METHOD AND SYSTEM OF HISTOLOGICAL CHARACTERISATION OF A LIVER

Field of the Invention

The present invention relates to a method for portal tract detection and characterisation. In particular, the present invention relates to a method and system to determine metrics of portal tracts, anatomical micro-components, and associated regions in liver histology slides, of multiple different stains.

Background

Portal tracts, also referred to as portal triads, are conglomerations of anatomical micro-components present in the liver. The anatomical micro-components of which the portal tracts are composed comprise a hepatic artery, a portal vein, and a bile duct surrounded by connective tissue. The micro-components facilitate the flow of nutrient rich blood (portal vein), oxygenated blood (hepatic artery) and bile (bile duct) through the liver.

Traditionally, liver microanatomy is divided into three meta-biologically differentiated acinar zones, based on blood supply and distance between a portal tract and its closest central veins. The acinar zones are found within the acinus, which is a diamond-shaped area within each hepatic lobule. The portal tracts exist at the corners of each acinus. The zone that is furthest from the central vein is denoted zone one and receives the most oxygenated blood and nutrients. The zone that is closest to the central vein is denoted zone three and receives the least oxygenated blood and nutrients.

The location of certain disease features within each of the acinar zones can be indicative of disease severity. This is evident with the presence of hepatocellular injury, inflammation, fibrosis and fat deposits found in the acinar zone three [S. Hübscher, Histological assessment of non-alcoholic fatty liver disease, Histopathology, 49 (2006) 450-465].

Portal tracts are important conglomerations to identify in liver histology slides because their structure and location inform pathologists' evaluation of patient health. For example, in Metabolic Associated SteatoHepatitis (MASH), portal inflammation has been associated with poorer clinical outcomes. Portal inflammation is defined as inflammation confined to the portal region of the liver tissue.

Portal tract detection is also an important part of biopsy sample quality control in liver pathology, both in clinical trials and in a routine diagnostic setting. Among other criteria (such as the minimum length of biopsy core, an absence of folding etc.), to be deemed adequate for assessment, a biopsy slide must have a sufficient number of portal tracts. The number required varies depending on the setting, but guidance issued by the British Society of Gastroenterology, Royal College of Pathologists and American Association for the Study of Liver Diseases specifies a range of 6-11 portal tracts per sample as being sufficient with some studies suggesting the presence of over 11 complete portal tracts constitutes an adequate sample [A. Boyd, O. Cain, A. Chauhan, G.J. Webb, Medical liver biopsy: background, indications, procedure and histopathology, Frontline gastroenterology, 11 (2020) 40-47; E. Cholongitas, M. Senzolo, R. Standish, L. Marelli, A. Quaglia, D. Patch, A.P. Dhillon, A.K. Burroughs, A systematic review of the quality of liver biopsy specimens, American journal of clinical pathology, 125 (2006) 710-721; D.C. Rockey, S.H. Caldwell, Z.D. Goodman, R.C. Nelson, A.D. Smith, Liver biopsy, Hepatology, 49 (2009) 1017-1044; A.R. Crawford, X.-Z. Lin, J.M. Crawford, The normal adult human liver biopsy: a quantitative reference standard, Hepatology, 28 (1998) 323-331].

Six full portal tracts can be found per linear centimeter of liver tissue [Crawford AR, Lin X-Z, Crawford JM. The normal adult human liver biopsy: a quantitative reference standard. Hepatology. 1998;28(2):323-331]. The longer the liver biopsy, the greater the number of portal tracts across the specimen and therefore the more reliable the grading and staging of liver disease progression. Many trials mandate the rejection of biopsy samples that do not meet these criteria.

Detection and identification of portal tracts is challenging because they vary greatly in their size, structure, shape and overall appearance. The location and angle of the biopsy needle insertion also affects the appearance of portal tracts on a slide.

The distribution and appearance of portal tracts is affected by most liver diseases, with the impact increasing with disease severity. This is apparent in the development of fibrotic bridges which grow gradually from portal tract to portal tract.

Fibrosis scoring is affected by the quantity, structure and location of collagen deposits in a connective tissue-stained liver histology slide. High scores are given to slides where bridging is present. Bridging is defined as the connection of two or more portal

tracts through a bridge of collagen.

Fibrosis regression is categorised via a scale (F0-4) where F0 indicates no fibrosis, F1 portal fibrosis without septa (bands of scar tissue), F2 portal fibrosis with few septa, F3 numerous septa without cirrhosis and F4 cirrhosis.

Fibrosis regression at later stages (F3 and F4) will exhibit rounded and/or interrupted bridges with or without aligned septal ends, suggesting where complete bridges were previously visible, as well as the repopulation of scarred areas with hepatocytes. The majority of fibroseptal stroma will show signs of hepatic repair complex with densely compacted stroma that is heavily stained and largely acellular [S. Khan, R. Saxena, Regression of hepatic fibrosis and evolution of cirrhosis: a concise review, Advances in anatomic pathology, 28 (2021) 408-414].

Fibrosis regression at earlier stages (F1 and F2) will show a lack of perisinusoidal/pericellular fibrosis.

Fibrosis regression is often a marker of drug efficacy in liver diseases [A.J. Sanyal, Q.M. Anstee, M. Trauner, E.J. Lawitz, M.F. Abdelmalek, D. Ding, L. Han, C. Jia, R.S. Huss, C. Chung, Cirrhosis regression is associated with improved clinical outcomes in patients with nonalcoholic steatohepatitis, Hepatology, 75 (2022) 1235-1246] even with AI-based metrics being utilised in clinical trial endpoints [R. Loomba, P. Bedossa, K. Grimmer, G. Kemble, E.B. Martins, W. McCulloch, M. O'Farrell, W.-W. Tsai, J. Cobiella, E. Lawitz, Denifanstat for the treatment of metabolic dysfunction-associated steatohepatitis: a multicentre, double-blind, randomised, placebo-controlled, phase 2b trial, The Lancet Gastroenterology & Hepatology, (2024)].

Fibrosis quality is also a potential surrogate endpoint that has been shown to be an accurate metric in Chronic Hepatitis B treatment [Y. Sun, W. Chen, S. Chen, X. Wu, X. Zhang, L. Zhang, H. Zhao, M. Xu, Y. Chen, H. Piao, Regression of Liver Fibrosis in Patients on Hepatitis B Therapy Is Associated With Decreased Liver-Related Events, Clinical Gastroenterology and Hepatology, 22 (2024) 591-601. e593].

The process of defining a boundary of anatomical micro-components present in the liver or a boundary of conglomerations of the micro-components such portal tracts and fibrotic regions is known as segmentation or delineation. The boundary can be represented in different ways, for example as a set of coordinates for a polygonal

representation of the boundary of each instance of one of the micro-components or conglomerations. They can also be represented as mask images where the pixel dimensions match those of the original image and the value of each pixel in the mask determines whether it is inside an instance of the structure of interest (foreground) or outside the structure (background). Delineations, whether as polygons or masks, can be created using manual processes (for example using software with a set of drawing tools) or using automated models (for example using convolutional neural networks).

The segmentation of portal tracts enables investigation into other metrics of disease progression. This includes the delineation between lobular and portal inflammation, fat deposition, and the location and extent of fibroses.

Regions with an increased density of white blood cells are associated with inflammation. A single contiguous region of tissue in which the density is consistently above a certain level is described as a focus of inflammation. Very small structures, such as the nuclei of white blood cells can be represented by masks as well as by a set of coordinates to represent the centres of each of the nuclei. Socha et al provide an example of how a focus of inflammation is measured and quantified (P. Socha, E. Shumbayawonda, A. Roy, C. Langford, P. Aljabar, M. Wozniak, S. Chełstowska, E. Jurkiewicz, R. Banerjee, K. Fleming, Quantitative digital pathology enables automated and quantitative assessment of inflammatory activity in patients with autoimmune hepatitis, Journal of Pathology Informatics, 15 (2024) 100372]).

Measures of steatosis, inflammation and fibrosis are generally reported as averages across the slide. This precludes measurement of heterogeneity of the presence of features across the slide. However, heterogeneity is a clinically important consideration, as different liver diseases exhibit different patterns of portal inflammation, lobular inflammation, and interface hepatitis.

Several commercially available automated methods of segmentation have been applied to liver histopathology in various capacities. Some use second harmonic generation (SHG) and two photon excitation fluorescence (TPFE) microscopy to differentiate between portal and lobular regions and to identify the portal tract and central vein to detect inflammation and quantify fibrosis (Y. Wei, M. Zhang, J. Zhang, X. Teng, Q. Yang, A. Leong, G. Ho, H. Lu, D. Xu, Clinical relevance of an animal model of non-alcoholic steatohepatitis (NASH) and digital pathology with artificial intelligence

(DP-AI) analyses of hepatic fibrosis, Journal of Hepatology, 77 (2022) S679-S680). This has been applied to assessing fibrosis dynamics in MASH patients with bridging fibrosis and fibrosis regression to evaluate drug treatment efficacy or lifestyle intervention using a qFibrosis metric (N.V. Naoumov, D.E. Kleiner, E. Chng, D. Brees, C. Saravanan, Y. Ren, D. Tai, A.J. Sanyal, Digital quantitation of bridging fibrosis and septa reveals changes in natural history and treatment not seen with conventional histology, Liver International, (2024); H.Y. Yuan, X.F. Tong, Y.Y. Ren, Y.Y. Li, X.L. Wang, L.L. Chen, S.D. Chen, X.Z. Jin, X.D. Wang, G. Targher, AI-based digital pathology provides newer insights into lifestyle intervention-induced fibrosis regression in MASLD: An exploratory study, Liver International, (2024)).

Another of the commercially available automated methods of segmentation assesses MASH/MAFLD in preclinical rodent models of NASH. The automated methodology relies on morphometric recognition and entails use of picrosirius red (PSR) stain for the quantification of fibrosis, although it is unclear if portal tracts are detected for analysing fibrosis. Haematoxylin and Eosin (H&E) slides and F4/80 (a widely used antibody marker for macrophages in murine models) are both used to quantify inflammation. However, a 2020 article suggests they do not split portal and lobular inflammation and have since started using significantly more immunohistochemistry (IHC) staining (M. De Rudder, C. Bouzin, M. Nachit, H. Louvegny, G. Vande Velde, Y. Julé, I.A. Leclercq, Automated computerized image analysis for the user-independent evaluation of disease severity in preclinical models of NAFLD/NASH, Laboratory investigation, 100 (2020) 147-160).

Separate portal and lobular AI models are used by Vanderbeck et al. in Wisconsin for detection of lobular inflammation (S. Vanderbeck, J. Bockhorst, D. Kleiner, R. Komorowski, N. Chalasani, S. Gawrieh, Automatic quantification of lobular inflammation and hepatocyte ballooning in nonalcoholic fatty liver disease liver biopsies, Human pathology, 46 (2015) 767-775] and Yu et al., for identifying and quantifying portal tracts [H. Yu, N. Sharifai, K. Jiang, F. Wang, G. Teodoro, A.B. Farris, J. Kong, Artificial intelligence based liver portal tract region identification and quantification with transplant biopsy whole-slide images, Computers in Biology and Medicine, 150 (2022) 106089).

Another notable model is the quantification of fibrosis based on portal tract areas based on intersection with septa and evaluation of zonal fibrotic regions (M. Noureddin, Z.

Goodman, D. Tai, E.L. Chng, Y. Ren, P. Boudes, H. Shlevin, G. Garcia-Tsao, S.A. Harrison, N.P. Chalasani, Machine learning liver histology scores correlate with portal hypertension assessments in nonalcoholic steatohepatitis cirrhosis, Alimentary Pharmacology & Therapeutics, 57 (2023) 409-417).

However, there is no current commercial or other solution that provides an interpretation framework for the assignments each system makes of portal tract segmentations within a sample.

Summary of the Invention

According to a first aspect of the invention there is a Method and System of Histological Characterisation of a Liver comprising all features of claim 1. Further features of the invention are disclosed in dependent claims.

According to an aspect of the invention there is a method to determine liver histology metrics, comprising:

use of segmentations of micro-components that are histological from a biological sample of a liver histology image;

spatially associate those of the micro-components that are within a preselected proximity of each other into a conglomeration of the micro-components that defines a histological component or metric of disease severity; and

association of each the micro-components that are within a preselected proximity of each other with a bile duct, portal vein, hepatic artery in a portal tract to put a confidence in the conglomeration of micro-components above a threshold.

The method provides an interpretation framework for assignments of conglomerations that comprise portal tract segmentations within a sample, comprising means to relate spatial analysis with segmentation procedures in order to explain which known biological elements of a portal tract are present in a resultant segmentation of the conglomeration. Spatial analysis to spatially associate the micro-components may include analysis of patterns of the micro-components or conglomerations or both.

One of the patterns may be a hexagonal arrangement of hepatic lobules. In the pattern there is a portal tract approximately at each one of the six peripheral corners of each

approximately hexagon shaped hepatic module. The pattern of the hexagonal arrangement of hepatic lobules appears as honeycomb of hexagons. Approximately at the centre of each hexagon is a central vein.

Another of the patterns may comprise nested rhombus shapes of acinar zones. Smaller rhombus shapes may be nested within larger rhombus shapes. All of rhombus shapes may have a first pair of opposite corners and a second pair of opposite corners. All of the rhombus shapes may share the first pair of opposite corners. There may be a portal tract at each one of two opposite corners of the first pair. There may be a central vein at each of the two opposite corners of the second pair of the largest rhombus shape. However the smaller rhombus shapes may not have opposite corners that share the central veins because the smaller rhombus shapes are nested within the largest rhombus shape. The nested rhombus shapes may correspond to acinar zones.

Another of the patterns may be a conglomeration in connective tissue of at least one portal vein, hepatic artery, and bile duct within a distance range of one another.

Micro-components or conglomerations or both that are detected and identified may be spatially associated to determine whether they fit within one or more of the patterns. The fit may be determined by relative direction and distance from micro-components and conglomerations previously detected and identified. The patterns may be known to dispose particular micro-components and conglomerations at a respective position within a range of distances and angles with respect to one or more of the micro-components in the pattern. The method of histological characterization may improve as it progresses in analysis of a histological slide to become quicker and more accurate in detecting each next micro-component or conglomeration from a pattern filled in with micro-components or conglomerations already detected.

A micro-component may comprise: tissue foreground, a lobule, a portal tract, connective tissue, a bile duct, an artery, a vein, a fat globule, a periportal space, a sinusoidal space, a limiting hepatic place, a liver cell type or a combination of them.

A confidence in the conglomeration may be determined from an existence of co-localising elements such as co-localised micro-components or micro-components within a preselected proximity of the conglomeration or a segmentation of the conglomeration. Proximity may be determined by a shortest distance from an edge of

the segmentation to an edge of segmentation of the micro-component.

Application of geometric, cartographic, or morphological operations may used to derive spatial domains or patterns from the portal tracts and the component features, wherein the spatial domains or patterns include at least one of lobular areas of the liver, hepatic zones, or interface regions;

Detection and identification of each micro-component or conglomeration of them, or pattern of them, or spatial domain of them may be used to derive liver disease features existing within liver lobules and neighbouring elements.

The detection and identification of the micro-components and conglomerations of them with confidence as the portal tracts in the biological sample, or the spatial domains or pattens of them may be used to provide measurement information or metrics.

Cartographic or morphological analysis or both may be used to spatially associate the micro-components relative to each other or relative to the conglomerations or both.

A presence or absence of spatially associated micro-components, conglomerations of them, or patterns of them may contribute to a metric or confidence score by which the likelihood of the portal tract being 'true'. In this way a candidate portal tract may be identified as being a genuine portal tract. Use of micro-components, conglomerations of the micro-components such as the portal tracts, or use the spatial domains may be in isolation of other spatial features of the biological sample on the histology slide. The portal tracts may be quantified to assess sample adequacy.

Preferably locations of inflammatory cells, ballooned cells, fibrosis, fat globule features, or the connective tissue, are integrated with the lobular areas and the portal tracts to provide spatially differentiated metrics of inflammatory cell number and densities within and external to portal tracts or to identify bridging fibrosis between the portal tracts.

Segmentation models may be used to identify component features of portal tracts. Micro-components or conglomerations of them or a part of them may be segmented with a neural network, convolutional neural network, transformer model, including vision transformer or combination of multiple such networks trained for the purpose. They may be segmented with a neural network comprising a segmentation foundation model, or an autoencoder or transformer capable of learning and producing

embeddings of arbitrary image features. Micro-components or conglomerations of them or a part of them may be segmented with non-neural network based image processing by thresholding, pixel classification, filtering, or feature detection.

Micro-components or conglomerations of them or a part of them may be segmented approximately with the use of a tile-based classification, wherein smaller tiles of a larger image are extracted and classified according to whether the tile contains the presence of each component feature. Micro-components, or conglomerations of them, or parts of the micro-components such as cells they comprise may be identified as bounding boxes, centroids or other summary sets of coordinates and these summary descriptors may spatially associated.

Micro-components, or conglomerations of them, or parts of the micro-components such as cells may be represented as location-based probabilities that the micro-component, conglomeration, or part exists at a location in a probability map. Spatial association of component features is performed for geometries derived from thresholding the probability map at a value. Micro-components or conglomerations of them may be identified through segmentation of their component cell types with a cell segmentation or detection model trained to predict cell types. Micro-components or conglomerations of them that are connective tissues or in connective tissue may be identified as connective tissue cells in proximity with one another or micro-components or conglomerations in proximity with each other. Micro-components or part of them may be detected or identified by the cell types they comprise. For example a bile duct may be identified by cholangiocyte cells in proximity with one another in a micro-component.

Portal tracts and the lobular areas of the liver may be delineated via combination of the component features of the portal tracts and tissue foreground features through logical predicates including taking a union, intersection, or difference. Interface regions may be delineated between portal tracts and lobular areas of the liver through constructive and destructive geometric operations of features including buffering, dilation or erosion. For example, A mid-line between two portal tracts and the lobular area closest to one of the two portal tracts may be found and further subdivided by a radial distance from the portal tract boundary to the other boundary. Zones for analysis in the liver histology image may be delineated by subdivision of the portal tracts and lobular areas of the liver.

The portal tracts, the lobular areas, and interface regions may be distinguished using the portal tract model by generating descriptive statistics.

A distribution of portal tract inflammation, lobular area inflammation, or interface inflammation may be expressed according to at least one of a number of inflammatory foci, a mean area of foci, a portal tract inflammatory cell density, an interface inflammatory cell density, or a lobular area inflammatory cell density.

Preferably identified are the spatial location of neutrophils, eosinophils, lymphocytes (including T and B cells), plasma cells, macrophages (including Kupffer cells), or other inflammatory cell types relative to portal tracts, lobular regions and other liver zones. A location or abundance of communities of cooperating cells defined by statistical frequency, nearest neighbour, 'bag of words' or other techniques, relative to portal tracts, lobular areas, or interface regions may be used.

The portal tracts may be assessed or combined with micro-component in the connective tissue, or the connective tissue or both to identify bridging fibrosis between portal tracts. Shape, length and other morphological characteristics of the connective tissue or of micro-components in connective tissue or both may be assessed relative to the portal tracts to assess collagen bridge formation.

The biological sample may be used to assess change to portal tract, lobular area, interface, or connective tissue over time, including for the assessment of reduction or increase in inflammation, fibrosis and scarring. The biological sample in a liver histology image may be a longitudinal sample. Scarring or fibrosis leading bridging between portal tracts may occur over time. A length or a width or both of bridging fibrosis between the portal tracts may be measured, whereby the progressive/regressive pattern of fibrosis in patients provides a metric of drug efficacy.

A number and density of portal tracts per patient, per slide, per sample, per tissue region, or per duplicate biopsy within a slide may be used as a quality control metric to assess sample adequacy for pathology assessment.

Spatial metrics based on portal tract or other liver micro-component types, locations, areas, boundaries or a combination of them or a pattern of them may be used to correlate against outcomes or histopathology scores. Preferably the spatial metrics produced by the method are used for fully-automated, digital scoring of liver disease.

The results of the segmentation that identified the component features of the portal tracts may be used to produce metrics relating to the component features, and to produce scoring related to illness, disease, or dysfunction associated with the component features.

According to an aspect of the invention there is a system comprising a computer program directly loadable into the memory of a computer for operating the method according to any preceding claim. The system may comprise the computer with the computer program loaded into the memory.

The invention will now be described by way of example only, with reference to the accompanying figures in which:

Brief Description of the Figures

Figure 1 shows background and foreground of liver tissue on a histology slide;

Figure 2 shows a portal tract in liver tissue on a liver histology slide;

Figure 3 shows a portal tract proximate co-localized anatomical micro-components;

Figure 4 shows a schematic of portal tracts and associated micro-components;

Figure 5 shows a schematic of patterns of micro-components in liver tissue; and

Figure 6 shows a flowchart of a method of histology anatomical interpretation.

Detailed Description of the Invention

Figure 1 shows a histology of liver tissue on a histology slide 700. Everything within the tissue outline is tissue foreground 17. There is nothing to see outside the tissue itself because the tissue background 18 is only the glass of the histology slide.

Figures 2 and 3 show portion a liver histology slide in which liver tissue is stained to highlight various different micro-components. An outline of a portal tract 101, 102, also known as a portal triad, is visible. In general, the various different micro-components comprised within a portal tract 101, 102 include at least one hepatic artery 41, 42, 45, at least one portal vein 51, 52, 55 and at least one bile duct 201, 202, 203, 204, 208. The particular portal tract 101 shown in Figure 2 comprises two hepatic arteries 41, 42,

two portal veins 51, 52, and one bile duct 201. In the portal tract 101 there is also connective tissue 31 that surrounds the various different micro-components: hepatic artery, portal vein, bile duct and holds the portal tract 101 together.

Connective tissue 31, 32, 33, 34, 35, 36, 37, 38, 39 is visible in Figures 1 to 5. Connective tissue is the stroma which surrounds the capsule of the liver (Glisson) consisting of connective tissue and vessels. Connective tissue forms a meshwork within the liver that provides integrity for hepatocytes and sinusoids. The portal tract 101-120 conglomeration of micro-components comprising bile ducts, portal veins, and hepatic arteries reside within connective tissue. The boundary of this connective tissue is the delimiting boundary of the portal tract.

There is an interface 161, 162 along the delimiting boundary between the liver tissue inside the portal tract 103, 104 and the liver tissue 35 outside the portal tract. The interface 161, 162 between the portal tract 103, 104 and the surrounding external liver tissue is difficult to see in the liver histology in Figures 2 and 3. The schematic Figure 4 shows the interface 16 around the portal tract 103, 104 by a dotted line to indicate there is a gradual and difficult to detect change between the liver tissue inside the portal tract 103, 104 and the external liver tissue 35.

Bile ducts 201-208 are visible in the histology of Figures 2 and 3 and shown schematically in Figure 4. The bile ducts are channels of the liver which facilitate the transport of bile. The bile ducts are a micro-component of portal tracts 101-120. Bile ducts are characterised by a lining of specialised and visually distinct epithelial cells known as cholangiocytes.

Hepatic arteries 41-45 are visible in the histology of Figures 2 and 3 and shown schematically in Figure 4. The hepatic arteries 41-45 are characterised by an outer layer consisting of collagen fibres, a middle layer of smooth muscle and an inner layer of endothelium lined with endothelial cells.

Portal veins 51-55 are visible in the histology of Figures 2 and 3 and shown schematically in Figure 4. The portal veins 51-55 begin behind the neck of the pancreas and supply oxygen-poor but nutrient-rich blood to the liver. Unlike systemic veins, portal veins 5 and contributing veins carry blood to the liver prior to the heart. Portal veins are characterised by a wide lumina lined with a single layer of flattened endothelial cells with larger portal vein branches having thin walls of fibrous connective

tissue.

Anatomical micro-components are visible in the liver histology slides of Figures 1, 2, and 3. They are also shown in the schematics of live histology shown in Figures 4 and 5. Anatomical micro-components refer to the constituents, and conglomerations of micro-components that make up the tissue foreground 17. Anatomical micro-components include, but are not limited to: portal tract 101-120, bile duct 201-207, connective tissue 31-37, hepatic artery 41-45, portal vein 51-55, central vein 61-64, acinus, hepatic lobule 81-84, hepatic zone, acinar zone 10, 11, 12, fibrotic bridge 151, 152, collogen deposit, interface region 161, 162, and co-localized anatomical micro-components such as steatosis 91, hepatocyte 92, inflammatory cell 93, ballooned cell 94.

A collagen deposit is an excessive or abnormal accumulation of collagen and other proteins in the extracellular matrix resulting from hepatic fibrosis. A fibrotic bridge 151 is shown in the schematic of Figure 4 connecting two portal tracts 103, 104. Collagen deposits are a hallmark of hepatic fibrosis. Collagen deposits are used to detect and identify fibrotic bridges 151 and to detect and identify portal tracts 101-120 in the foreground 17 liver tissue in a histology slide as shown in Figure 1.

Figure 5 shows a schematic of a liver tissue histology. The schematic is intended to visually explain the complex arrangement of the various different micro-components and conglomerations of micro-components in the liver tissue in the liver histology slides of Figure 1, Figure 2, and Figure 3. From Figure 5, patterns in the complex arrangement emerge and are more easily seen than in the liver histology slides.

One of the patterns in Figure 5 is a hexagonal arrangement of hepatic lobules 81-84. The hepatic lobules 81-84 are a micro-component the liver tissue histology. There is one of the portal tracts 105-120 approximately at each one of the six peripheral corners of each approximately hexagon shaped hepatic module 81-84. The pattern of the hexagonal arrangement of hepatic lobules 81-84 appears as honeycomb of hexagons. Approximately at the centre of each hexagon is a central vein 61-64.

It should be appreciated that the complex arrangement of portal tracts 105-120 in the liver tissue in the liver histology slides is not precisely hexagonal. Nor do the hepatic lobules 81-84 have a precisely hexagonal shape. The portal tracts 105-120 in the liver microanatomy are distributed in a meandering biological manner. Furthermore, the

central vein 61-64 is only approximately at the centre of hepatic lobules 81-84 in the liver tissue. In histology slides of liver tissue, the portal tracts 105-120 appear to be approximately at corners of a hexagon to a person or machine trained to see the honeycomb pattern. The schematic in Figure 5 is an aid in training to find in a histology slide, portal tracts 1, central veins 61-64, and other micro-components of the liver tissue. The approximately hexagonal pattern also aids in confirming that a finding of a conglomeration of micro-components is probably a finding of a portal tract 105-120 because of the location of the conglomeration at approximately a corner position of a hexagon apparent from other already found portal tracts 105-120.

Another of the patterns that may be seen in Figure 5 is a pattern of nested rhombus shapes of three acinar zones 10, 11, 12. The largest of the three is the third acinar zone 12. The second largest is second zone two 11 which is nested within third acinar zone 12. The smallest is first zone 10 which is nested within the second zone 11. All three of the rhombus shapes have a first pair of opposite corners 181, 182 and a second pair of opposite corners. All three of the rhombus shapes share the first pair 181, 182 of opposite corners. There is a portal tract at each one of two opposite corners 181, 182 that all three of the rhombus shapes share.

The three rhombus shapes of the three acinar zones 10, 11, 12 do not share the second pair of opposite corners because the three rhombus shapes are nested. The largest of the three rhombus shapes is the third acinar zone 12, and there is a central vein 61, 63 at each one of the second pair of opposite corners 171, 172 of the third acinar zone 12. The central veins 61, 63 are in adjacent hepatic lobules 82, 84. The second pair of opposite corners 173, 174 of the second acinar zone 11 are located on an imaginary straight line through the second pair of opposite corners 171, 171 of the third acinar zone 12. That is the imaginary straight line passes through the central veins 61, 63. The second pair of opposite corners 175, 176 of the first acinar zone 10 are located on the imaginary straight line so that the three acinar zones 10, 11, 12 are nested. The opposite corners 173, 174 of the second pair of the second acinar zone 11 are disposed between respective ones of the opposite corners 171, 172, 175, 176 of the second pair of the first acinar zone 10 and the third acinar zone 12 so that the three acinar zones 10, 11, 12 are nested.

It should be appreciated that the complex arrangement of adjacent central veins 6, 61, adjacent portal tracts 181, 182, and second pairs of opposite corners 171, 172, 173,

174, 175, 176 in the liver tissue in the liver histology slides is not precisely rhombus shaped. The corners are curved, rather that sharp. The outlines of each rhombus are meandering, rather that straight. The nested rhombus pattern is not precisely concentric. The schematic in Figure 5 showing the nested rhombus pattern is an aid in training to find in a histology slide, portal tracts 181, 182, central veins 61, 63, hepatic lobules 82, 84, acinar zones 10, 11, 12 and other micro-components of the liver tissue. The patterns are devised to aid a person or machine to detect, recognise, or identify a particular micro-component in the pattern. The aid is useful because simply segmenting a micro-component or conglomeration of micro-components in liver tissue of a liver technology slide usually provides insufficient information to recognize or identify with sufficient probability of accuracy.

The acinar zone that is furthest from the central vein 61, 63 that is denoted acinar zone one 10 receives the most oxygenated blood and nutrients. The acinar zone that is closest to the central vein 61, 63 and denoted acinar zone three 12 receives the least oxygenated blood and nutrients. The acinar zone two 11 between acinar zone one 10 and acinar zone three 12 receives an intermediate amount of oxygenated blood and nutrients.

In Figure 6 a flowchart of a method of histology anatomical interpretation 600 is shown. By way of illustrative example, portal tracts 101-120 in histological images of histological slides 700 (for example H&E, Masson's Trichrome, Picrosirius red or any other stain) are identified and characterised according to the following schema.

1. Creating or using a semantic, instance or other segmentation model for each of liver tissue:

1.1. segmentation of foreground tissue 604;

1.2. segmentation of connective tissue 606;

and particular micro-components 602, especially portal tract micro-components 608:

1.3. segmentation of bile ducts 610;

1.4. segmentation of hepatic arteries 612; and

1.5. segmentation of portal veins 614.

2. The objects of these distinct segmentation problems have a known or expected spatial relationship. Analyses are conducted according to the method and system of histological characterisation to produce segmentations of the compound tissue conglomerations of micro-components of the liver that are portal tracts. The segmentations, either all of them, or several or one or more subsets thereof, are spatially associated to determine confidence or probability that a region of connective tissue 31-39 is a portal tract 101-120. For example, in an embodiment a region of connective tissue is only considered a portal tract provided it contains a defined or preselected number of bile ducts, portal veins, or hepatic arteries either individually or in total.

3. Semantic segmentations of micro-components or conglomerations of them are processed into a portal tract prediction/s. Candidates for portal tract instances 618 are identified or selected from an intersection of tissue foreground 604 and connective tissue 606. The term 'portal tract' (PT) 101-120 to refer to an area of segmented connective tissue (CT) 31-36 that intersects with one or more instance of the following:

a) a segmented bile duct (BD) 201-208.

b) a segmented hepatic artery (HA) 41-45.

c) a segmented portal vein (PV) 51-55.

4. Where micro-component or conglomeration {a|b|c} intersects with CT but lies partially outside (i.e. partially within 624) the connective tissue segmented area, the boundary of the portal tract is the union 626 of the of connective tissue and micro-component or conglomeration {a|b|c}.

5. The intersection of an object with connective tissue is calculated with an associated distance threshold on the boundary of connective tissue. For example, if a portal vein was detected near to a region of connective tissue (e.g. within 5, 10, or 20 microns), but not intersecting with the raw connective tissue, then that portal vein is still considered a part of the portal tract, and the final portal tract is the union of the connective tissue and the portal vein. This is a check 620 of whether the micro-component object intersects or is within a distance threshold of a candidate PT boundary.

6. Micro-components satisfying criteria with a candidate portal tract include those fully within the candidate portal tract 628 and those unioned 626 the candidate portal tract are counted 630. Candidate portal tracts are assigned a confidence score 632 according to the number of distinct features associated with the bounding connective tissue.

a) +1 for >= 1 bile duct

b) +1 for >= 1 portal vein

c) +1 for >= 1 hepatic artery

7. Thus, confidence is assigned for each candidate portal tract region based on which components of the portal triad (portal vein, bile duct or hepatic artery) have been identified as present in the region:

0 = No elements of the portal triad.

1 = A single element, either bile ducts, portal veins, or hepatic arteries.

2 = Any combination of two out of the three components of the portal triad

3 = All components of the portal triad.

Higher scores correspond to higher likelihood predictions. The confidence score provides an interpretable rationale for an image region being defined as a portal tract and can be used to filter candidate portal tracts on the number of associated features, which may be considered a proxy for the likelihood that a candidate conglomeration of micro-components is actually a portal tract.

A preselected confidence score is used to ensure that the probability a candidate portal tract is genuine exceeds a minimum threshold 634. If the confidence score of the candidate portal tract is below the minimum, then the conglomeration of elements and connective tissue they are in is determined to be in connective tissue outside of a portal tract 636. If the confidence score of the candidate portal tract is below the minimum, then it is determined that a portal tract has been detected and identified and includes the conglomeration of elements and connective tissue of the candidate portal tract 636.

Information of the identified portal tract including its location in the tissue foreground is

combined 638 with information determined from the tissue foreground such as from segmentation of central veins in the tissue foreground. This enables hepatic lobule regions to be found such as acinar zones 640.

Because there are patterns of portal tracts and central veins in the hepatic lobule regions, as explained previously, that indicate where portal tracts are likely to be, the patterns may be fed back to the step of finding candidate portal tract instances 618. The patterns may be fed back to a step 620 such as checking whether the position of the micro-component or a conglomeration of them intersects within a distance or angle range of a position in one of the patterns. This causes the method of histological characterisation to improve as it progresses in analysis of a histological slide to become quicker and more accurate in detecting portal tracts, central veins, and hepatic lobule regions including but not limited to acinar zones of the tissue on the slide.

Geometric analysis 636 is performed on the portal tracts detected and identified 642. Ancillary liver regions 644 such as those of steatosis 91, hepatocyte 92, inflammatory cell 93, ballooned cell 94, and fibrotic bridge 15 may be detected and identified. The geometric analysis 636 may be combined with the connective tissue determined to be outside the portal tract either by confidence analysis 636 or intersection or within a distance threshold 622 to find ancillary regions outside the portal tract but associated with it.

The invention has been described by way of examples only. Therefore, the foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents may be resorted to, falling within the scope of the claims.

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Claims:

1. A method to determine liver histology metrics, comprising:

use of segmentations of micro-components that are histological from a biological sample of a liver histology image;

spatially associate those of the micro-components that are within a preselected proximity of each other into a conglomeration of the micro-components that defines a histological component or metric of disease severity; and

association of each the micro-components that are within a preselected proximity of each other with a bile duct, portal vein, hepatic artery to put a confidence in the conglomeration of components as a portal tract above a threshold.

2. A method according to claim 1 wherein a presence or absence of the micro-components that are within the preselected proximity of each other contributes to a metric or confidence score of the likelihood of the conglomeration of components as portal tract being 'true'.

3. A method according to claim 1 or 2 wherein use of the micro-components, the conglomeration, and the spatial domains is in isolation of other spatial features of the biological sample.

4. A method according to any preceding claim wherein the conglomerations identified as portal tracts are quantified to assess sample adequacy.

5. A method according to any preceding claim wherein locations of inflammatory cells, ballooned cells, fibrosis, fat globule features, or the connective tissue, are integrated with hepatic lobules and the conglomerations as portal tracts to provide spatially differentiated metrics of inflammatory cell number and densities within and external to portal tracts or to identify bridging fibrosis between the portal tracts.

6. A method according to any preceding claim wherein segmentation models are used to identify the micro-components of portal tracts or the conglomeration as a portal tract.

7. A method according to claim 6 wherein the micro-components or conglomeration are

segmented with a neural network, convolutional neural network, transformer model, including vision transformer or combination of multiple such networks trained for the purpose.

8. A method according to claim 6 wherein the micro-components or conglomeration are segmented with a neural network comprising a segmentation foundation model, or an autoencoder or transformer capable of learning and producing embeddings of arbitrary image features.

9. A method according to claim 6 wherein the micro-components or conglomeration are segmented with non-neural network-based image processing by thresholding, pixel classification, filtering, or feature detection.

10. A method according to any of claims 6 to 9 to wherein micro-components or conglomeration are segmented approximately with the use of a tile-based classification, wherein smaller tiles of a larger image are extracted and classified according to whether the tile contains the presence of each micro-component.

11. A method according to any preceding claim wherein segmentations of the micro-components or conglomerations are identified as bounding boxes, centroids or other summary sets of coordinates and these summary descriptors are spatially associated.

12. A method according to any preceding claim wherein micro-components or conglomeration are represented as location-based probabilities that the component feature exists at a location in a probability map, and spatial association of micro-components or conglomerations is performed for geometries derived from thresholding the probability map at a value.

13. A method according to any preceding claim wherein the micro-components or conglomeration are identified through segmentation of their component cell types with a cell segmentation or detection model trained to predict cell types.

14. A method according to claim 13 wherein the micro-components or parts of the conglomeration that are connective tissues are identified as connective tissue cells in proximity with one another.

15. A method according to any preceding claim wherein the conglomeration as the portal

tract and hepatic lobules of the liver are delineated via combination of the micro-components of the conglomeration and tissue foreground features through logical predicates including taking a union, intersection, or difference.

16. A method according to any preceding claim wherein interface regions are delineated between the conglomeration as a portal tract and hepatic lobules of the liver through constructive and destructive geometric operations of features including buffering, dilation or erosion.

17. A method according to any preceding claim wherein acinar zones for analysis in the liver histology image are delineated by subdivision of the conglomerations as a portal tracts and lobular areas of the liver.

18. A method according to claim 17 wherein a mid-line between two portal tracts and the lobular area closest to one of the two portal tracts is found and further subdivided by a radial distance from the portal tract boundary to the other boundary.

19. A method according to any preceding claim distinguishes the portal tracts, the lobular areas, and interface regions using the portal tract model by generating descriptive statistics.

20. A method according to any preceding claim expresses a distribution of portal tract inflammation, lobular area inflammation, or interface inflammation according to at least one of a number of inflammatory foci, a mean area of foci, a portal tract inflammatory cell density, an interface inflammatory cell density, or a lobular area inflammatory cell density.

21. A method according to any preceding claim identifies the spatial location of neutrophils, eosinophils, lymphocytes (including T and B cells), plasma cells, macrophages (including Kupffer cells), or other inflammatory cell types relative to portal tracts, lobular regions and other liver zones.

22. A method according to any preceding claim identifies a location and abundance of communities of cooperating cells defined by statistical frequency, nearest neighbour, ‘bag of words’ or other techniques, relative to portal tracts, lobular areas, or interface regions.

23. A method according to any claim combining the portal tracts with the component

features that are connective tissues to identify bridging fibrosis between portal tracts.

24. A method according to any preceding claim measuring shape, length and other morphological characteristics of the component features that are connective tissue relative to the portal tracts to assess collagen bridge formation.

25. A method according to any claim wherein the biological sample in a liver histology image is a longitudinal sample, to assess change to portal tract, lobular area, interface, or connective tissue over time, including for the assessment of reduction or increase in inflammation, fibrosis and scarring.

26. A method according to any preceding claim wherein a length and width of bridging fibrosis between the portal tracts are measured, whereby the progressive/regressive pattern of fibrosis in patients provides a metric of drug efficacy.

27. A method according to any preceding claim wherein a number and density of portal tracts per patient, per slide, per sample, per tissue region, or per duplicate biopsy within a slide is used as a quality control metric to assess sample adequacy for pathology assessment.

28. A method according to any preceding claim using spatial metrics based on portal tract and other liver sub-compartment locations/areas/boundaries to correlate against outcomes or histopathology scores.

29. A method according to claim 28 wherein the spatial metrics produced by the method are used for fully-automated, digital scoring of liver disease.

30. A method according to any preceding claim wherein the results of the segmentation that identified the component features of the portal tracts are used to produce metrics relating to the component features, and to produce scoring related to illness, disease, or dysfunction associated with the component features.

31. A system comprising a computer program directly loadable into the memory of a computer for operating the method according to any preceding claim.

32. The system according to claim 30 comprising the computer with the computer program loaded into the memory.