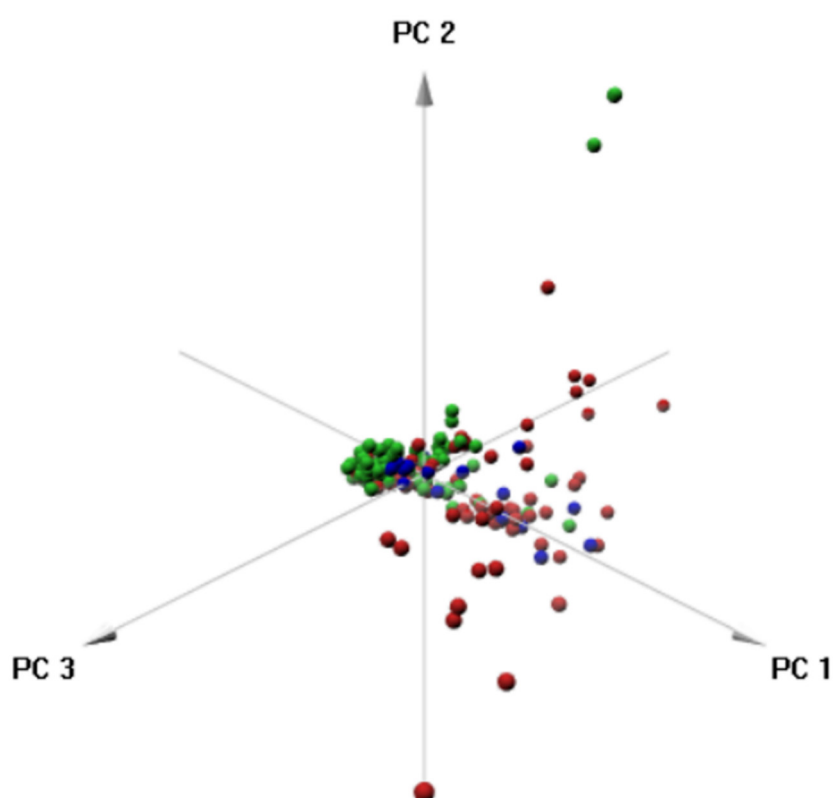


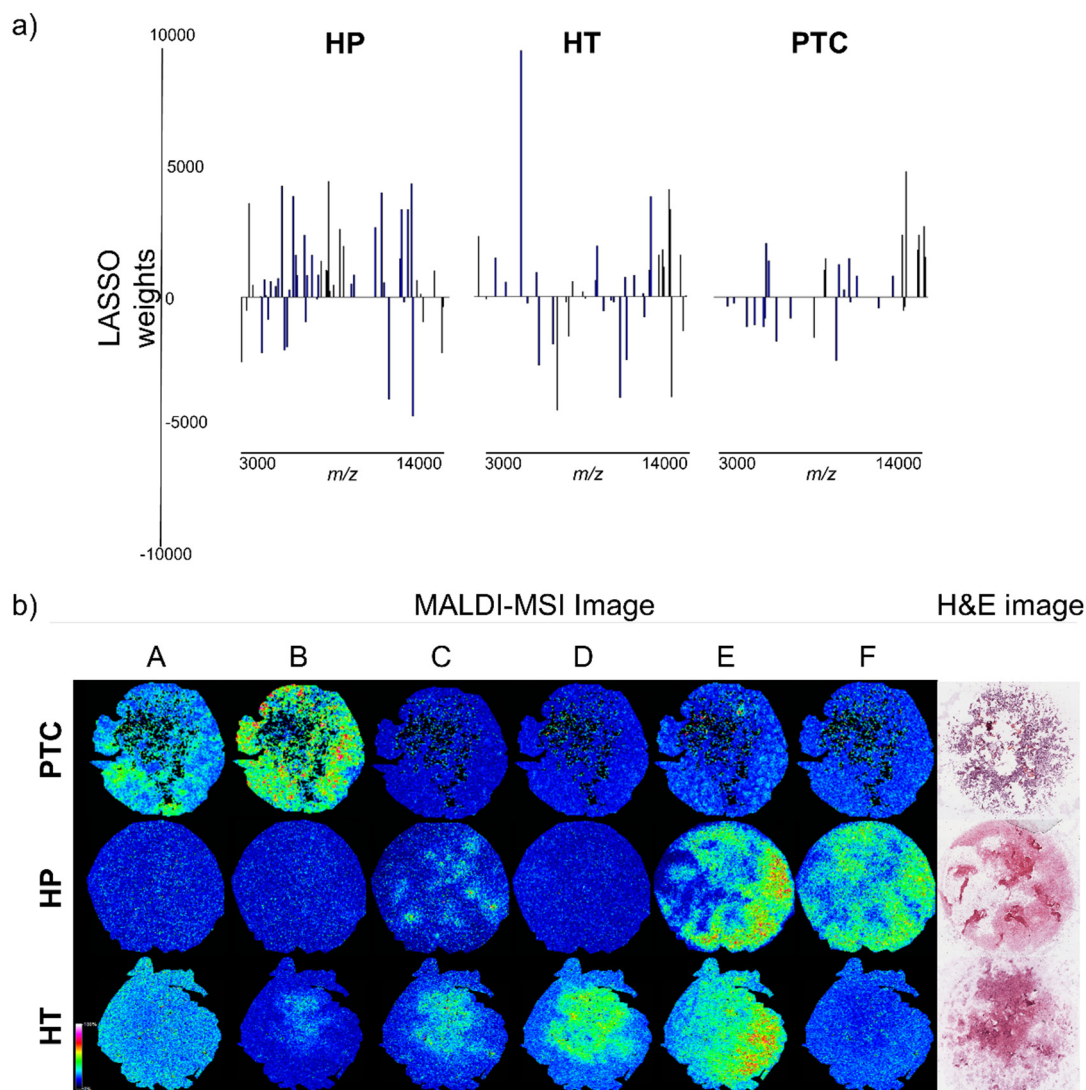
## Article

# Cytomolecular Classification of Thyroid Nodules Using Fine-Needle Washes Aspiration Biopsies

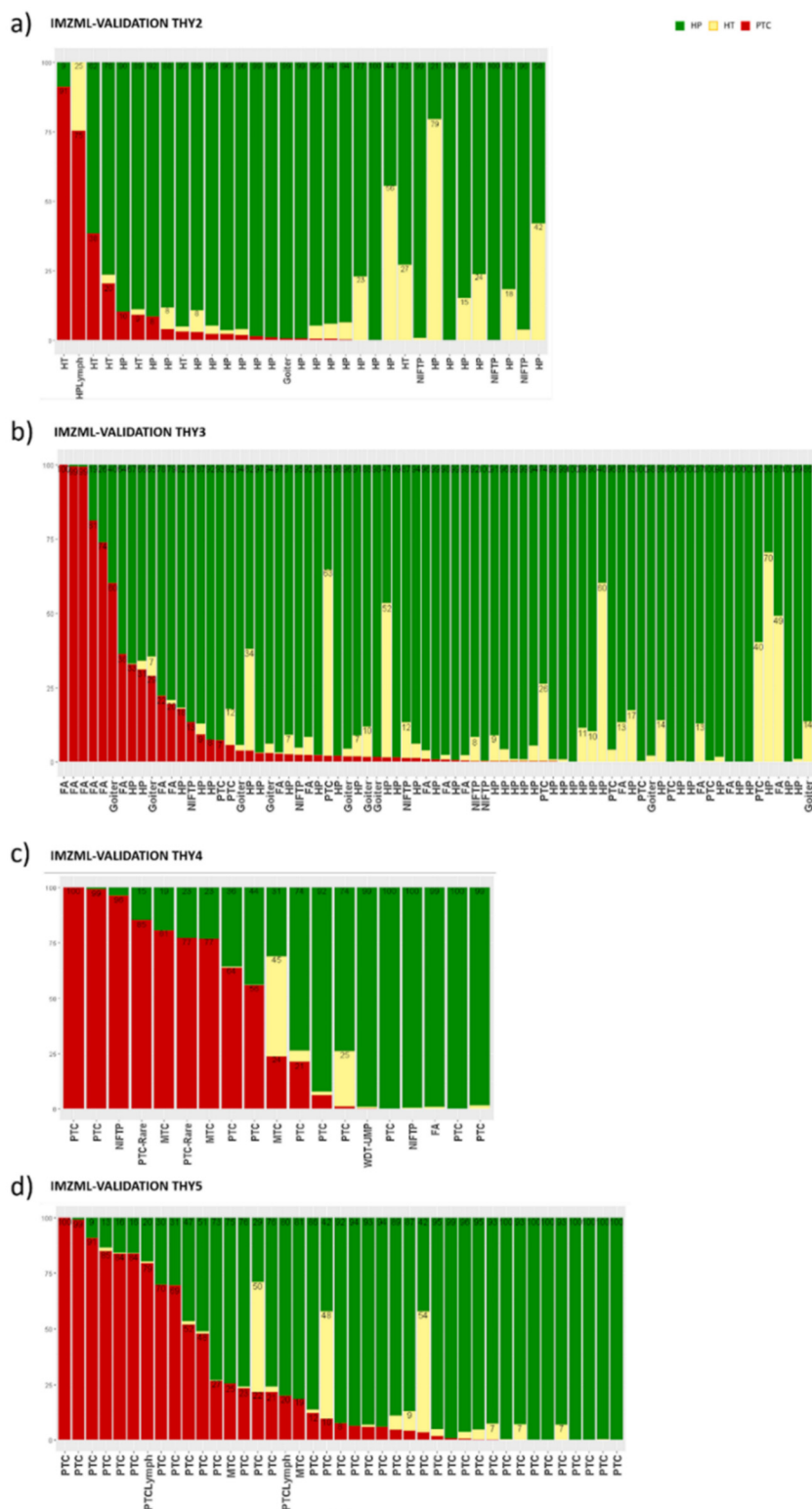
## Supplementary Figures



**Figure S1.** Principal component analysis on 240 thyroid FNA using the overall average spectrum of each sample. Green: benign entities (i.e. HP, HT, B-Goiter), Red: malignant nodules (i.e. PTC, MTC, rare parathyroid tumour), Blue: borderline diagnosis (i.e. NIFTP, FA, WDT-UMP) and Lymph node.



**Figure S2.** MALDI-MSI analysis of FNA needle washes and statistical analysis. **(a)** 95  $m/z$  features selected by the Lasso model with the corresponding weights in HP, HT and PTC samples. **(b)** HP, HT and PTC MALDI-MSI images of six  $m/z$  and their H&E images.



**Figure S3.** Stacked bar charts of the percentage of pixels in each FNA of the validation set classified based on pixel-by-pixel (IMZML) approach as HP (green), HT (yellow) and PTC (red) in the (a) 33 TIR2/THY2, (b) 77 TIR3/THY3, (c) 19 TIR4/THY4 and (d) 41 TIR5/THY5 samples. Each nodule was classified as malignant when the percentage of red pixels was > 7%.

## Supplementary Tables

Table S1. Cytological characteristics of thyroid nodules.

Characteristic	Total (n=240)	THY2 (n=83)	Cytology		
			THY3 (n=77)	THY4 (n=19)	THY5 (n=61)
<b>Diagnosis</b>					
<b>Benign</b>					
HP	96	59	37	0	0
HP-lymph	1	1	0	0	0
B-goiter	13	4	9	0	0
HT	16	16	0	0	0
FA	18	0	17	1	0
NIFTP	10	3	5	2	0
WDT-UMP	1	0	0	1	0
<b>Malignant</b>					
PTC	76	0	9	10	57
PTC-lymph	2	0	0	0	2
MTC	5	0	0	3	2
Rare parathyroid tumour	2	0	0	2	0
<b>Nodule Localization</b>					
Right Lobe	122	45	40	12	25
Left Lobe	112	35	36	7	34
Isthmus	3	2	1	0	0
Lymph node	3	1	0	0	2
<b>Nodule Size (mm)</b>					
Mean (SD)	20.7 (11.3)	21.3 (10.4)	23.7 (13.6)	16.1 (6.8)	17.6 (9.1)
Range	5-70	5-60	5-70	8-35	8-50
<b>Sample cellularity*</b>					
Poor	87	18	44	7	18
Good	94	40	24	7	23
Optimal	59	25	9	5	20

Legend: Goiter, HP-lymph node reported as HP; PTC-lymph and Rare parathyroid tumour, for the purpose of the analysis considered malignant as a PTC.

\*Sample cellularity: poor (sample with 20-30% of thyrocytes cells), good (sample with 30-70%) and optimal (sample with >70%).

HP: hyperplastic, HT: Hashimoto thyroiditis, FA: follicular adenoma, PTC: papillary thyroid carcinoma, MTC: medullary thyroid carcinoma, WDT-UMP: well differentiated tumor of uncertain malignant potential, NIFTP: Non-Invasive Follicular Thyroid neoplasm with Papillary-like nuclear features.

**Table S2.** Predicted diagnosis based on the 605 ROIs of the training set according to histopathology/follow-up.

Histopathology/ follow-up	Prediction			
	Total	Benign	HT	Malignant
HP	381	HP	5	PTC
HT	50	12	38	0
PTC	174	5	2	167

HP: hyperplastic, HT: Hashimoto thyroiditis, PTC: papillary thyroid carcinoma.

**Table S3.** Performances of the classifier on the three MALDI-MSI approaches in the training set (n=70).

	TP	FP	TN	FN	% Sensitivity (95%CI)	% Specificity (95%CI)	% PPV (95%CI)	% NPV (95%CI)	AUC	% Accuracy
Pixel-by-pixel	20	2	48	0	100.0 (83.2-100.0)	96.0 (86.3-99.5)	90.9 (70.8-98.9)	100 (92.6-100)	1.00	97.1
ROIs	20	0	50	0	100.0 (83.2-100.0)	100 (92.9-100)	100 (83.2-100)	100 (92.9-100)	1.00	100
Average spectra	19	0	50	1	95.0 (75.1-99.9)	100 (92.9-100)	100 (82.4-100)	98.0 (89.6-100)	0.98	98.6

Legend: TP=True Positive, FP=False Positive, TN=True Negative, FN=False Negative, PPV=Positive Predictive Value, NPV=Negative Predictive Value, 95%CI=95% Confidence Interval, AUC=Area Under the Curve

**Table S4.** Predicted diagnosis based on the 3 levels classification strategy in the pixel-by-pixel analysis according to histopathology/follow-up for a) the training set and b-e) the validation set by the Bethesda reporting system.

a) Training (n=70)					Prediction			
Histology/FUP	Total	Benign	Gray Zone	Malignant				
Benign								
HP	40	35*	3	2				
HT	10	10	0	0				
Malignant								
PTC	20	0	0	20				

\*3 Goiter.

b) TIR2 (n=33)					Prediction			
Histology/FUP	Total	Benign	Gray Zone	Malignant				
Benign								
HP	24	21*	2	1#				
HT	6	2	1	3				
FA	0	0	0	0				
NIFTP	3	3	0	0				
WDT-UMP	0	0	0	0				

\*1 Goiter, #1 HP-lymph node.

c) TIR3 (n=77)		Prediction		
Histology/FUP	Total	Benign	Gray Zone	Malignant
<b>Benign</b>				
HP	46	39*	2	5#
HT	0	0	0	0
FA	17	9	0	8
NIFTP	5	4	1	0
WDT-UMP	0	0	0	0
<b>Malignant</b>				
PTC	9	8	1	0
MTC	0	0	0	0

\*7 Goiter, #2 Goiter.

d) TIR4 (n=19)		Prediction		
Histology/FUP	Total	Benign	Gray Zone	Malignant
<b>Benign</b>				
HP	0	0	0	0
HT	0	0	0	0
FA	1	1	0	0
NIFTP	2	1	0	1
WDT-UMP	1	1	0	0
<b>Malignant</b>				
PTC	12	5	0	7°
MTC	3	0	0	3

°2 rare parathyroid tumour.

e) TIR5 (n=41)		Prediction		
Histology/FUP	Total	Benign	Gray Zone	Malignant
<b>Malignant</b>				
PTC	39	20	3	16#
MTC	2	0	0	2

#2 PTC-lymph node.

HP: hyperplastic, HT: Hashimoto thyroiditis, FA: follicular adenoma, PTC: papillary thyroid carcinoma, MTC: medullary thyroid carcinoma, WDT-UMP: well differentiated tumor of uncertain malignant potential, NIFTP: Non-Invasive Follicular Thyroid neoplasm with Papillary-like nuclear features.