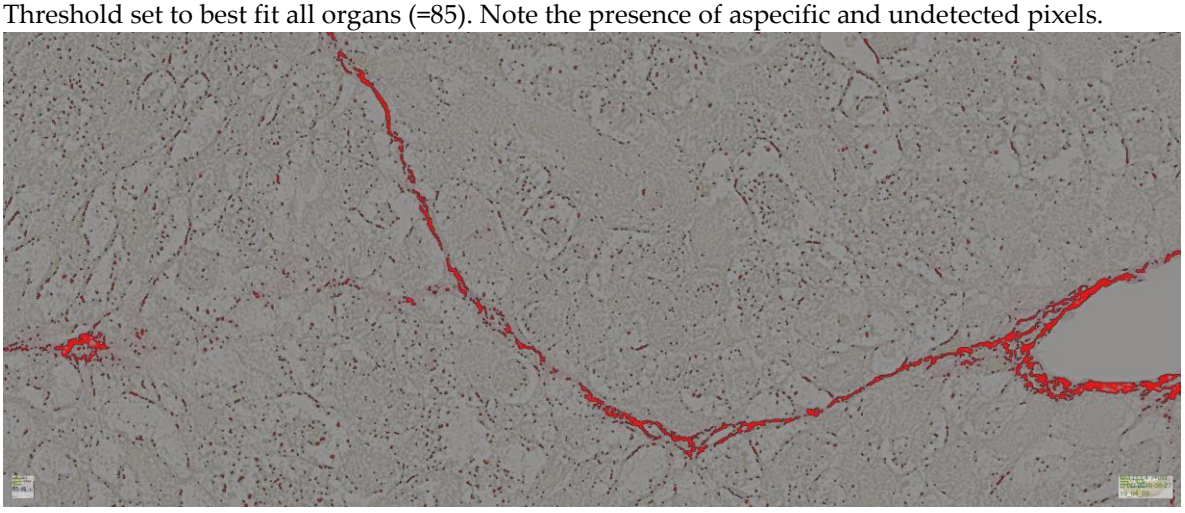
A. RGB-based method

1. Detection filter (RGB-G)



2. Result with RGB-based method

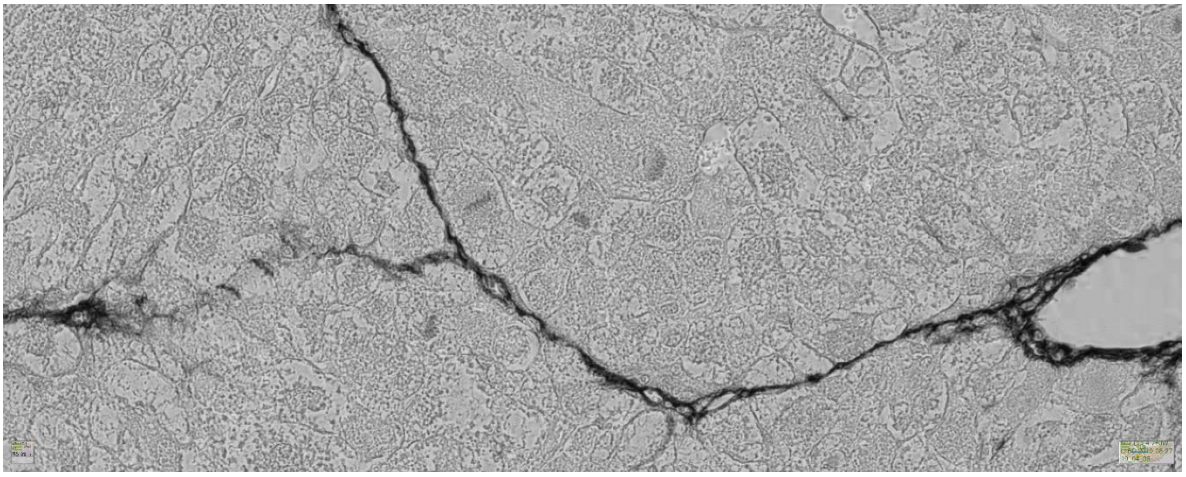
Threshold set to best fit all organs (=85). Note the presence of aspecific and undetected pixels.



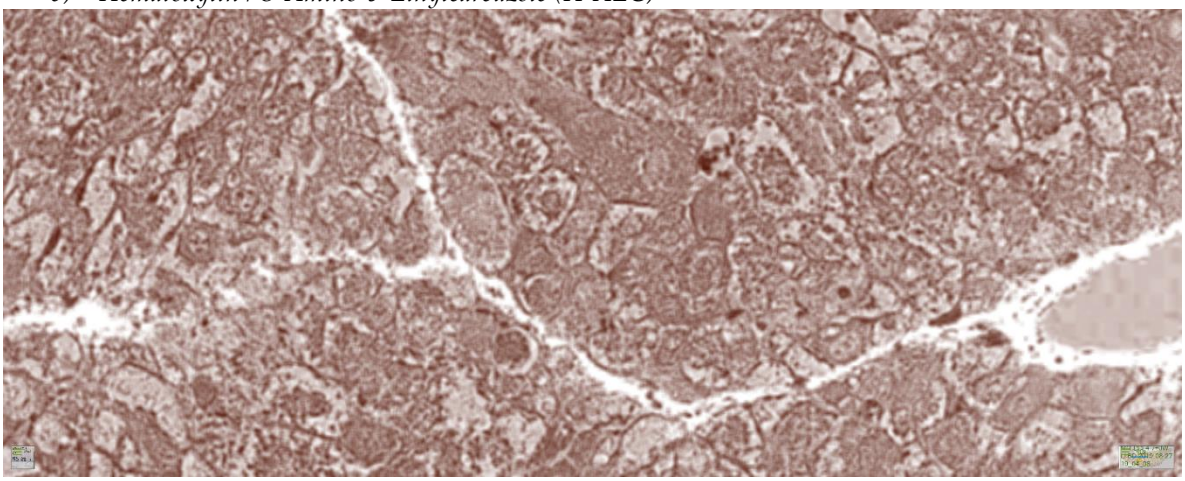
B. Optimized method

1. Pre-filters

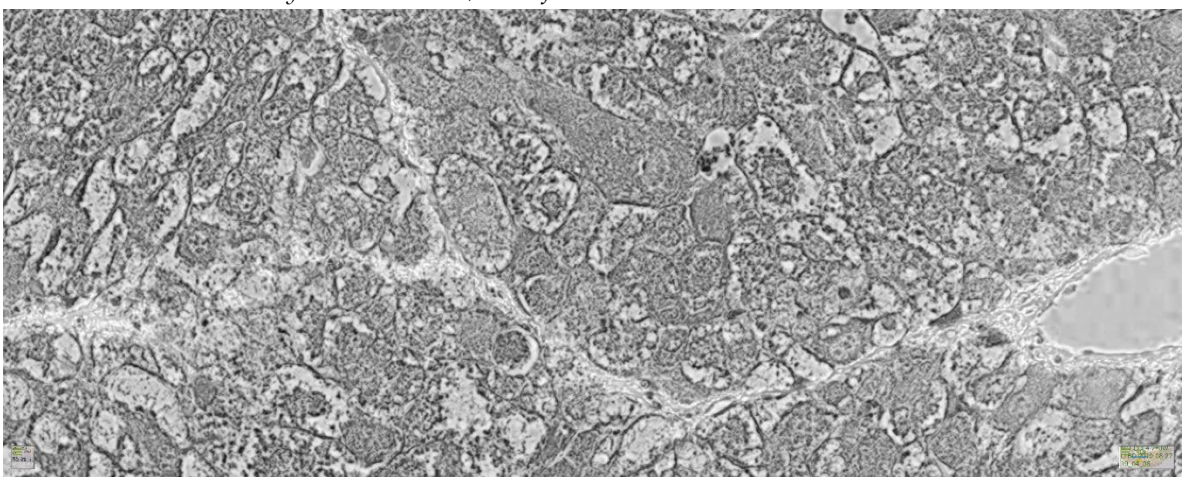
a) RGB-G (mean)



b) Hematoxylin / 3-Amino-9-Ethylcarbazole (H-AEC)

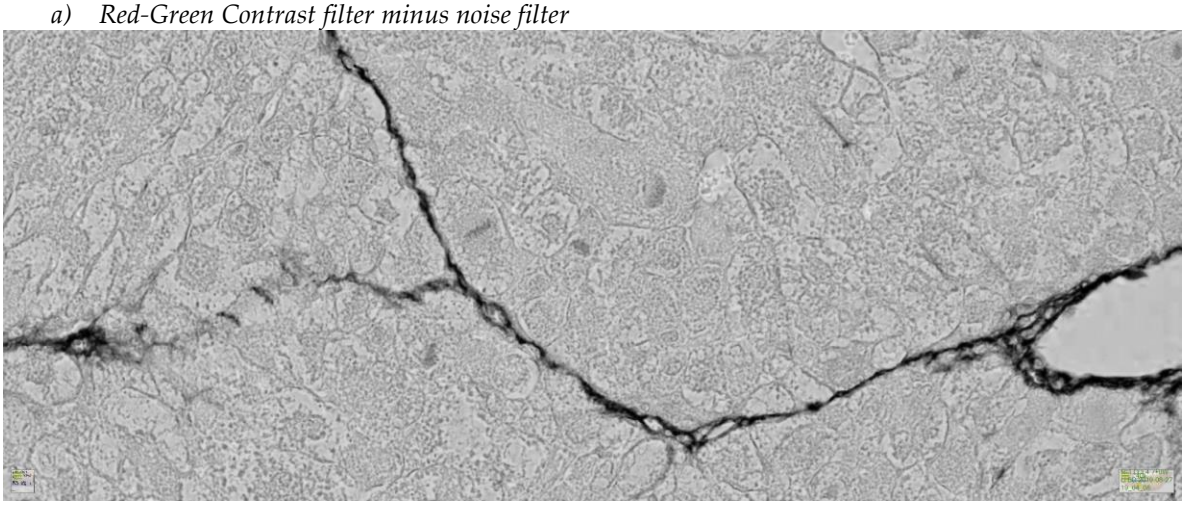


c) H-AEC divided by RGB-G (mean); noise filter

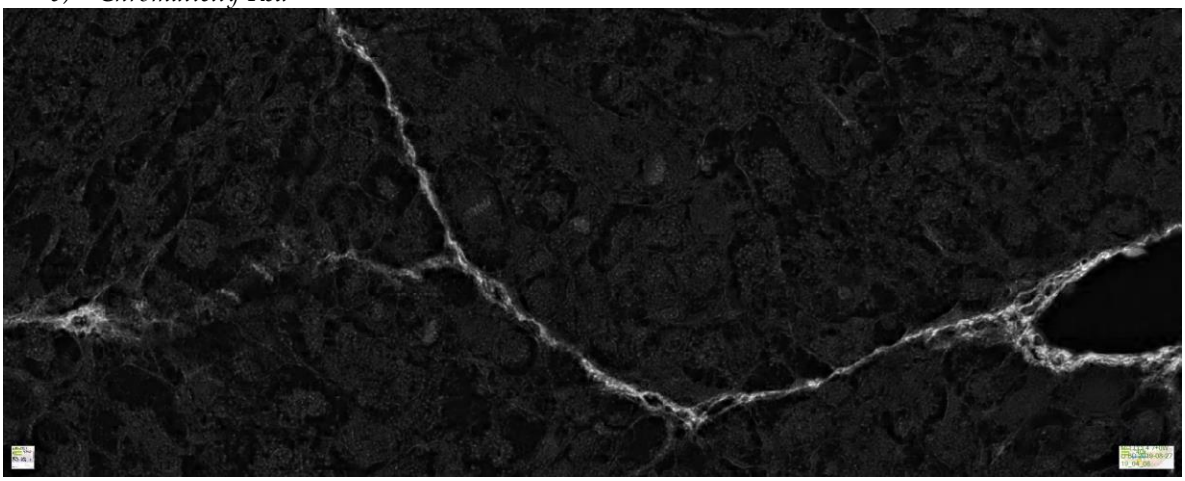


2. Detection filters

a) Red-Green Contrast filter minus noise filter

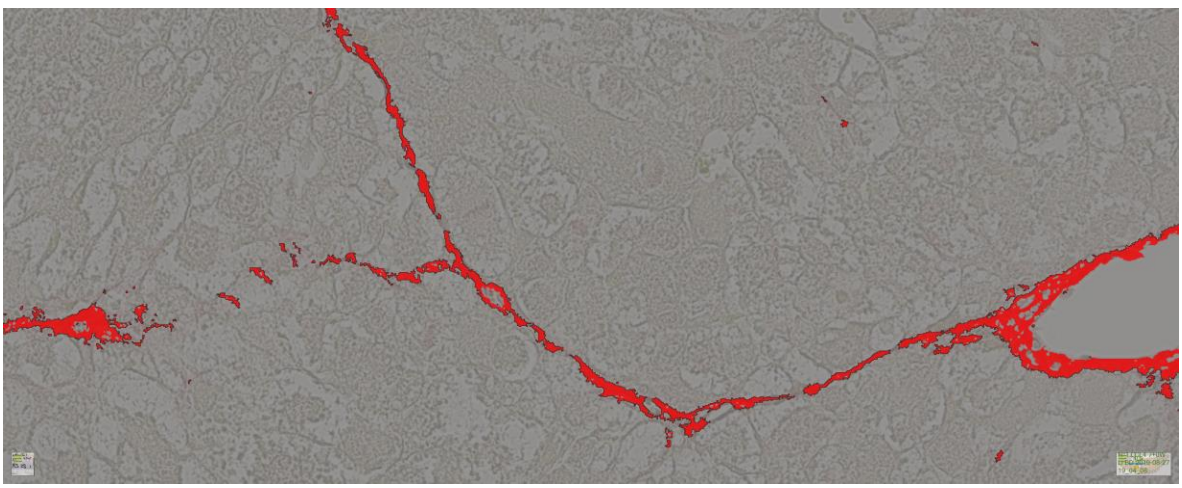


b) Chromaticity Red



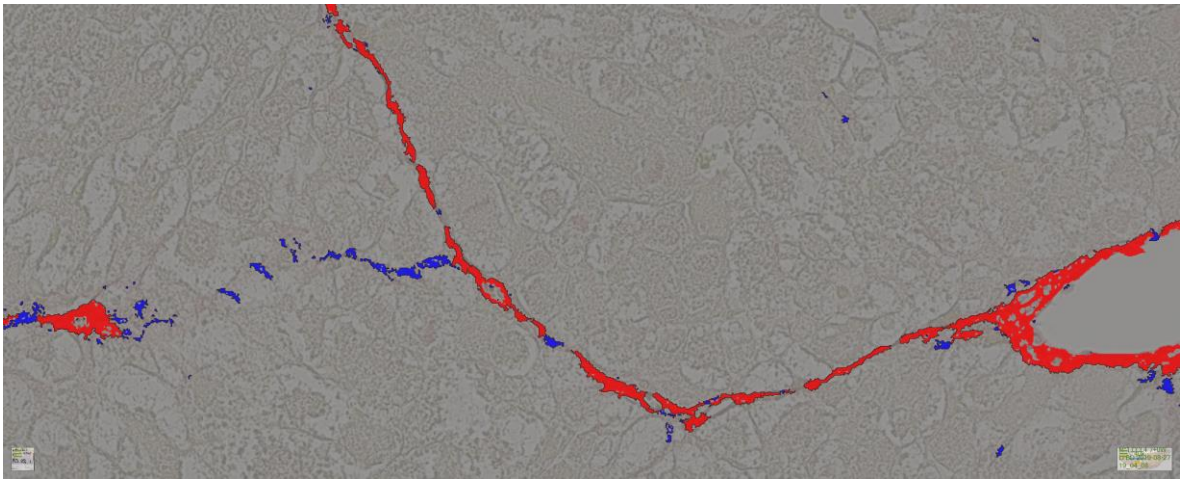
3. Result with PSR optimized method

Threshold set to best fit all organs (= 90). Note the good sensitivity (correct detection of faintly stained pixels) and specificity (no unstained pixel included) with these parameters. An example is given in the three studied organs in **Supplement material 3**. If needed, threshold can easily be adjusted per organ according to specific background. See below the great dynamic range of this method.



4. PSR optimized method: subsequent fiber classification

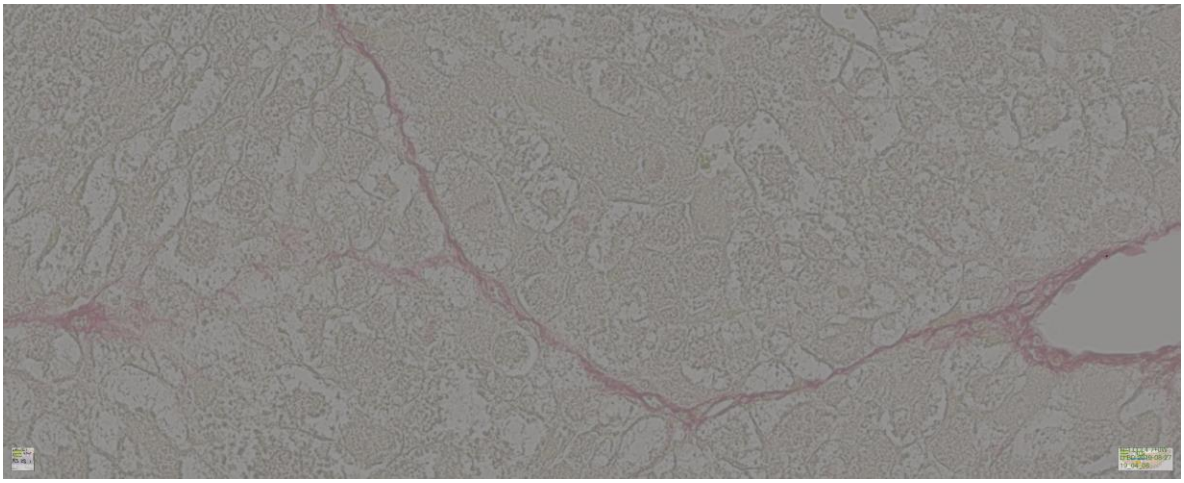
Having detected PSR staining, subsequent segmentation was achieved by watershed (Chromaticity red used as reference) and separate objects functions (size). Each segment was classified as *scattered fibers* (blue) or *compact fibers* (red) based on the mean intensity.



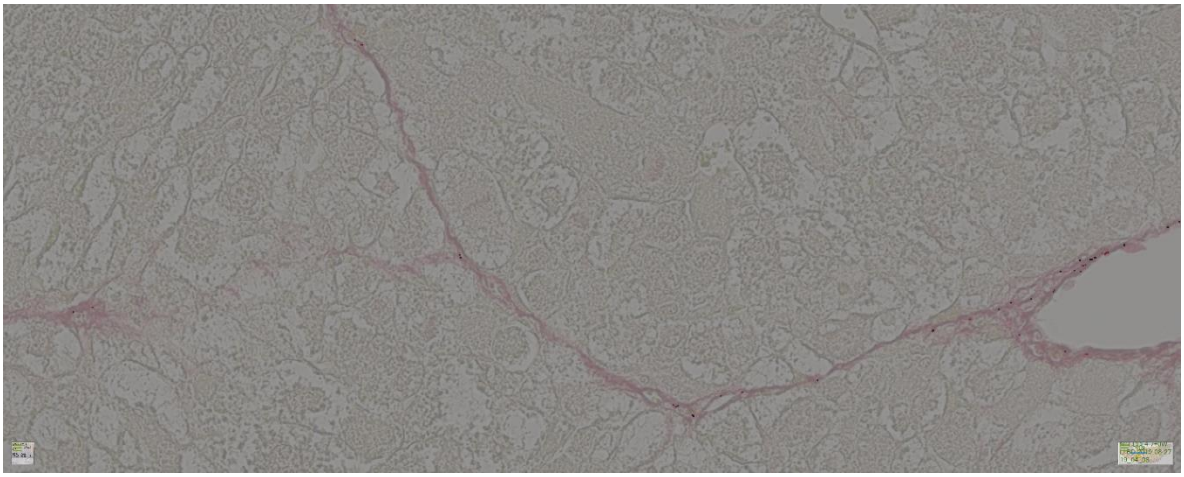
C. Dynamic range of the two detection methods

A. RGB method

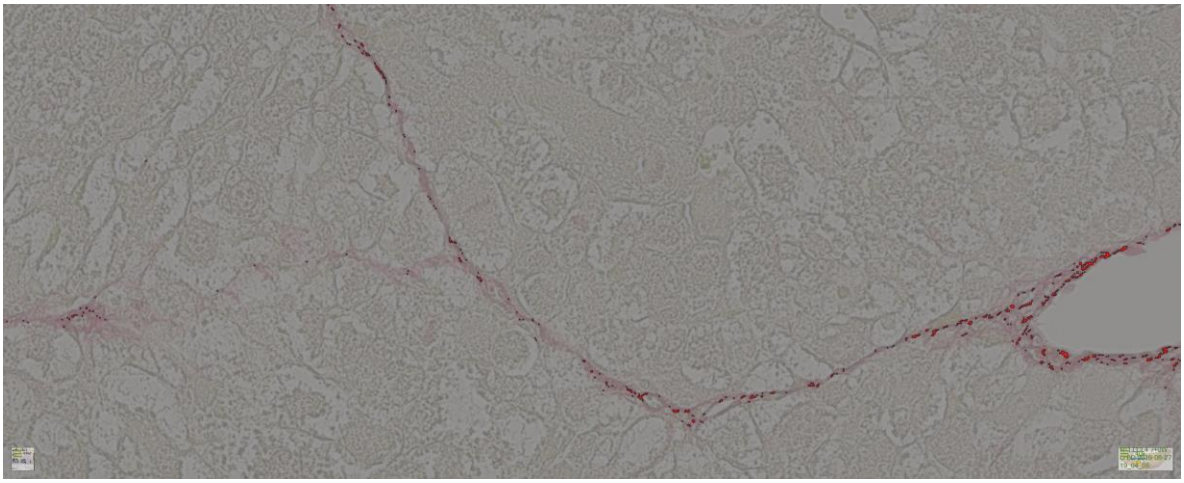
threshold = 5



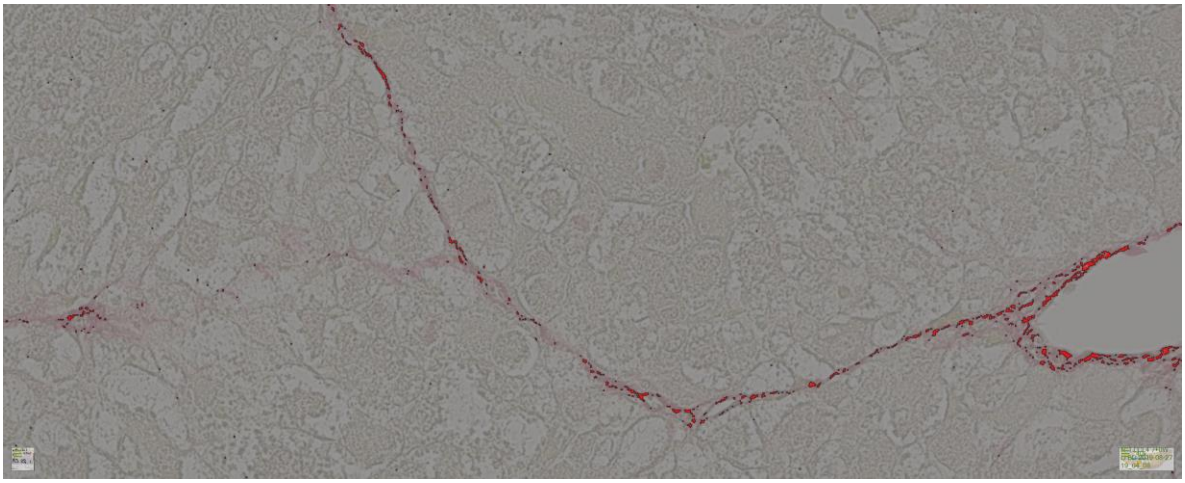
threshold = 20



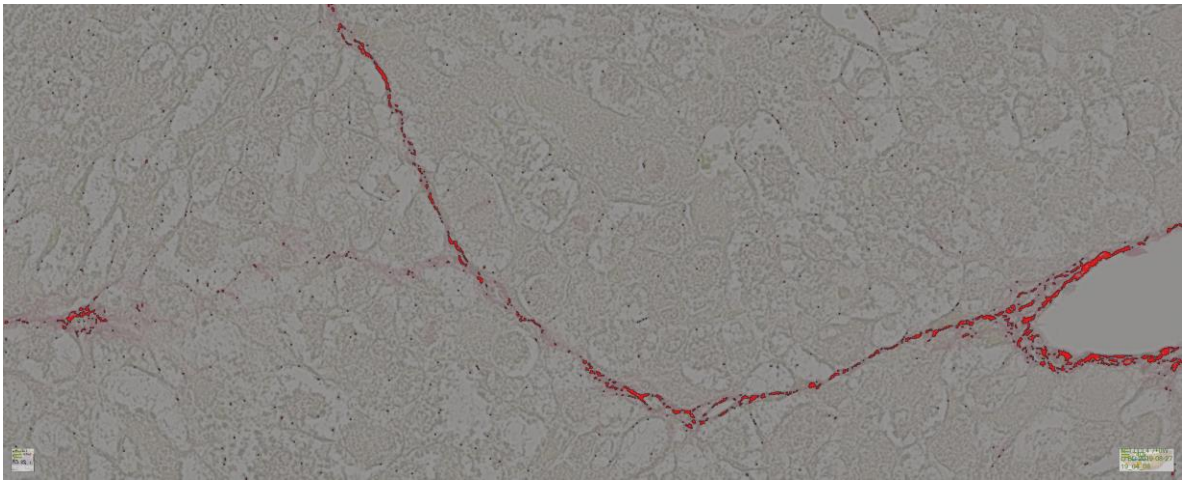
threshold = 40



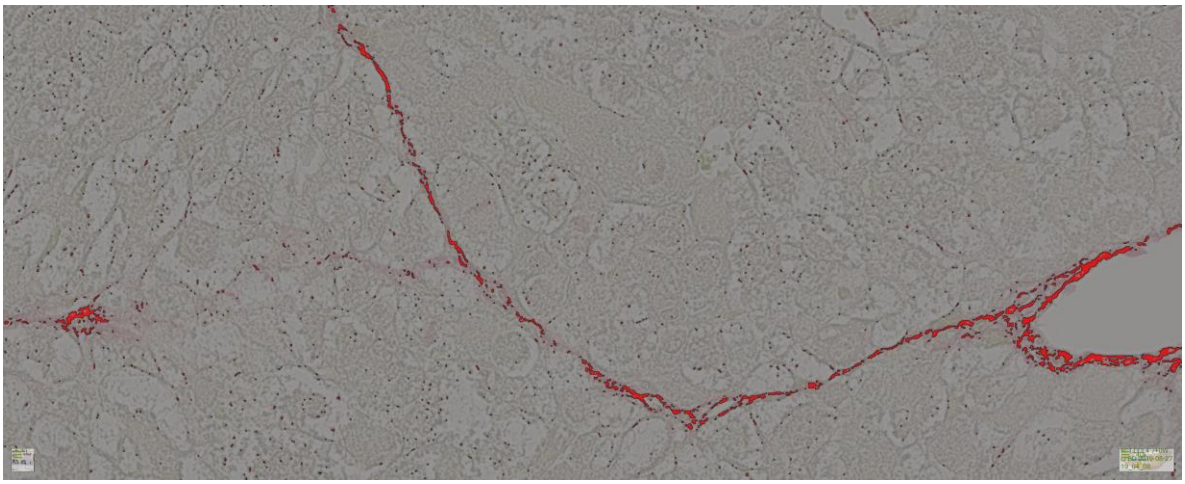
threshold = 50



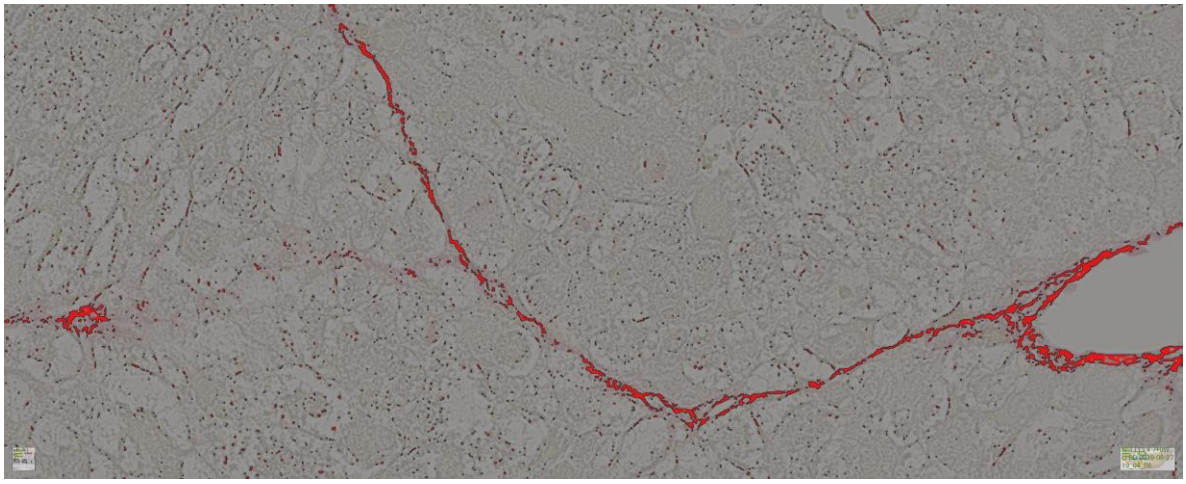
threshold = 60



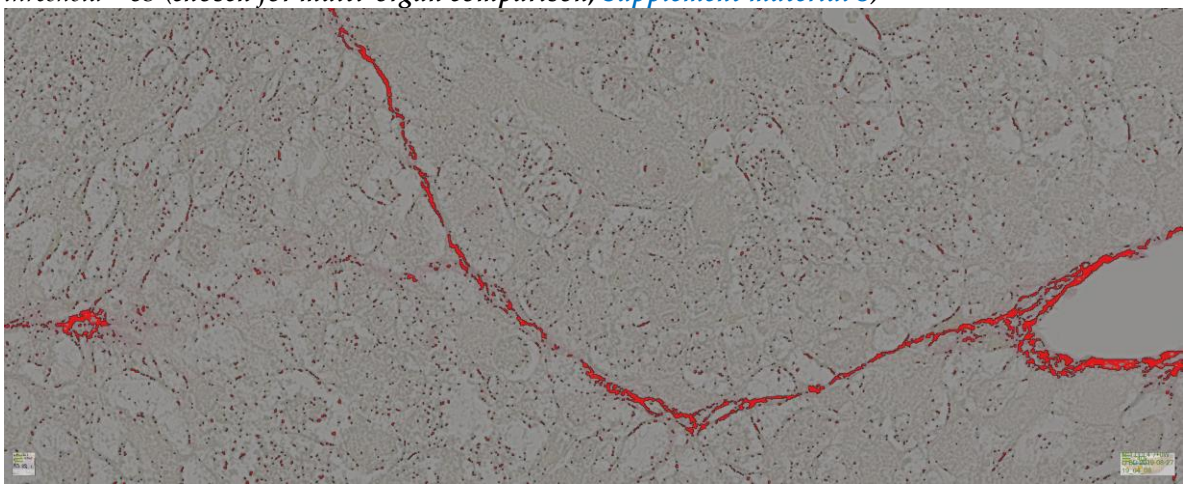
threshold = 70



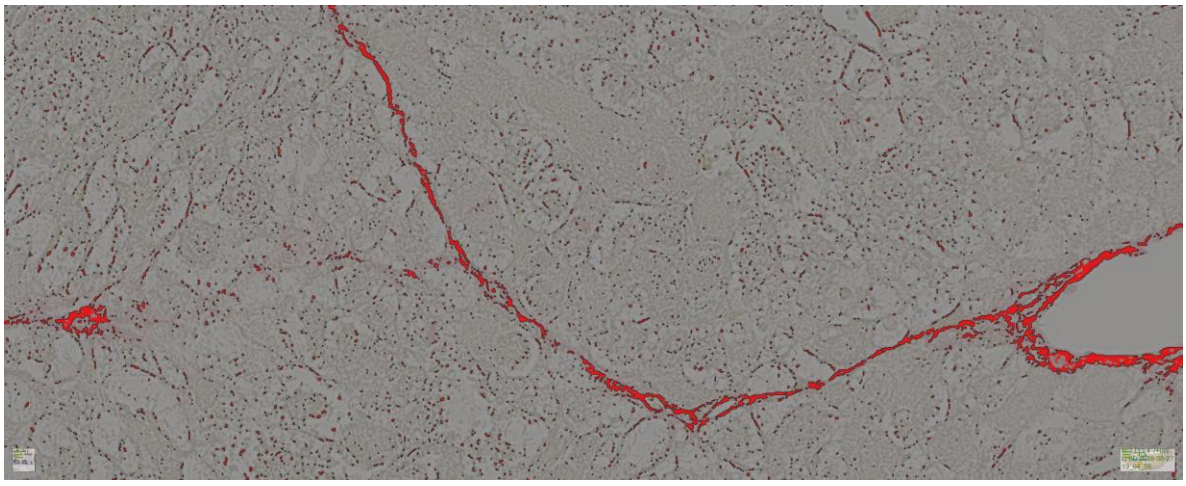
threshold = 80



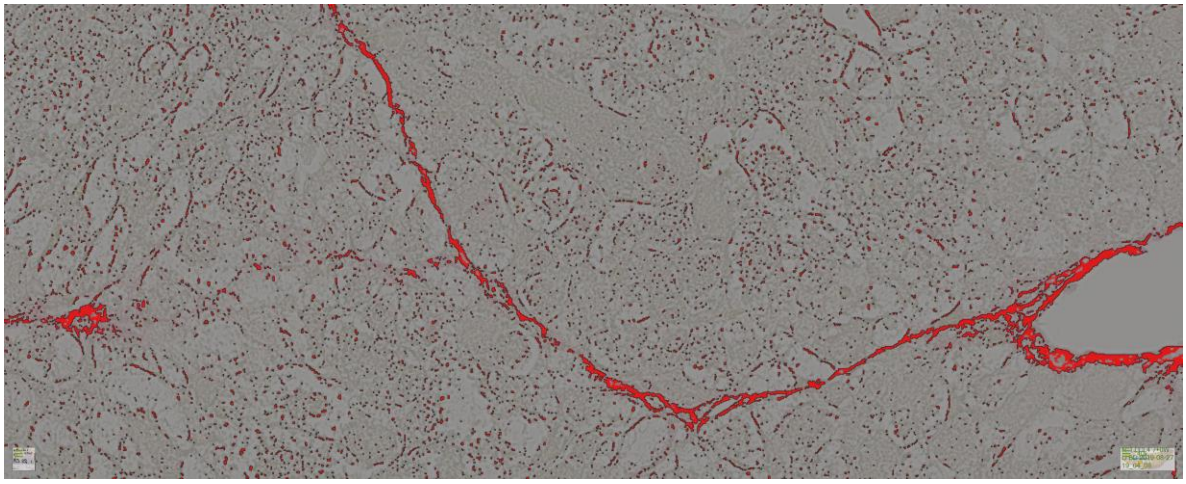
threshold = 85 ***(chosen for multi-organ comparison, Supplement material 3)***



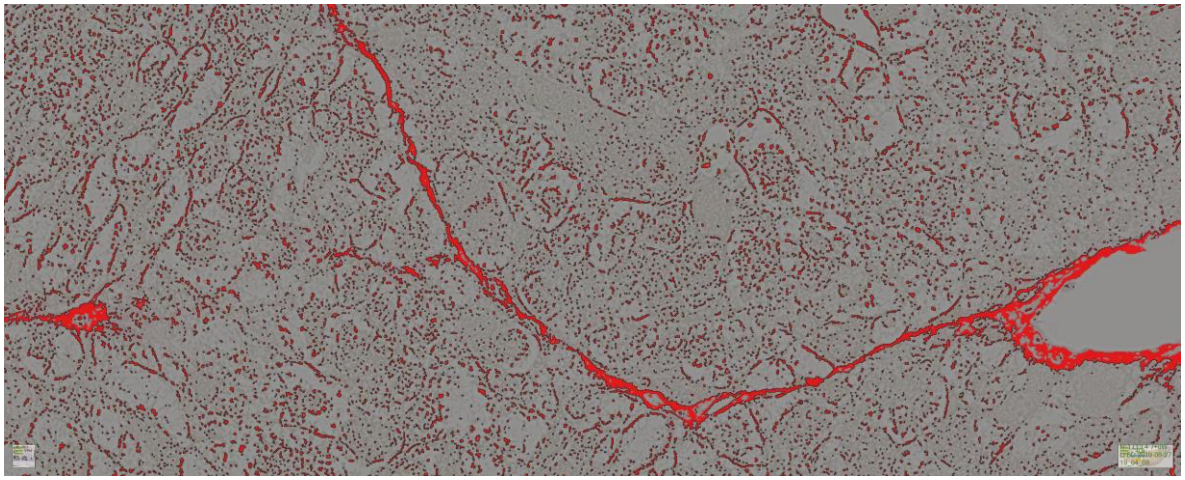
threshold = 90



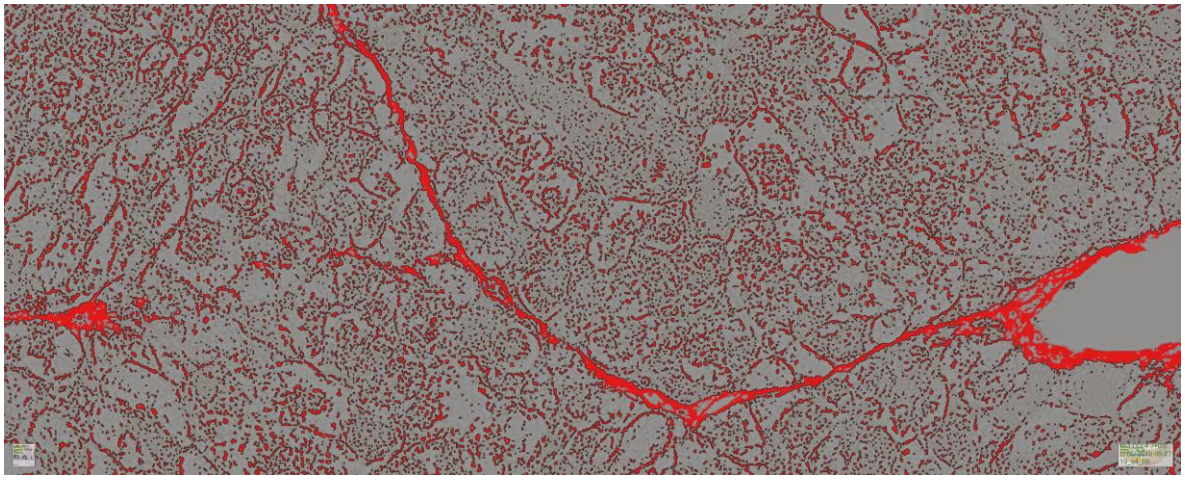
threshold = 100



threshold = 120

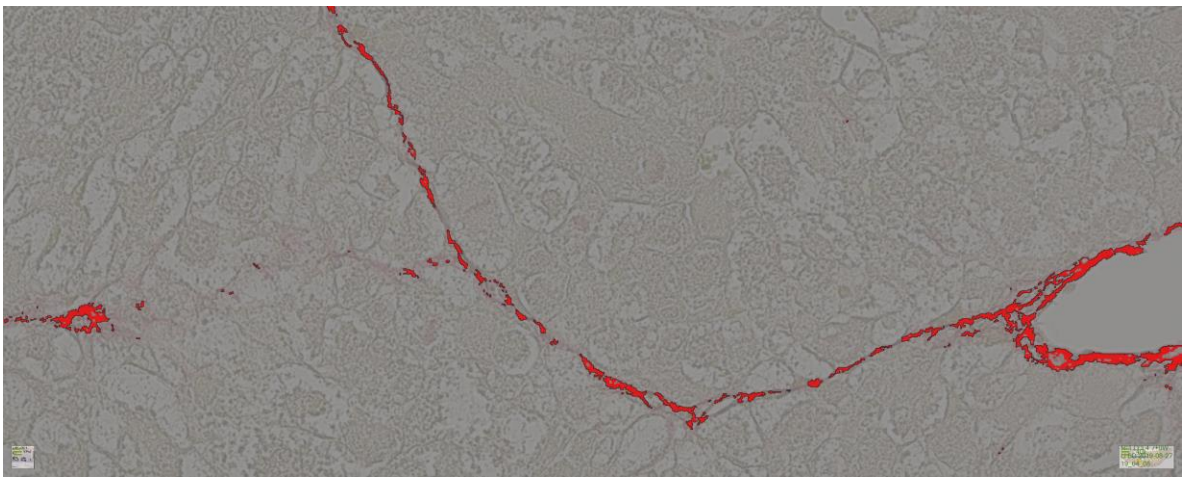


threshold = 140



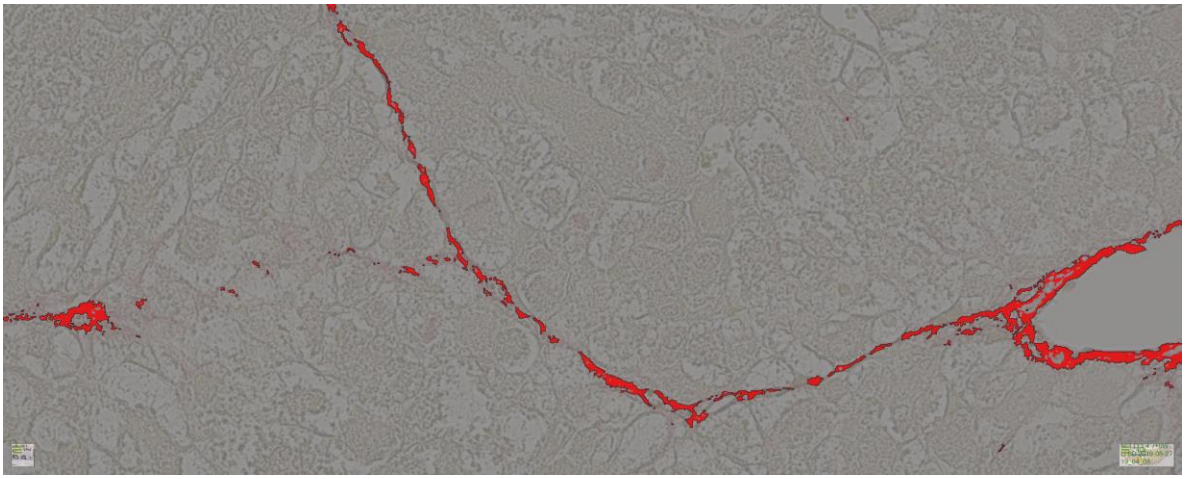
66 ***B. PSR optimized method***

67 *threshold = -20*



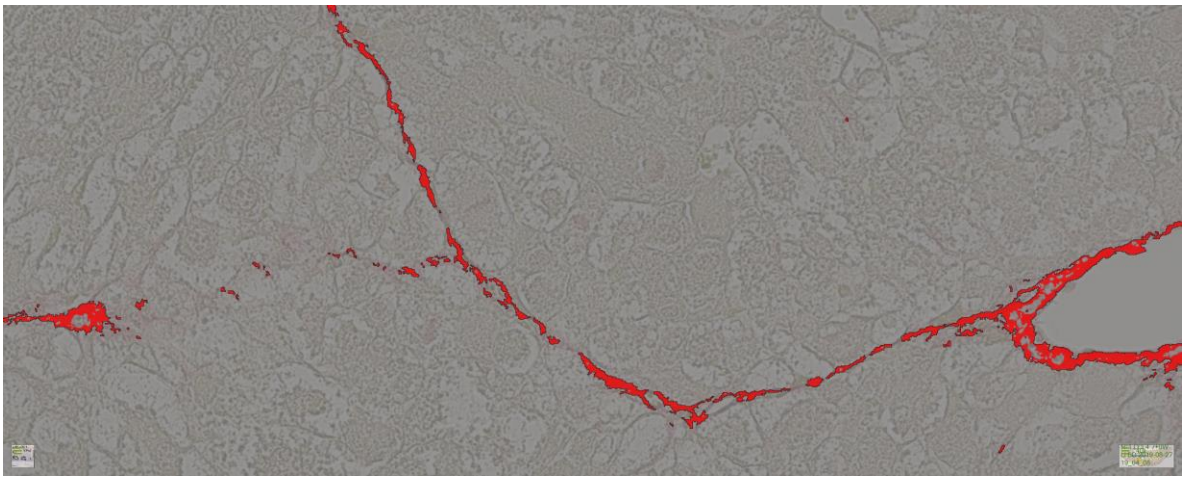
68

69 *threshold = 0*



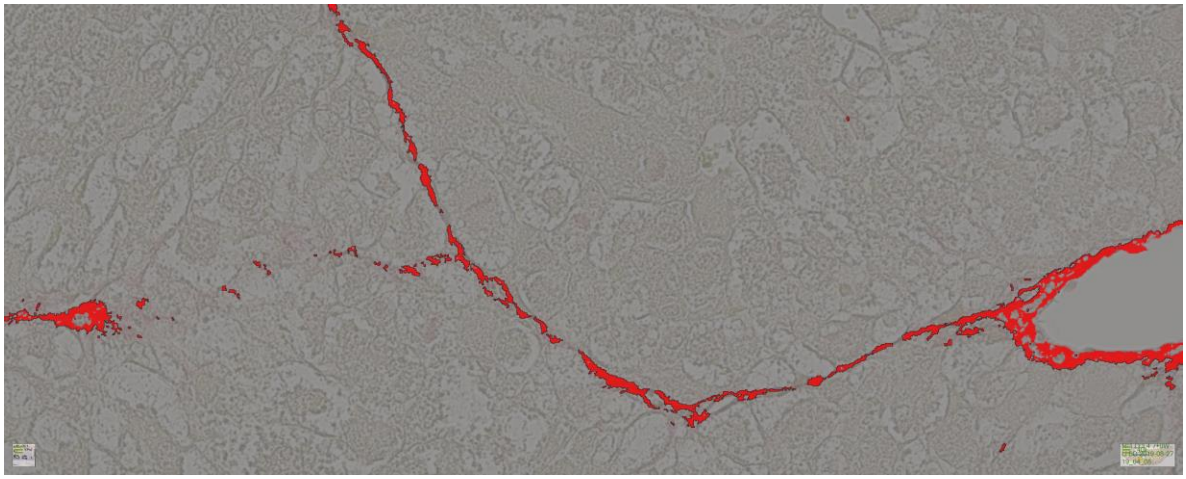
70

71 *threshold = 20*

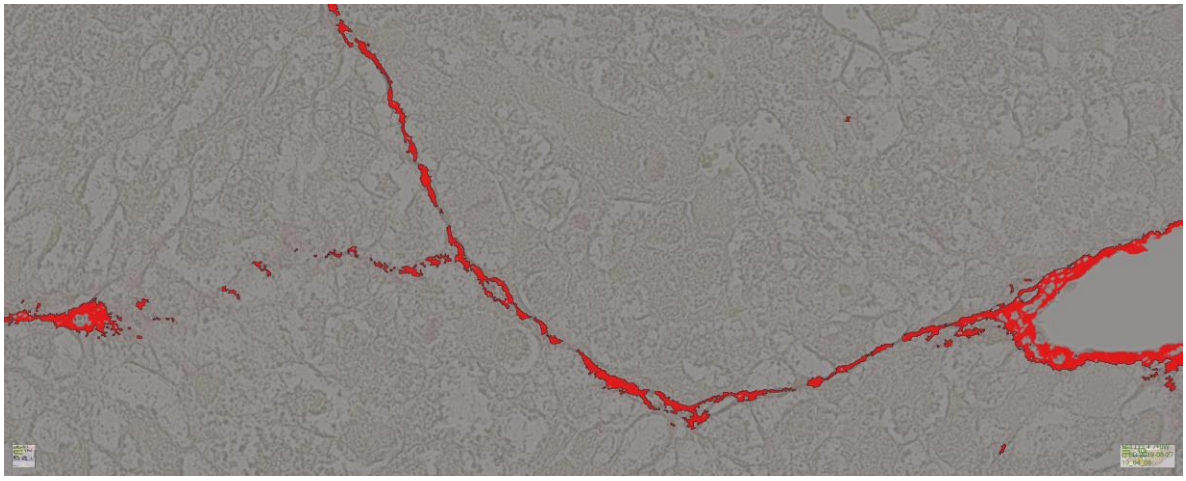


72

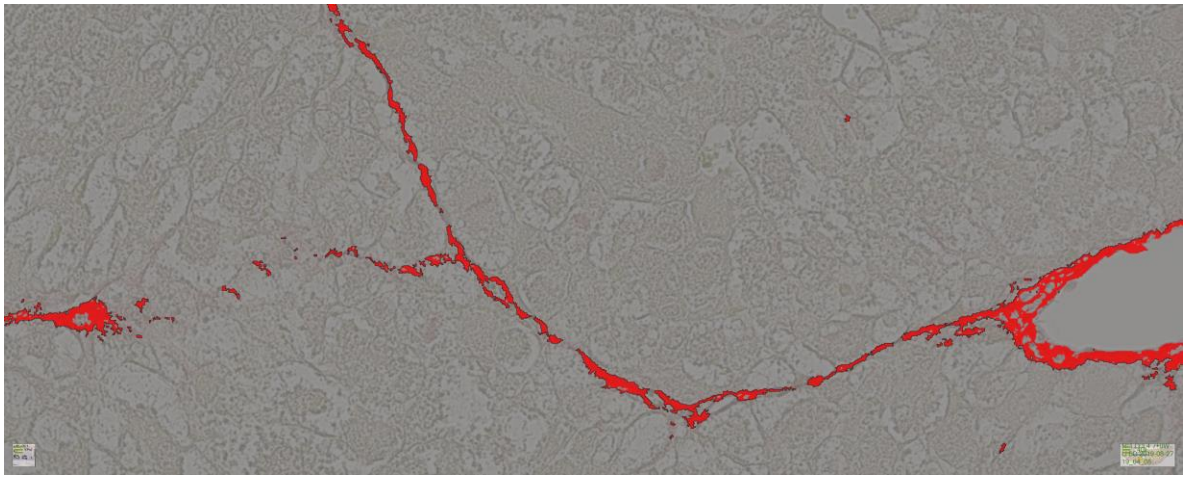
threshold = 30



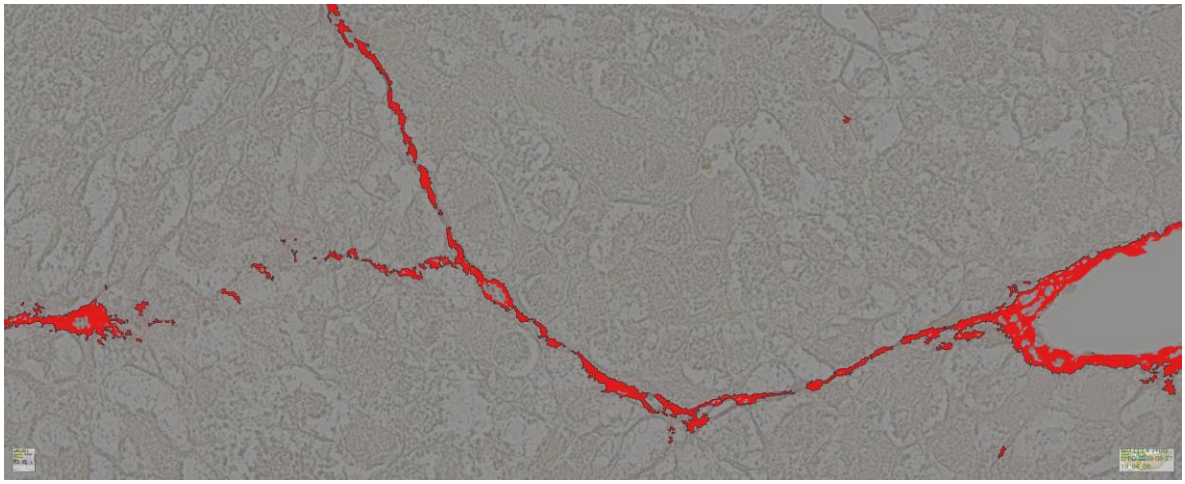
threshold = 40



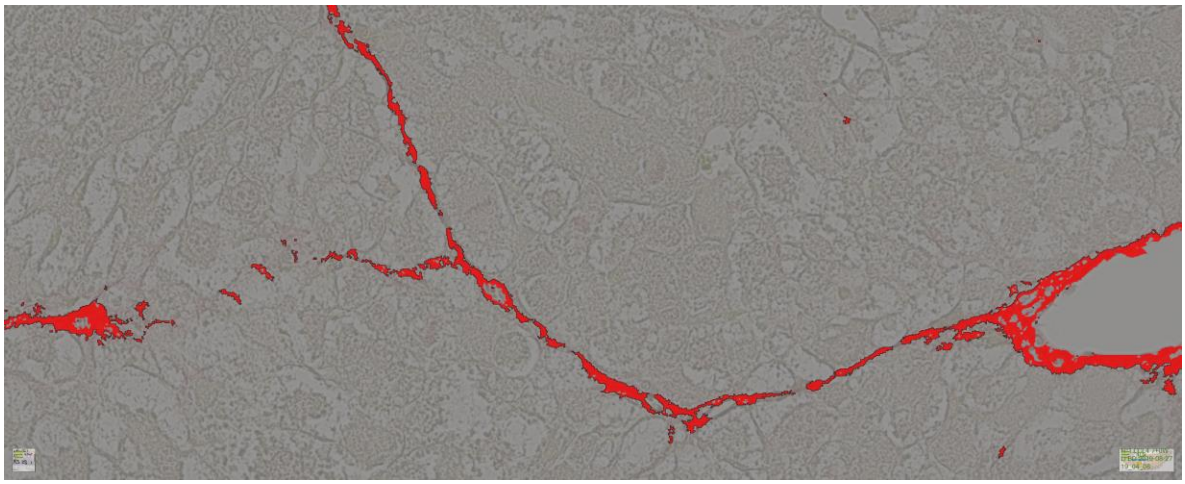
threshold = 50



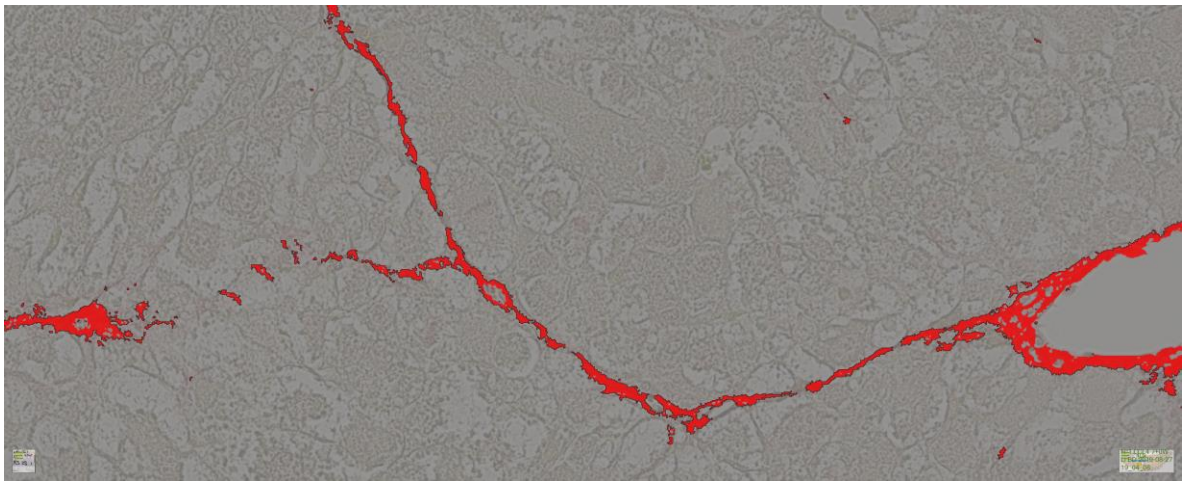
threshold = 60



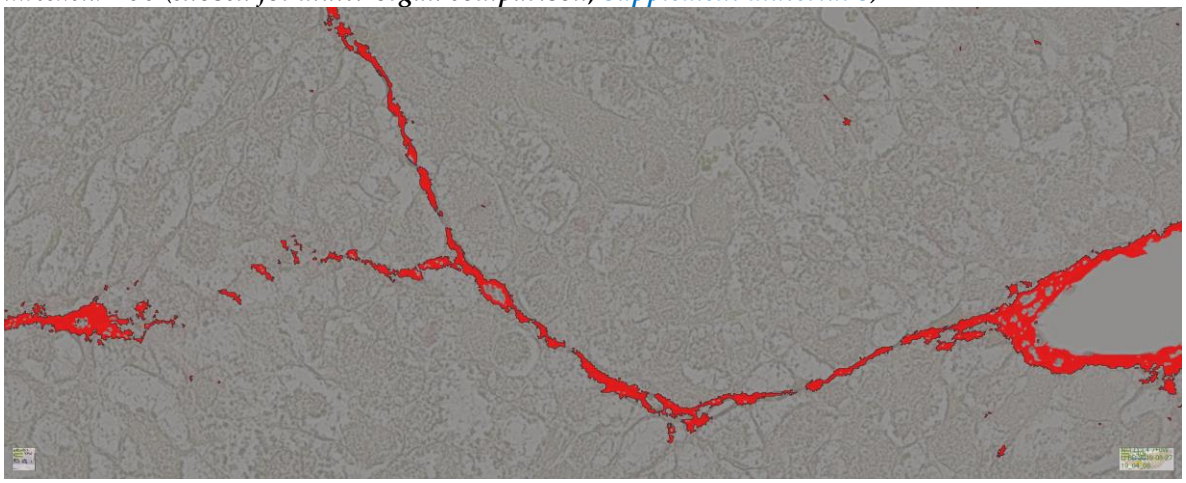
threshold = 70



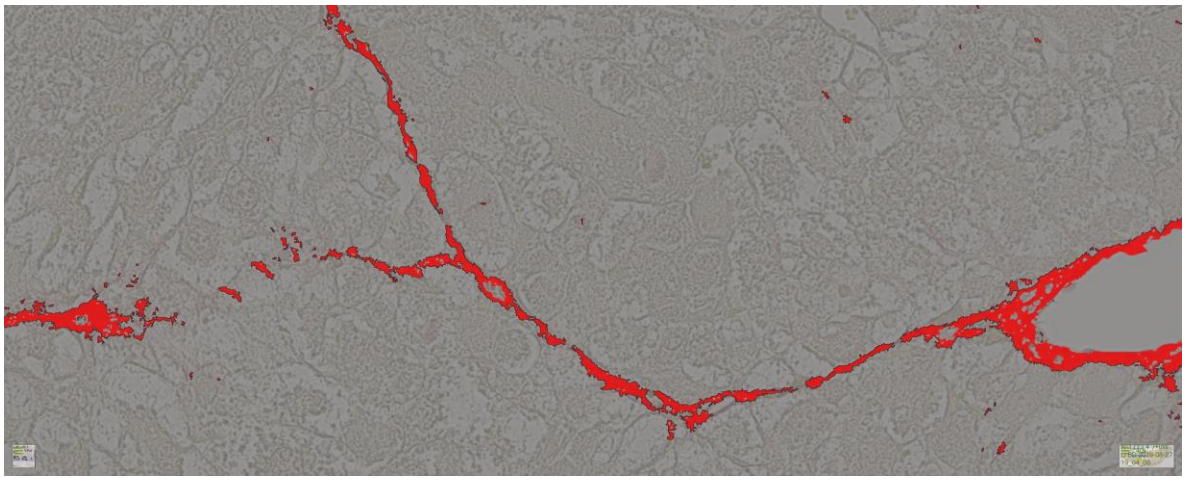
threshold = 80



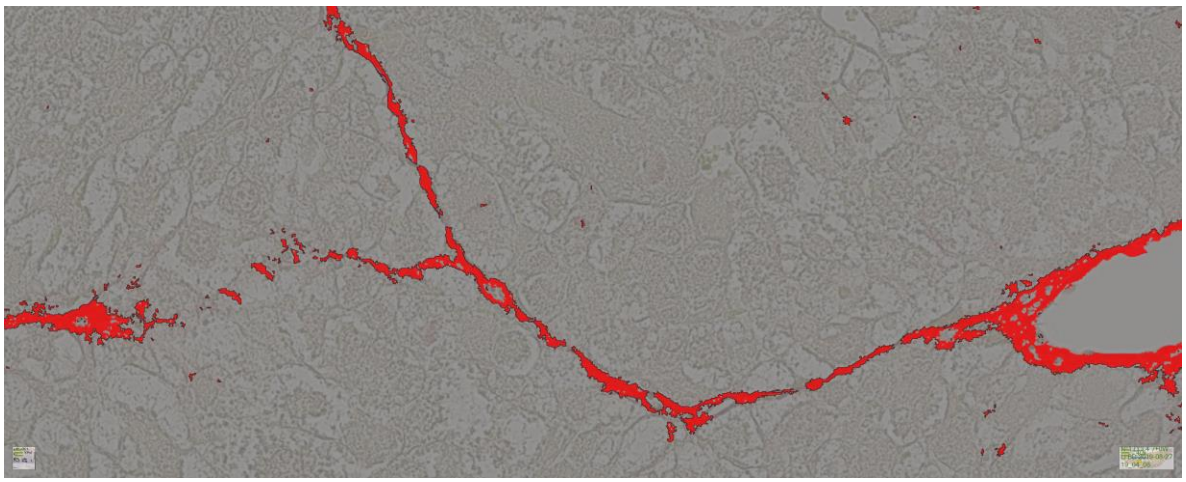
threshold = 90 ***(chosen for multi-organ comparison, Supplement material 3)***



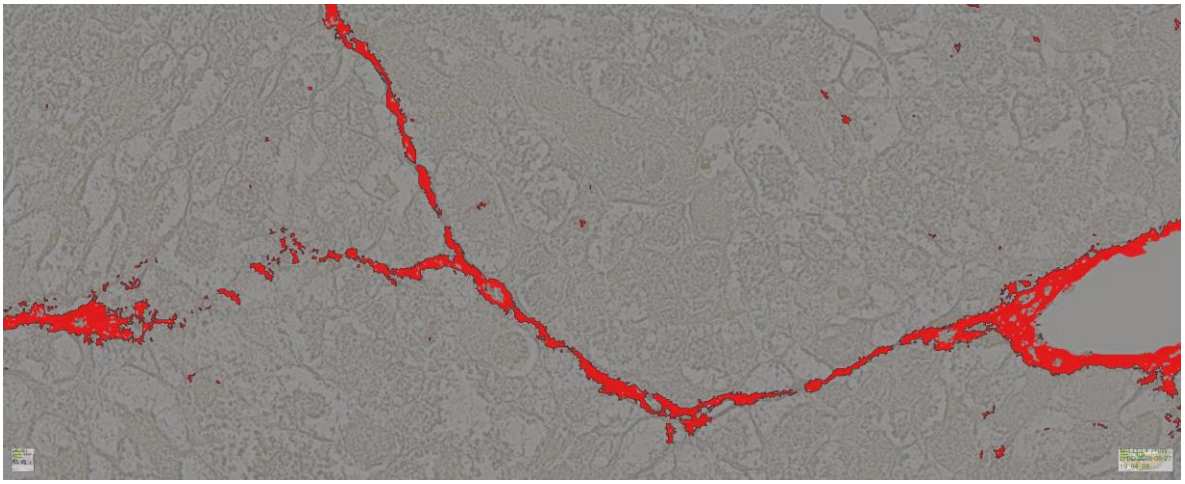
threshold = 100



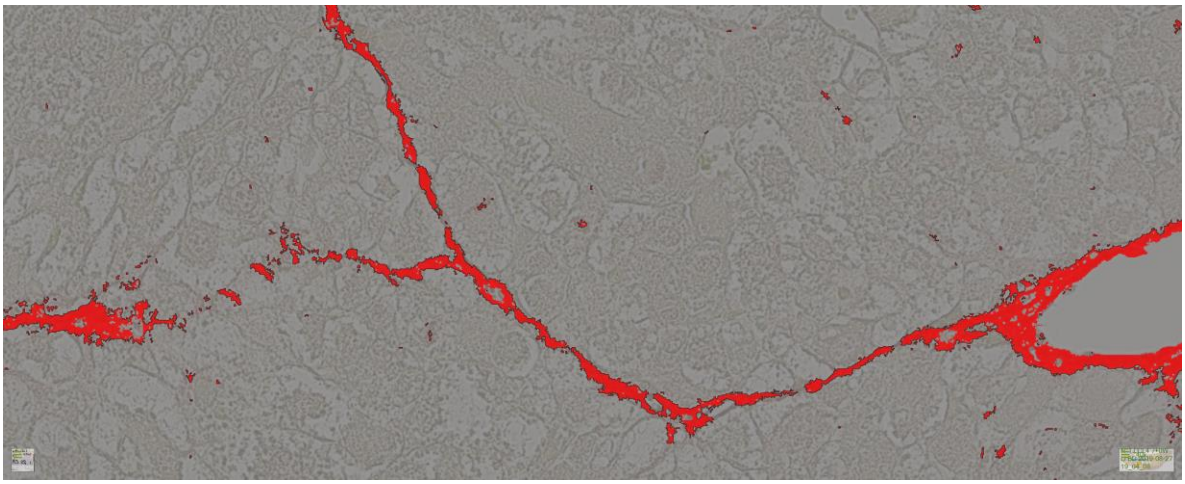
threshold = 110



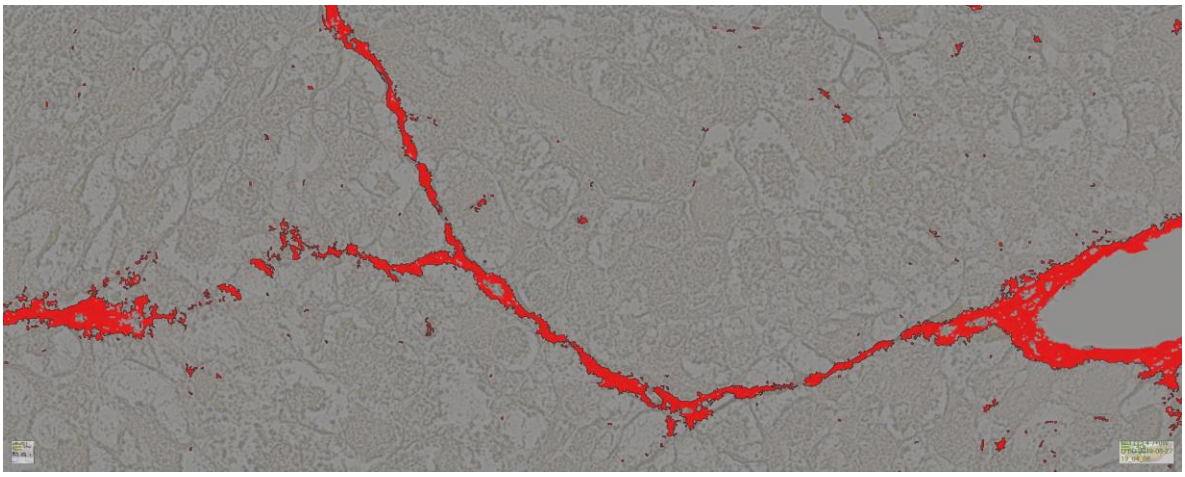
threshold = 120



threshold = 130



threshold = 140



D. Comparison of the detection methods

An example is given for the detection of discrete liver fibrosis. Signal is either detected using the PSR_{RGB} or the PSR_{OPT} filtering. Using a common threshold defined to best fit all organs, PSR_{RGB} provided incomplete (yellow arrow) or irrelevant results (white arrow) (**B**). The accuracy of detection was improved with PSR_{OPT} method (**C**). Based on PSR_{OPT} detection method, it was possible to segment the fibers and to apply an automated classification based on the staining intensity and pixel homogeneity (**D**).