Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Figure S1 Raw Raman spectra showing mean ± standard deviation.

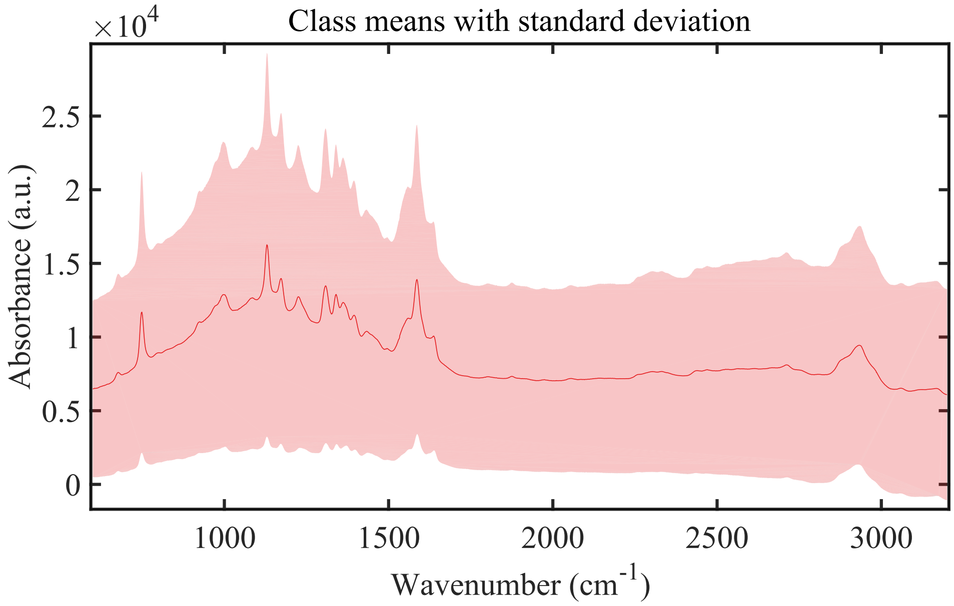


Table S1 Primary wavenumbers in loading plots derived from principal component analysis of the Raman spectral dataset (Cancer vs. Control tissue)

	Raman shift (cm^{-1})	**Tentative assignments**
PC1	1595	Phenylalanine & Tyrosine
	738	Cytosine/Uracil Ring Breathing of DNA/RNA
	1121	C-N Stretching of proteins
	1644	C=O Stretching of Collagen or C=C Stretch of Fatty Acids
	1297	CH_3/δ(CH_2) Twisting, Wagging, Collagen, Amide III, & -CH_2- Bending
	1179	C-H Bending Tyrosine
	1405	C-N Stretching
	1436	CH_2 Deformation
	1332	Nucleic Acid
	1233	Asymmetric Phosphate Stretching
PC2	1305	CH_3/δ(CH_2) Twisting, Wagging, Collagen, Amide III, & -CH_2- Bending
	1011	Phenylalanine
	1578	Tryptophan v(CN) & Amide II, v(C=C) Porphyrin & Tyrosine
	1441	CH_2 Deformation
	1472	CH2 Bending/CH_3 Bending, C-H Deformations
	1228	Asymmetric Phosphate Stretching
	752	Cytosine/Uracil Ring Breathing of DNA/RNA
	1692	C=O Stretching
	1547	v(C=C)
	1152	C-C, C-N Stretching
PC3	1647	C=O Stretching of Collagen or C=C Stretch of Fatty Acids
	754	Cytosine/Uracil Ring Breathing of DNA/RNA
	1132	C-N Stretching
	1600	Phenylalanine & Tyrosine
	1341	CH Deformation
	1444	CH Deformation
	1009	Phenylalanine
	1383	C-N Stretching in Quinoid Ring-Benzoid
	1309	CH_3/δ(CH_2) Twisting, Wagging, Collagen, Amide III, & -CH_2- Bending
	1690	C=O Stretching

Table S2 Score plots in the LD1 space derived from the Raman spectral dataset at different cancer stages

One-way ANOVA with Dunnett's Multiple Comparison Test	**LD1**
T1 vs. Control	$P < 0.0001$
T2 vs. Control	$P < 0.0001$
T3 vs. Control	$P < 0.0001$
T1 vs. T2	$P > 0.05$
T1 vs. T3	$P < 0.05$
T2 vs. T3	$P < 0.05$

Table S3 Primary wavenumbers in cluster vectors derived from principal component analysis-linear discriminant analysis of the Raman spectral dataset at different cancer stages (Others compared to T0)

	Raman shift (cm^{-1})	Tentative assignments
T1	1132	C-N Stretching
	1591	Phenylalanine & Tyrosine
	750	Cytosine/Uracil, Ring Breathing
	1314	Nucleic Acid
	1343	CH Deformation
	1266	Amide III (α-Helix)/Tryptophan/Collagen
	1199	Tryptophan & Phenylalanine ν($C-C_6H_5$)
	1007	Phenylalanine
	1373	*vs* (CH_3) Phospholipids
	1527	Tryptophan ν(CN) & Amide II, *ν*(C=C) Porphyrin & Tyrosine
T2	1132	C-N Stretching
	750	Cytosine/Uracil, Ring Breathing
	1471	CH_2 Bending/CH_3 Bending
	1314	Nucleic Acid
	1176	C-H Bending Tyrosine
	1591	Phenylalanine & Tyrosine
	1799	C=O Stretching
	1343	CH Deformation
	1258	C-N in Plane Stretching
	1000	Phenylalanine
T3	1132	C-N Stretching
	750	Cytosine/Uracil, Ring Breathing
	1591	Phenylalanine & Tyrosine
	1314	Nucleic Acid
	1343	CH Deformation
	1265	Amide III (α-Helix)/Tryptophan/Collagen
	875	C-C-N+ Symmetric Stretching in lipid
	1527	Tryptophan ν(CN) & Amide II, *ν*(C=C) Porphyrin & Tyrosine
	1176	C-H Bending Tyrosine
	1007	Phenylalanine

Table S4 Ratio of Raman intensity derived from the Raman spectral dataset of different cancer stages

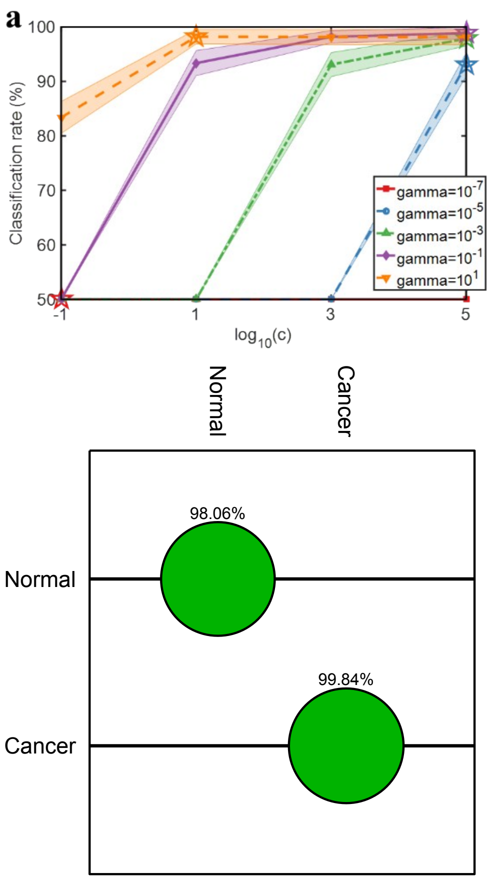
Ratio	One-way ANOVA with Dunnett's Multiple Comparison Test	
Saturated lipid	Control vs. T1	****
	Control vs. T2	****
	Control vs. T3	*
	T1 vs. T2	ns
	T1 vs. T3	***
	T2 vs. T3	***
Unsaturated lipid	Control vs. T1	***
	Control vs. T2	****
	Control vs. T3	**
	T1 vs. T2	****
	T1 vs. T3	ns
	T2 vs. T3	***
Protein to lipid	Control vs. T1	****
	Control vs. T2	****
	Control vs. T3	****
	T1 vs. T2	ns
	T1 vs. T3	ns
	T2 vs. T3	ns
Nucleic acid to lipid	Control vs. T1	****
	Control vs. T2	****
	Control vs. T3	****
	T1 vs. T2	ns
	T1 vs. T3	ns
	T2 vs. T3	ns
Protein to nucleic acid	Control vs. T1	***
	Control vs. T2	ns
	Control vs. T3	**
	T1 vs. T2	ns
	T1 vs. T3	ns
	T2 vs. T3	ns

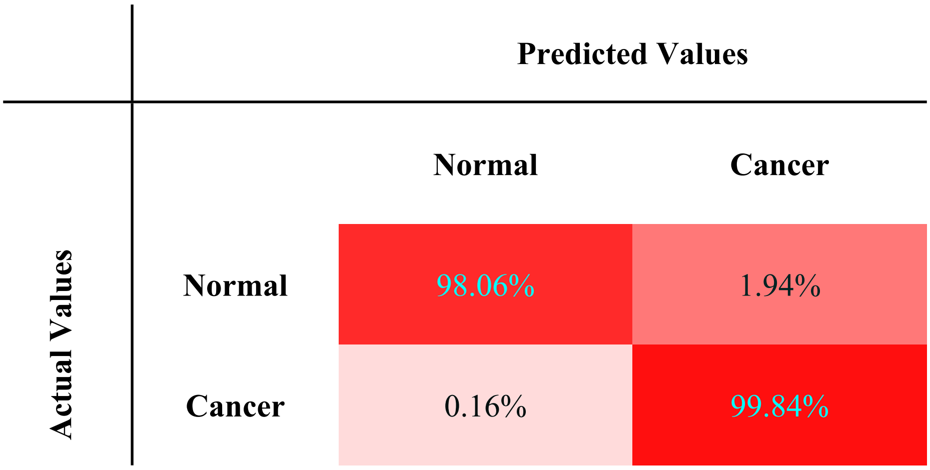
****: P＜0.0001; ***: P＜0.001; **: P＜0.01; *: P≤0.05; ns: P＞0.05

Table S5 Primary wavenumbers in cluster vectors derived from principal component analysis-linear discriminant analysis of the Raman spectral dataset of adenocarcinomas and squamous carcinomas.

	Raman shift (cm^{-1})	**Tentative assignments**
Adenocarcinomas vs. Squamous carcinomas	1134	C-N Stretching
	896	Phosphodiester
	1455	CH_2 Stretching/CH_3 Asymmetric Deformation
	823	Out-of-Plane Ring Breathing, Tyrosine
	1364	Tryptophan
	1628	Amide I
	866	Phosphatidic Acid
	1170	C-H In-Plane Bending Mode of Tyrosine
	751	Symmetric Breathing of Tryptophan
	1331	Phospholipids

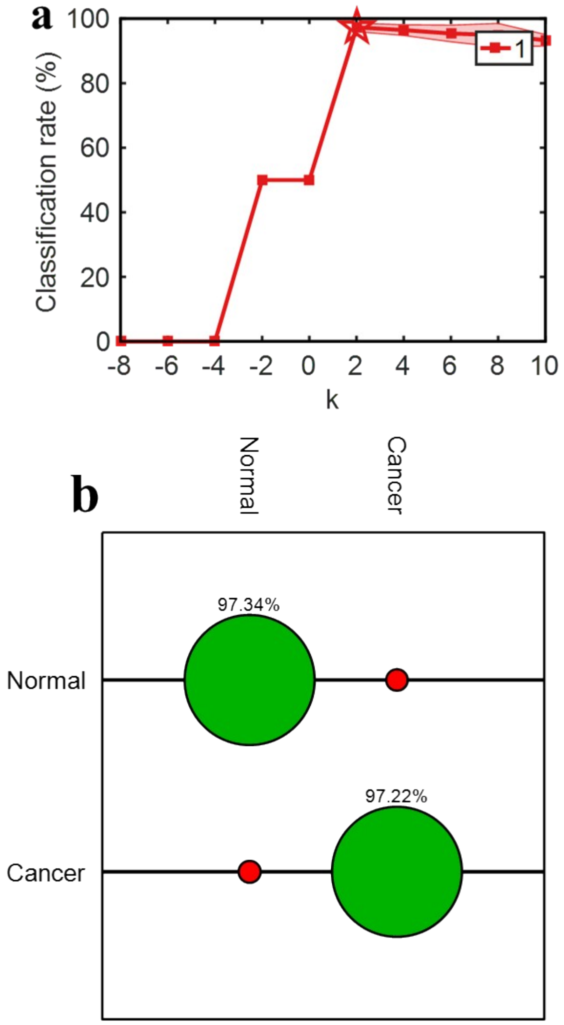
Figure S2 Classification rate of Raman spectral data at different cancer stages calculated via the **SVM** method.

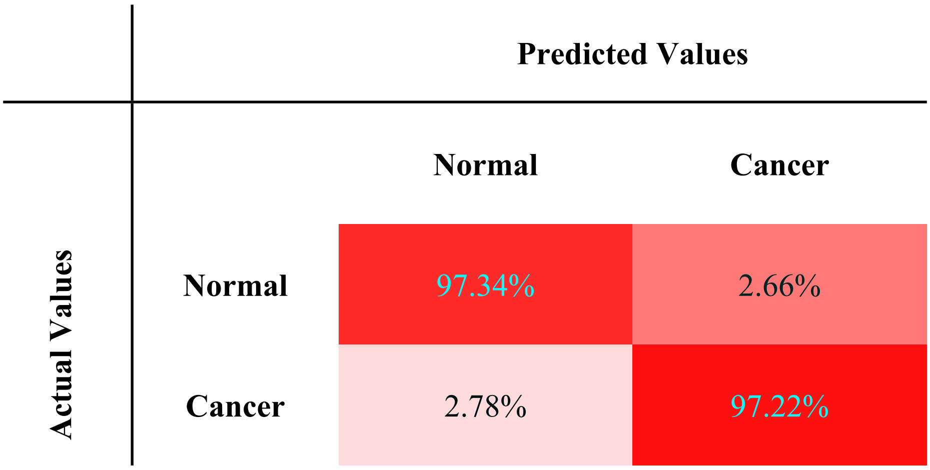




		Predicted Values	
		Normal	Cancer
Actual Values	Normal	98.06%	1.94%
	Cancer	0.16%	99.84%

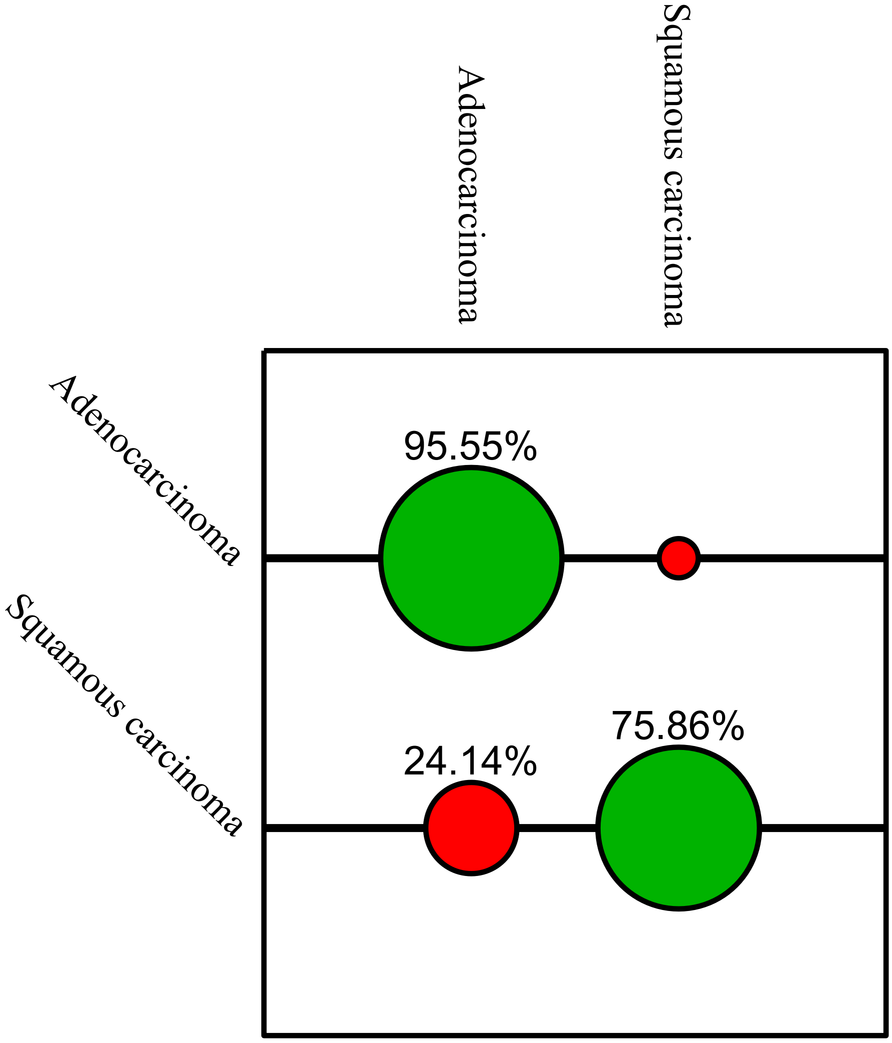
Figure S3 Classification rate of Raman spectral data at different cancer stages, determined via the **kNN** method.

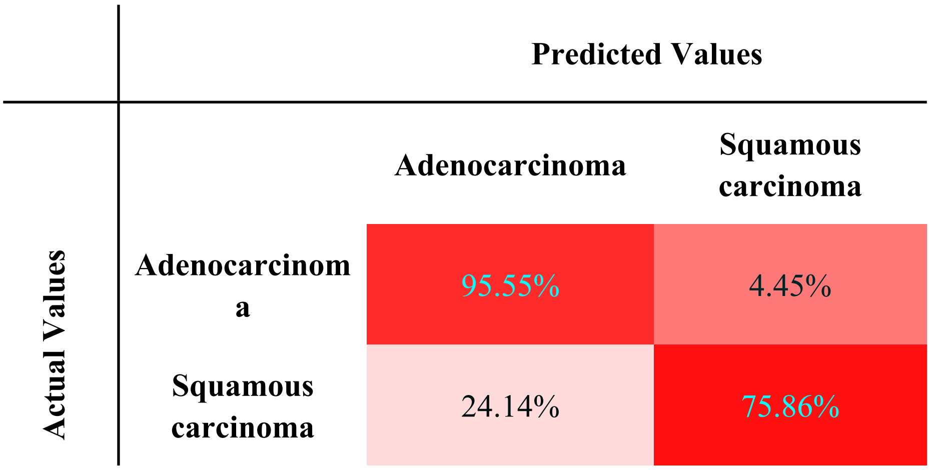




		Predicted Values	
		Normal	**Cancer**
Actual Values	**Normal**	97.34%	2.66%
	Cancer	2.78%	97.22%

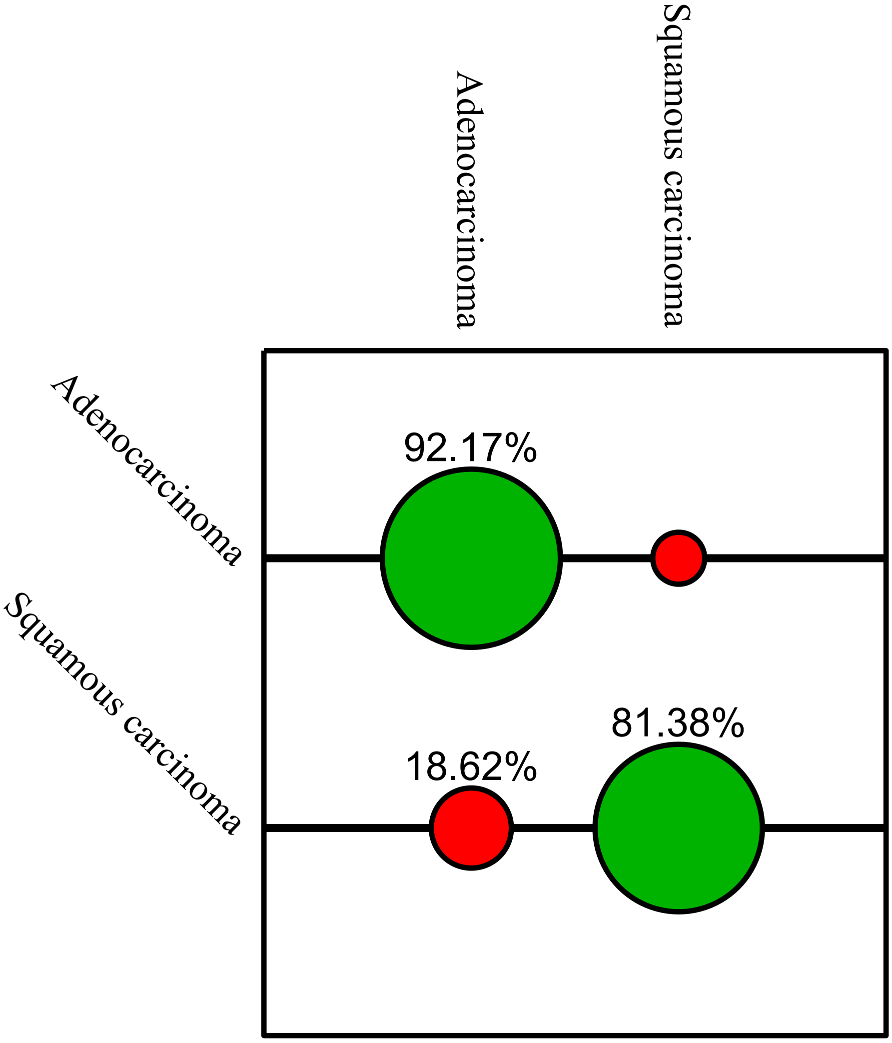
Figure S4 Classification rate of Raman spectral data of different cancer types determined via the **SVM** method.

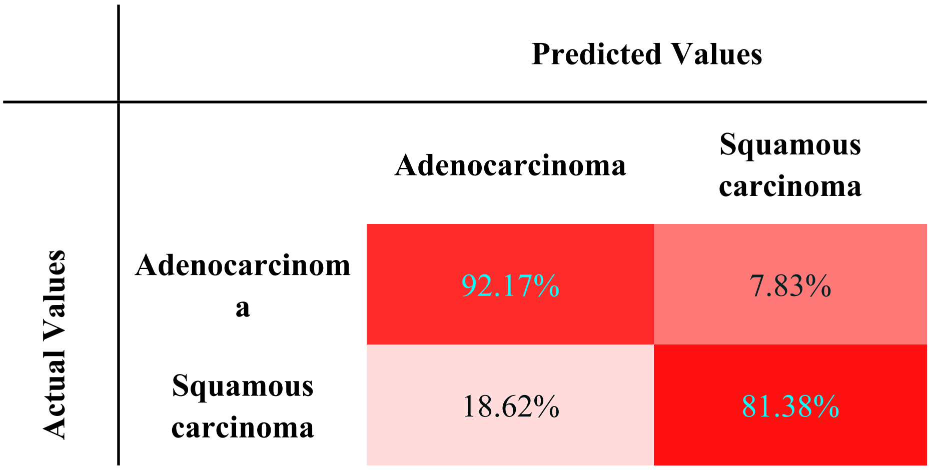




		Predicted Values	
		Adenocarcinoma	**Squamous carcinoma**
Actual Values	**Adenocarcinoma**	95.55%	4.45%
	Squamous carcinoma	24.14%	75.86%

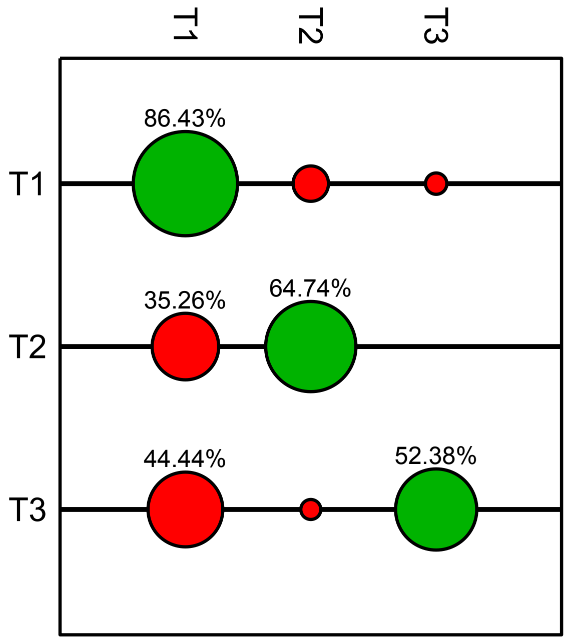
Figure S5 Classification rate of Raman spectral data of different cancer types determined via the **kNN** method.





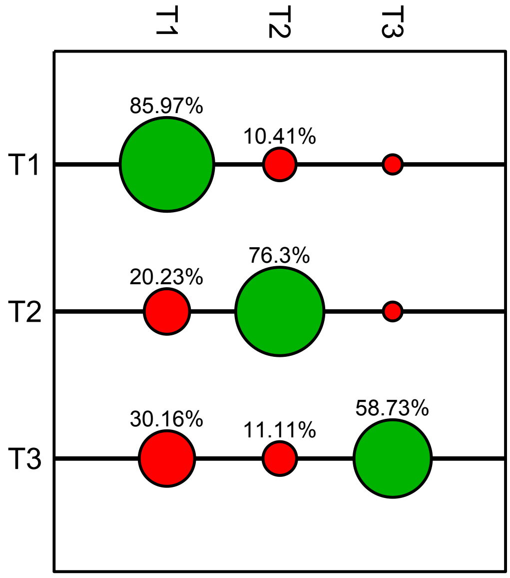
		Predicted Values	
		Adenocarcinoma	**Squamous carcinoma**
Actual Values	**Adenocarcinoma**	92.17%	7.83%
	Squamous carcinoma	18.62%	81.38%

Figure S6 Classification rate of Raman spectral data at different cancer stages, determined via the **SVM** method.



		Predicted Values		
		T1	**T2**	**T3**
Actual Values	**T1**	86.43%	9.95%	3.62%
	T2	35.26%	64.74%	0%
	T3	50.09%	17.67%	50.34%

Figure S7 Classification rate of Raman spectral data at different cancer stages, determined via the **kNN** method.



		Predicted Values		
		T1	**T2**	**T3**
Actual Values	**T1**	85.97%	10.41%	3.62%
	T2	20.23%	76.30%	3.47%
	T3	30.16%	11.11%	58.73%