# Novel image analysis and immunohistochemistry advances to accompany pathologist driven NAFLD diagnosis

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Poster n°526

## 1| Introduction

Global prevalence of non-alcoholic fatty liver disease (NAFLD) is 25.24% in 2016 [1], a percentage that has rapidly increased over the past decade. There exist two types of NAFLD, non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). Both subtypes are associated with lipid accumulation in the liver, the latter being more severe with inflammatory cell infiltration, fibrosis and subsequent hepatocyte damage and impaired organ function. The current gold standard for NAFLD diagnosis is liver biopsies evaluated by experienced pathologists who assign scores for several features (fibrosis, steatosis, inflammation and ballooning). However, documented inter-pathologist variability in scoring and semi-quantitative nature of the scoring system itself highlight the need for new methods to ensure the unbiased and consistent assessment of disease.

## 2| Aim

Develop image analysis solutions using Visiopharm® software to provide a more quantitative and reproducible analysis of the liver contexture based on:

- Fibrosis, steatosis, ballooning and inflammation analysis using Masson's Trichrome (MT) and hematoxylin and eosin (HE) staining.
- Quantification of CD45 for inflammation, CD138 for plasma cells detection, and adipophilin to help in steatosis detection.
- Quantification of CK8/ CK18 expression to help in the identification of ballooned cells.

## 3| Method

### HE & Masson's Trichrome

- A digital pathology approach involving 30 needle core biopsies stained with HE and MT has been developed.
- A pathologist evaluated the classic diagnostic parameters on these matched stains for each liver sample to serve as the gold standard.
- A threshold-based application has been developed with Visiopharm® software to quantify fibrosis and steatosis on Masson's Trichrome stained slides with help from the pathologist's annotations.

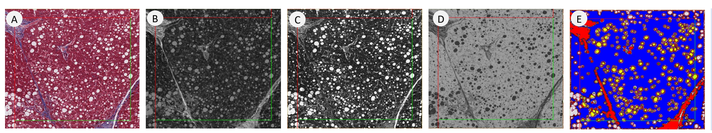


***Figure 1| Overview of implemented feature used in MT APP to classify image pixels into different classes according to their color.***

(A) Masson's Trichrome stained image.
(B) Visiopharm Feature (Contrast-Red-Blue) used to separate Fibrosis Vs Tissue.
(C) Visiopharm Feature (Green) used to separate Tissue Vs Image Background.
(D) Visiopharm Feature (Contrast Red-Green) used to separate Lipids droplets Vs Tissue.
(E) Visiopharm segmentation mask showing Fibrosis in Red, Tissue in Blue, Micro droplet (<65µm²) in Green and Macro droplet (> 65µm²) in Yellow.20X magnification

- A pre-Build APP (#10154) based on deep learning, has been used as a start point to enhance the analysis workflow on HE staining according to the Kleiner scoring system [2]. This APP was tailored on a predefined sampling by annotating several examples for inflammation nuclei, ballooning cells and hepatocytes with and without lipids. Annotations were reviewed by a pathologist before using them to continue the algorithm training.

- Statistical analysis were performed at CRM using JMP software to evaluate the correlation of image analysis results with the pathologist results.

### Multiplex Panels

- A deep learning APPs have been developed for each IHC panel. Manual annotations were required to train the algorithm how to distinguish hepatocytes that were positive for each marker from those that were negative.

## 4| Results & Conclusions

### Masson's Trichrome Analysis

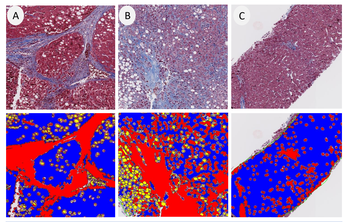


***Figure 2| A Threshold Based Approach for MT quantification***

**On the Top**: MT stained liver tissues. **On the bottom**: Visiopharm segmentation mask showing Fibrosis in Red, Tissue in Blue, Micro droplet (<65µm²) in Green and Macro droplet (>65µm²) in Yellow.
(A): Slide ID 22.01.0154V.001/01.002, (B): Slide ID 22.01.0156V.004/01.002, (C) Slide ID 22.01.0156V.009/01.002.20X magnification

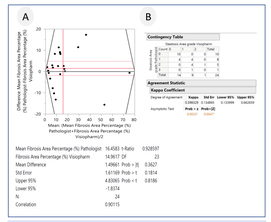


***Figure 3| Statistical analysis with JMP software***

(A) Matched pairs analysis for fibrosis comparison between pathologist and image analysis data.

(B) Contingency table for steatosis grade comparison between pathologist and image analysis data.

Statistical analysis demonstrates a good correlation of fibrosis evaluation between Visiopharm APP and pathologist analyses (Correlation = 90%). However, agreement between both method for steatosis evaluation is not satisfactory (Kappa = 0.39).

### CD45/ CD138/ Adipophilin IHC

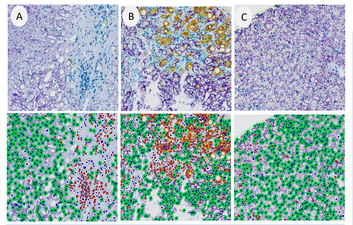


***Figure 4| Deep learning approach for CD45/ CD138/ Adipophilin Panel***

**On the Top**: CD45 (Teal)/ CD138 (Purple)/ Adipophilin (Yellow) chromogenic Multiplex IHC stained liver tissues. **On the bottom**: Visiopharm segmentation mask showing positive cells for CD45 in Red, CD138 in Green, Adipophilin in Orange and negative cells in white. Heptaocytes nuclei are labelled in blue. (A): Slide ID 22.09.0068V.001/01.003, (B): Slide ID 22.09.0069V.001/01.003, (C) Slide ID 22.09.0070V.002/01.003.20X magnification.

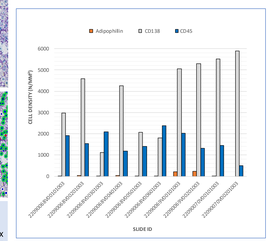


***Figure 5| Data obtained using Visiopharm APP for CD45/CD138/Adipophilin quantification***

Good accuracy is obtained for cell segmentation and positivity assessment of CD45, CD138 whereas Adipohillin staining can be underestimated in some regions due to a membranous vesicular pattern (difficult to assign to a cell). All samples have an equivalent inflammation degree. CD138 is more expressed in 4 out of 10 samples which suggest that these high levels are associated to patients with NAFLD [3].

### HE analysis

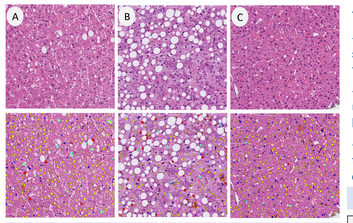


***Figure 6| Deep learning approach for HE quantification***

**On the Top**: HE stained liver tissues. **On the bottom**: Visiopharm segmentation mask showing Hepatocytes with lipids in red, Hepatocytes without lipids in yellow, Inflammation nuclei in blue, inflammatory foci in orange, potential ballooned cell in Cyan. (A): Slide ID 22.07.0006V.002/01.001, (B): Slide ID 22.07.0006V.007/01.001, (C) Slide ID 22.07.0006V.014/01.001.20X magnification

The output variables are:

- Inflammation score: A score in the interval [0,3] using the foci density.

- Steatosis score: A score in the interval [0,3] using the percentage of hepatocytes with lipids.

-Ballooning score (Experimental): A score in the interval [0,1] using the ballooning cell density.

***Table 1| Scoring table used by Visiopharm APP***

| Foci Density (N/mm²) | Inflammation Score | Hepatocytes with lipids (%) | Steatosis Score | Ballooning Density (N/mm²) | Ballooning score (Experimental) |
|---|---|---|---|---|---|
| < 0 | 0 | 0-5 | 0 | < = 10 | 0 |
| < = 20 | 1 | 5-33 | 1 | | |
| < = 40 | 2 | 33-66 | 2 | > 10 | 1 |
| > = 20 | 3 | 66-100 | 3 | | |

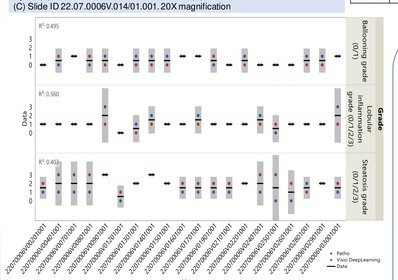


***Figure 7| Statistical analysis with JMP software***
Comparison of ballooning, inflammation and steatosis grade between image analysis and pathologist results on 20 livers tissues.

Overall, the comparison between pathologist and Image analysis shows an intermediate satisfaction level for all NAS scoring features ($R^2 > 0.4$).

QCs on image analysis masks reveals that some errors occurred throughout the cell classification process especially for inflammatory nuclei which can sometimes lead to overestimation.

Note that these are preliminary results of the APP development and optimization steps are ongoing to improve accuracy and robustness by adding more annotated features to train the algorithm and sourcing additional NAFL/NASH samples.

### CK8/CK18 IHC

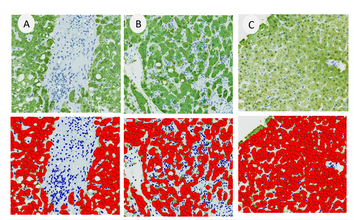


***Figure 8| Deep Learning approach for CK8/CK18 Panel***

***On the Top**: CK8/CK18 chromogenic Multiplex IHC stained liver tissues.*
***On the bottom**: Visiopharm segmentation mask showing positive cells for CK8/CK18 in Red and negative cells in blue. (A): Slide ID 22.09.0068V.001/01.001, (B): Slide ID 22.09.0069V.001/01.001, (C) Slide ID 22.09.0070V.002/01.001.20X magnification*

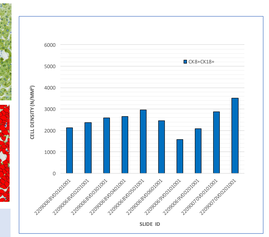


***Figure 9| Data obtained using Visiopharm APP for CK8/CK18 quantification***

The obtained results show a good accuracy of cell segmentation and positivity assessment for CK8/ CK18 positivity. Moderate to strong expression level is detected overall samples. The loss of CK8/CK18 expression allows for a facilitated identification of ballooned hepatocytes that may increase the accuracy of distinguishing NASH from NAFL.

### Conclusion

We demonstrate an image analysis approach to provide a toolbox for pathologists to aid in a more rapid and reproducible classification of NAFLD based on multiple combination of non-invasive staining/markers.

Future works will focus on optimizing our image analysis APPs to validate this method for future use in clinical trials.

## 5| References

[1] Younossi ZM, K. AB, and al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016, 64:73-84.
[2] Kleiner, D. E, and al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology, 2005, 41 (6), 1313–1321.
[3] Yusuf Yilmaz, F.E, and al. Hepatic expression and serum levels of syndecan 1 (CD138) in patients with nonalcoholic fatty liver disease, Scandinavian Journal of Gastroenterology, 2012, 47:12, 1488-1493.

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