Electronic Supplementary Material (ESI) for Analyst.

Enhancing the accuracy of Mid-Infrared Spectroscopy Liver Steatosis quantification using Digital Image Analysis as Reference

Iván Rienda[a,*], Isabel Ten-Domenech[b,*], Erika Moro[c], Marta Moreno-Torres[c,d,e], Judith Pérez-Rojas[a], Eugenia Pareja[d,f], Álvaro Pérez-Rubio[f], Juan R. Trullenque[f], Ramiro Jover[c,d,e], Bernhard Lendl[g], David Perez-Guaita[h], Julia Kuligowski[b], Jose V. Castell[c,d,e], Guillermo Quintás[i]

[a]*Pathology Department, Hospital Universitario y Politécnico La Fe, Valencia, Spain*
[b]*Neonatal Research Group, Health Research Institute La Fe, Valencia, Spain*
[c]*Departamento de Bioquímica y Biología Molecular, Universidad de Valencia, Valencia, Spain*
[d]*Unit for Experimental Hepatology, Health Research Institute La Fe, Valencia, Spain*
[e]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain*
[f]*Servicio de Cirugía General y Aparato Digestivo, Hospital Universitario Dr. Peset, Valencia, Spain*
[g]*Institute of Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria*
[h]*Departamento de Química Analítica, Universidad de Valencia, Valencia, Spain*
[i]*Health and Biomedicine, Leitat Technological Center, Valencia, Spain*
** Contributed equally.*
**Corresponding author: gquintas@leitat.org

Table SM1. Set of morphological properties used for the characterization of each 8-connected component (object) in the binary images. (The MathWorks Inc., (2022). Imaging Processing Toolbox™ (R2022a). Retrieved November 29, 2022, from https://uk.mathworks.com/help/images/ref/regionprops.html).

Property	Description
Eccentricity	Eccentricity of the ellipse that has the same second-moments as the region, returned as a scalar. The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length.
Area	Number of pixels in the region.
Circularity	Roundness of objects. The circularity value is computed as $(4*Area*\pi)/(Perimeter^2)$.
Perimeter	Distance around the boundary of the region returned as a scalar. Computed as the distance between each adjoining pair of pixels around the border of the region.
Equivalent Diameter	Diameter of a circle with the same area as the region, returned as a scalar. Computed as $sqrt(4*Area/\pi)$.
Extent	Ratio of pixels in the region to pixels in the total bounding box, returned as a scalar. Computed as the Area divided by the area of the bounding box.
Major axis length	Length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region.
Minor axis length	Length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.
Solidity	Proportion of the pixels in the convex hull that are also in the region. Computed as Area/ConvexArea.

Table SM2. Main descriptors of the images used for the development of the models for the quantification of steatosis in the HE images, including the image size, the classification provided by the pathologist, the area percentages of macro- and micro-steatosis, and the number of macrosteatotic, microsteatotic, and non-steatotic objects included.

Image	Size (pixels)	Steatosis grade	% macro	%micro	No steat. (train/test)	micro-steat. (train/test)	macro-steat. (train/test)
1	12600x14090	3	23.83	0.65	5894 (4477/1507)	5685 (4225/1460)	13293 (9993/3300)
2	6801x7367	3	27.77	0.27	5968 (4480/1488)	1377 (1034/343)	6904 (5176/1728)
3	8736x9719	1	0.76	0.11	10381 (7764/2617)	754 (574/180)	786 (598/188)
4	16373x14731	0	0.08	0.05	21306 (16008/5298)	803 (593/210)	312 (243/69)

Table SM3 (1/2). Results from the analysis of 33 H&E-stained images of liver samples using the q-SVM method.

patient_nr	Patient code	S	A	F	.svs file	%total steat.	%macro	%micro	%CC	PPV	NPV	Specificity	Sensitivity	Steat as Steat (TP)	Steat as non-steat (FN)	Non-steat as Non-steat (TN)	Non-steat as Steat (FP)
1	S301	S1	A2	F0	3-01 HE_001	1.647	1.567	0.080	99.479	0.996	0.990	0.982	0.998	9841	179	43636	101
2	S302	S3	A4	F2	3-02 HE_001	15.110	14.769	0.342	99.267	0.992	0.995	0.978	0.998	29535	654	79916	154
3	S303	S0	A0	F0	3-03 HE_001	0.101	0.062	0.039	99.762	0.999	0.962	0.976	0.998	1483	36	37984	58
4	S304	S0	A0	F0	3-04 HE_001	0.256	0.202	0.053	99.737	0.998	0.986	0.964	0.999	4929	182	90520	70
5	S305	S1	A1	F0	3-05 HE_001	3.009	2.883	0.127	99.645	0.996	0.997	0.980	1.000	6859	139	37243	18
6	S306	S0	A0	F0	3-06 HE_001	0.507	0.326	0.180	99.795	0.999	0.982	0.979	0.999	7117	154	132894	134
7	S307	S1	A1	F0	3-07 HE_001_001	0.792	0.556	0.235	99.661	0.997	0.994	0.985	0.999	14697	219	75027	86
8	S308	S1	A1	F1C	3-08 HE_001	0.741	0.661	0.080	99.778	0.998	0.984	0.969	0.999	3704	117	76257	61
9	S309	S1	A0	F0	3-09 HE_001	1.867	1.504	0.363	99.685	0.997	0.995	0.978	0.999	33065	759	261865	174
10	S310	S1	A0	F0	3-10 HE_001	1.790	1.604	0.186	99.439	0.995	0.994	0.979	0.998	21958	467	85051	137
11	S312	S1	A1	F0	3-12 HE_001	1.800	1.375	0.425	99.604	0.996	0.996	0.974	0.999	21700	589	149817	93
12	S315	S1	A0	F0	3-15 HE_001_001	1.118	0.869	0.249	99.158	0.996	0.967	0.981	0.994	12416	246	67187	430
13	S321	S3	A3	F0	3-21 HE_001	7.990	7.220	0.770	99.220	0.992	0.991	0.976	0.997	21998	548	72308	193
14	S326	S0	A0	F0	3-26 HE	0.047	0.024	0.023	99.809	0.999	0.969	0.963	0.999	310	12	11159	10
15	S328	S0	A0	F0	3-28 HE_001	0.499	0.318	0.181	99.367	0.998	0.909	0.964	0.995	2906	108	59880	292
16	S333	S1	A2	F0	3-33 HE_001	1.318	1.084	0.234	99.619	0.997	0.986	0.972	0.999	6323	181	63934	88
17	S334	S1	A2	F1a	3-34 HE_001	1.302	1.176	0.126	98.862	0.989	0.985	0.937	0.997	2576	173	15924	40
18	S337	S1	A2	F0	3-37 HE_001	0.435	0.250	0.185	99.690	0.997	0.995	0.977	0.999	3673	85	30091	20
19	S338	S1	A2	F0	3-38 HE_001	1.499	1.262	0.238	99.681	0.996	0.999	0.982	1.000	10776	200	55738	13
20	S343	S2	A2	F0	3-43_001	12.537	12.212	0.325	98.736	0.981	0.996	0.974	0.997	20958	560	28939	79

Table SM3 (2/2 cont.). Results from the analysis of 33 H&E-stained images of liver samples using the q-SVM method.

patient_nr	Patient code	S	A	F	.svs file	%total steat.	%macro	%micro	%CC	PPV	NPV	Specificity	Sensitivity	Steat as Steat (TP)	Steat as non-steat (FN)	Non-steat as Non-steat (TN)	Non-steat as Steat (FP)
21	S344	S0	A0	F0	3-44_001	0.074	0.039	0.035	99.326	0.994	0.993	0.989	0.996	552	6	922	4
22	S345	S1	A1	F0	3-45 HE_001	1.196	0.733	0.463	99.492	0.997	0.978	0.973	0.997	4037	114	36093	91
23	S346	S1	A1	F0	3-46 HE_001	0.825	0.636	0.189	99.432	0.996	0.978	0.963	0.998	1712	66	16493	38
24	S348	S2	A3	F0	3-48 HE_001	5.051	4.423	0.628	99.399	0.992	0.999	0.982	1.000	16477	298	35275	15
25	S350	S1	A2	F1a	3-50 HE_001	1.157	0.837	0.321	99.702	0.998	0.985	0.969	0.999	5013	160	74278	77
26	S355	S2	A2	F0	3-55 HE_001	5.122	4.588	0.534	99.345	0.993	0.997	0.969	0.999	45204	1459	199607	156
27	S356	S0	A0	F0	3-56 HE_001	0.382	0.265	0.117	99.721	0.998	0.988	0.975	0.999	3580	93	45704	45
28	S357	S2	A2	F1c	3-57 HE_001	1.810	1.739	0.071	99.510	0.996	0.985	0.959	0.999	3567	151	38075	54
29	S360	S2	A2	F1c	3-60 HE_001	12.268	11.834	0.434	99.281	0.993	0.993	0.979	0.998	22759	498	69434	170
30	S361	S0	A1	F0	3-61 HE_001	1.079	0.769	0.311	99.564	0.997	0.982	0.980	0.998	6886	137	52956	125
31	S363	S0	A1	F0	3-63 HE_001	0.385	0.215	0.170	99.609	0.997	0.991	0.972	0.999	5499	160	48505	52
32	S365	S1	A3	F0	3-65 HE_002	2.786	2.547	0.239	99.655	0.997	0.987	0.964	0.999	15846	596	218485	215
33	S368	S2	S1	A0	3-68 HE_001	3.472	3.199	0.273	99.153	0.990	0.996	0.969	0.999	6001	191	19047	23