Appendix 1 Supplementary information

Flow of Raman microchip preparation

Dewaxing protocol

Lung cancer samples were processed using Formalin-Fixed Paraffin-Embedded (FFPE), the sections were required to have a Raman microchip with a thickness of 6 μm. After lifting the sections out of the water, the Raman microchip was agitated for 1 min in each of the baths: 2 xylene substitutes baths, 3 100% ethanol baths and 3 distilled water baths. Prior to Raman measurements, the slides were dried naturally at room temperature for 20 min to avoid water residue on the slides, and this step was performed without any additional chemical treatment.

Preparation of polished aluminum slides

A 304 aluminum plate was precision-cut into aluminum substrates measuring 75 mm × 25 mm × 1 mm. These substrates underwent the removal of a thin protective film prior to usage, followed by a thorough rinse with distilled water and subsequent rinses with 100% ethanol. After a final rinse with distilled water, the slides were left to air-dry naturally. The polished aluminum slides were then carefully stored in a clean and dry slide box for future use.

Flow of collecting data using Micro-Raman spectrometer

The Raman microchip was initially positioned on the Raman microscope stage, and the Leica microscope's white light was activated, utilizing a 5× objective lens. Adjust the sample surface to the system's focal plane, a setting achievable through the system joystick, ensuring alignment with identifiable tissue features visible on all tissue sections, referencing both H&E and IHC images. Capture a 5× image snapshot, saving it as a JPEG via the "Live Video" and "Save Image" tab. Subsequently, switch to a 20× objective lens, adjusting the WiRE software accordingly. Set the appropriate focal plane, capture the image, and repeat these steps with the 50× short working distance objective. Save each image individually, then arrange and photograph them to create a composite image covering a 200 μm × 200 μm area. Randomly select 100 to 200 data points within this composite image for data collection on the pathology. In the WiRE 5.4 software, configure the acquisition center to 1200 cm-1 (fingerprint area), set cumulative acquisition to 3 times, with an acquisition time of 3 s, and a laser power of 50%. Close the measurement window and select "Run" to initiate the acquisition process. Throughout this process, caution should be exercised to avoid any impact on the table supporting the Raman microscope.

Classification model evaluation indicators

In binary classification problems, model performance evaluation is crucial to ensure the reliability of the model in practical applications. In order to have a comprehensive understanding of the model's prediction effect, we need a series of scientifically rigorous evaluation indicators. These indicators are set up to comprehensively assess the performance of the model from different perspectives, to help researchers better understand the model behavior, and to guide the improvement and selection of the model.

Meaning and purpose of evaluation indicators

Sensitivity: The proportion of samples that are truly positive that are correctly predicted to be positive. Measures the model's ability to capture positive examples.

Specificity: The proportion of true negative cases that are correctly predicted to be negative. Measures the model's ability to exclude negative cases.

Accuracy: The proportion of correctly predicted samples out of the total number of samples. To improve the overall predictive accuracy of the model.

F1-score: The reconciled average of Precision and Recall, combining the effects of both. To balance the model's performance on positive and negative cases.

Precision: The proportion of samples predicted to be positive cases that are actually positive cases. To improve the accuracy

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of positive case prediction and reduce the risk of misclassification.

Recall: The proportion of true positive cases that are correctly predicted. To improve the identification of positive cases and reduce the number of true positive cases that are not captured.

Calculation formula for evaluation indicators

Accuracy = (TP + TN)/(TP + FP + TN + FN)
Sensitivity = TP/(TP + FN)
Specificity = TN/(TN + FP)
F1-score = 2 * (Precision * Recall)/(Precision + Recall)
Precision = TP/(TP + FP)
Recall = TP/(TP + FN)

Annotation

True positive (TP): true class. The true class of the sample is positive and the result recognized by the model is also positive.

False negative (FN): false negative class. The true class of the sample is a positive class, but the model recognizes it as a negative class.

False positive (FP): false positive category. The true category of the sample is negative, but the model recognizes it as positive.

True negative (TN): The true category of the sample is negative and the model recognizes it as negative.

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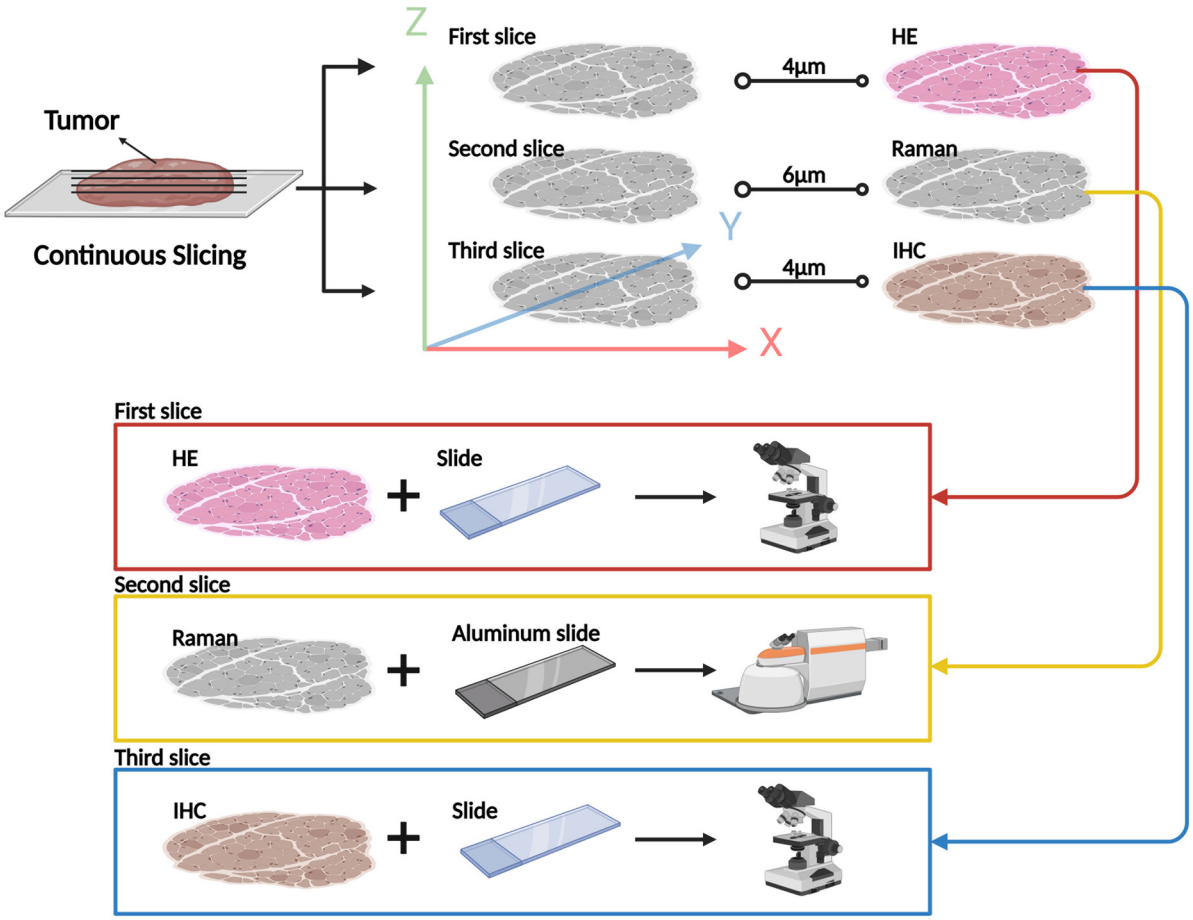

Figure S1 In this work, the serial section method was used for H&E staining, immunohistochemistry, and unstained sections were reserved for Raman spectroscopy acquisition to ensure consistency of information between adjacent sections and to minimize the impact of sample selection bias on the results. H&E, hematoxylin and eosin; IHC, immunohistochemistry.

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Table S1 Information on all patients/subjects

Patient number	Gender	Age, years	Rapid pathology	Pathology gold standard	Sampling	Training or testing
1	Male	62	MIA	MIA	Tumor	Randomly
2	Male	55	MIA	MIA	Tumor	Randomly
3	Female	57	IAC	IAC	Tumor	Randomly
4	Male	62	IAC	IAC	Tumor	Randomly
5	Female	71	IAC	IAC	Tumor	Randomly
6	Female	62	IAC	IAC	Tumor	Randomly
7	Female	60	IAC	IAC	Tumor	Randomly
8	Male	60	IAC	IAC	Tumor	Randomly
9	Female	69	IAC	IAC	Tumor	Randomly
10	Female	55	MIA	MIA	Tumor	Randomly
11	Female	51	IAC	IAC	Tumor	Randomly
12	Female	67	IAC	IAC	Tumor	Randomly
13	Male	67	IAC	IAC	Tumor	Randomly
14	Female	59	IAC	IAC	Tumor	Randomly
15	Male	60	IAC	IAC	Tumor	Randomly
16	Male	62	IAC	IAC	Tumor	Randomly
17	Female	54	IAC	IAC	Tumor	Randomly
18	Male	58	IAC	IAC	Tumor	Randomly
19	Male	72	IAC	IAC	Tumor	Randomly
20	Male	57	IAC	IAC	Tumor	Randomly
21	Female	54	IAC	IAC	Tumor	Randomly
22	Male	65	IAC	IAC	Tumor	Randomly
23	Male	56	IAC	IAC	Tumor	Randomly
24	Female	52	IAC	IAC	Tumor	Randomly
25	Female	57	IAC	IAC	Tumor	Randomly
26	Male	65	IAC	IAC	Tumor	Randomly
27	Male	62	IAC	IAC	Tumor	Randomly
28	Male	53	MIA	MIA	Tumor	Randomly
29	Female	26	MIA	MIA	Tumor	Randomly
30	Female	74	IAC	IAC	Tumor	Randomly
31	Male	56	IAC	IAC	Tumor	Randomly
32	Female	50	IAC	IAC	Tumor	Randomly
33	Female	57	IAC	IAC	Tumor	Randomly
34	Female	51	IAC	IAC	Tumor	Randomly
35	Male	69	IAC	IAC	Tumor	Randomly
36	Female	51	IAC	IAC	Tumor	Randomly
37	Male	69	IAC	IAC	Tumor	Randomly
38	Female	65	IAC	IAC	Tumor	Randomly
39	Male	58	IAC	IAC	Tumor	Randomly
40	Male	60	IAC	IAC	Tumor	Randomly
41	Female	50	IAC	IAC	Tumor	Randomly
42	Male	57	IAC	IAC	Normal tissue	Randomly
43	Male	71	IAC	IAC	Normal tissue	Randomly
44	Male	61	IAC	IAC	Normal tissue	Randomly
45	Male	60	IAC	IAC	Normal tissue	Randomly
46	Male	62	IAC	IAC	Normal tissue	Randomly
47	Female	59	IAC	IAC	Normal tissue	Randomly
48	Male	75	IAC	IAC	Normal tissue	Randomly
49	Female	67	IAC	IAC	Normal tissue	Randomly
50	Female	62	IAC	IAC	Normal tissue	Randomly
51	Female	71	IAC	IAC	Normal tissue	Randomly
52	Male	60	IAC	IAC	Normal tissue	Randomly
53	Male	51	IAC	IAC	Normal tissue	Randomly
54	Male	55	MIA	IAC	Tumor	Testing
55	Female	62	MIA	IAC	Tumor	Testing
56	Female	59	MIA	IAC	Tumor	Testing
57	Male	55	MIA	IAC	Tumor	Testing
58	Female	74	MIA	IAC	Tumor	Testing
59	Female	65	MIA	IAC	Tumor	Testing

MIA, microinvasive adenocarcinomas; IAC, invasive adenocarcinoma.

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Table S2 The classification results are assessed by comparing the predicted labels generated by the model with the true labels

Confusion matrix		True label	
		Positive	Negative
Predicted label	Positive	TP	FP
	Negative	FN	TN

TP, true positive; FP, false positive; FN, false negative; TN, true negative.

Table S3 Essential features and peak attributions employed for tissue type classification and corresponding Raman peaks (1,2)

Feature (cm^{-1})	Raman peak assignment	Biological information	Increase/decrease peaks			
			Normal tissue	LADC	MIA	IAC
852	Ring C-C bend	Protein (proline, tyrosine)	↑	↓	↑	↓
937	C-C stretch mode	Protein (proline, valine, a-helix)	↑	↓	↓	↑
1004	C-C aromatic ring stretch	Protein (phenylalanine)	↓	↑	↑	↓
1032	C-H bend mode	Protein (phenylalanine)	↑	↓	↑	↓
1209	C-C_6H_5 stretch mode	Protein (phenylalanine, tryptophan, tyrosine)	↑	↓	↑	↓
1238	Amide III (C-N, N-H bend)	Protein	↑	↓	↑	↓
1308	CH_3/CH_2 twist mode	Collagen and lipid	—	—	—	—
1341	Guanine; C-H	DNA/RNA; proteins and carbohydrates	↓	↑	↑	↓
1451	CH (CH_2) bend mode	Protein and lipid	↑	↓	—	—
1671	Amide I (C=O, C-N and N-H bend)	Protein	↓	↑	↓	↑

LADC, lung adenocarcinoma; MIA, microinvasive adenocarcinomas; IAC, invasive adenocarcinoma.

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