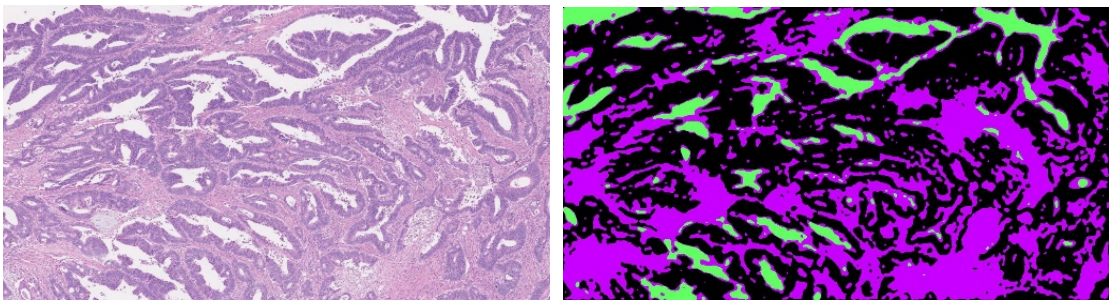
Materials Figure for

Rapid multi-dynamic algorithm for gray image analysis of the stroma percentage on colorectal cancer

Supplementary Figure

A **B**



Green- Necrosis (num1) Black -Tumor (num2) Purple-Stroma (num3)

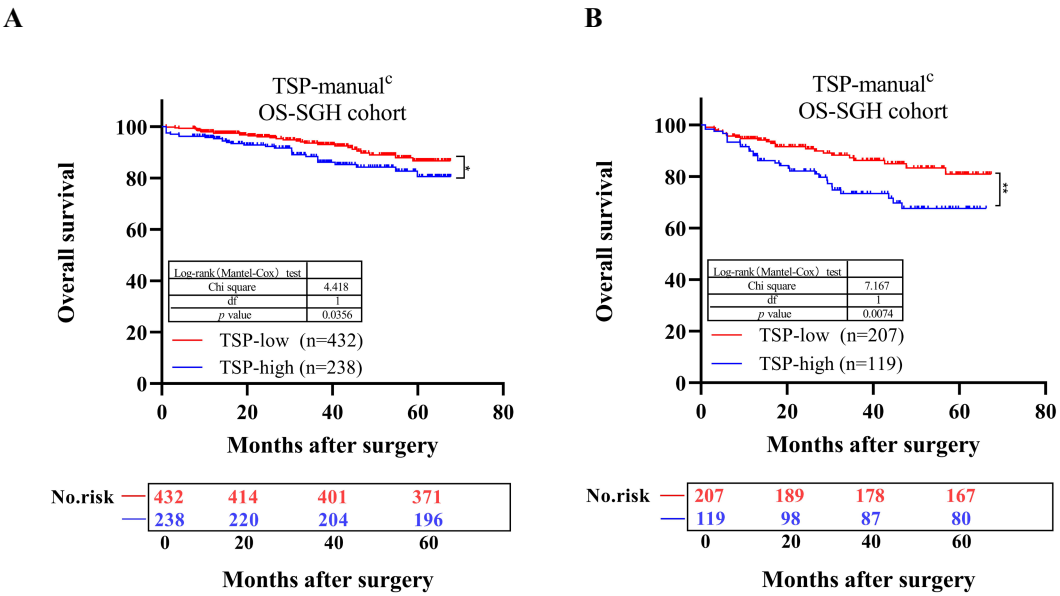
Supplementary Figure 1. The regions with tissues segmented by the MATLAB for the calculation of TSP-cad.

(A)Original Red-Green-Blue(RGB) style (B)Image was annotated using tumor, stroma and necrosis labels.

The result was num1= 0.0863 num2= 0.5459 num3=0.368

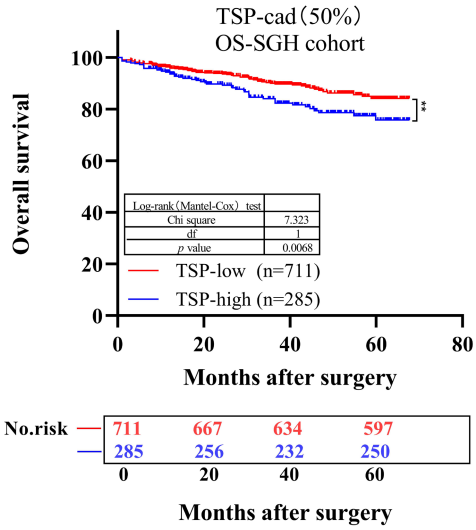
we considered the tumor and stroma proportions to be complementary.

TSP=Stroma/(Tumor+Stroma)*100%=num3/(num2+num3)*100%=0.368/(0.5459+0.368) *100%=40.27%

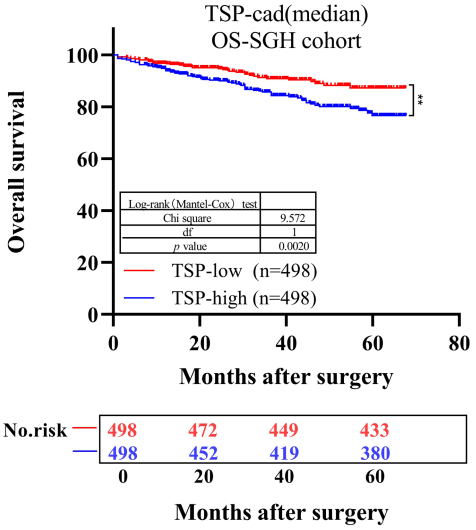


Supplementary Figure 2. Kaplan-Meier survival analysis curves of SGH chort for TSP-low verse TSP-high. (A) represent the OS of the left sided and rectum tumors (B) represent the OS of the right sided tumors.

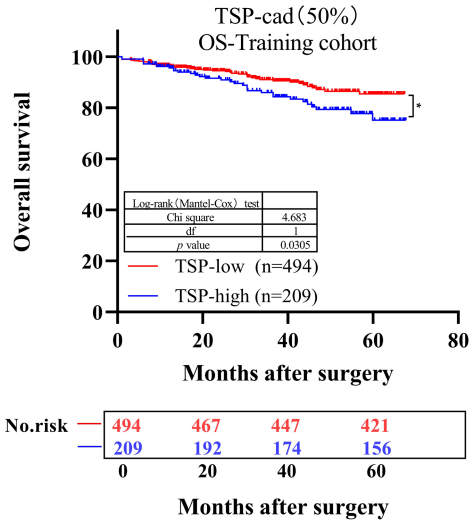
A


TSP-cad（50%）
OS-SGH cohort
Overall survival
100
80
60
40
20
0
0
20
40
60
80
Months after surgery
Log-rank（Mantel-Cox） test
Chi square 7.323
df 1
p value 0.0068
TSP-low (n=711)
TSP-high (n=285)
**
No.risk
711 667 634 597
285 256 232 250
0 20 40 60
Months after surgery

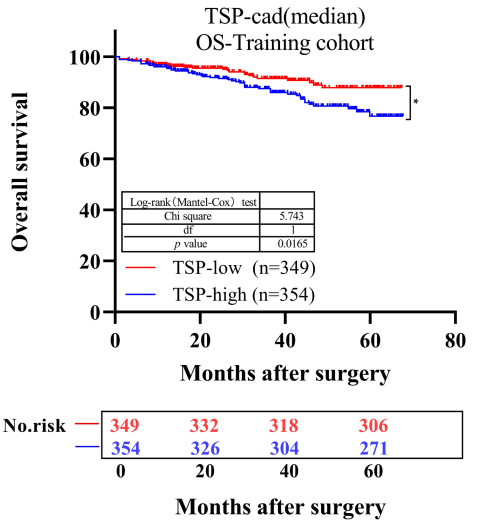
B


TSP-cad(median)
OS-SGH cohort
Overall survival
100
80
60
40
20
0
0
20
40
60
80
Months after surgery
Log-rank（Mantel-Cox） test
Chi square 9.572
df 1
p value 0.0020
TSP-low (n=498)
TSP-high (n=498)
**
No.risk
498 472 449 433
498 452 419 380
0 20 40 60
Months after surgery

C

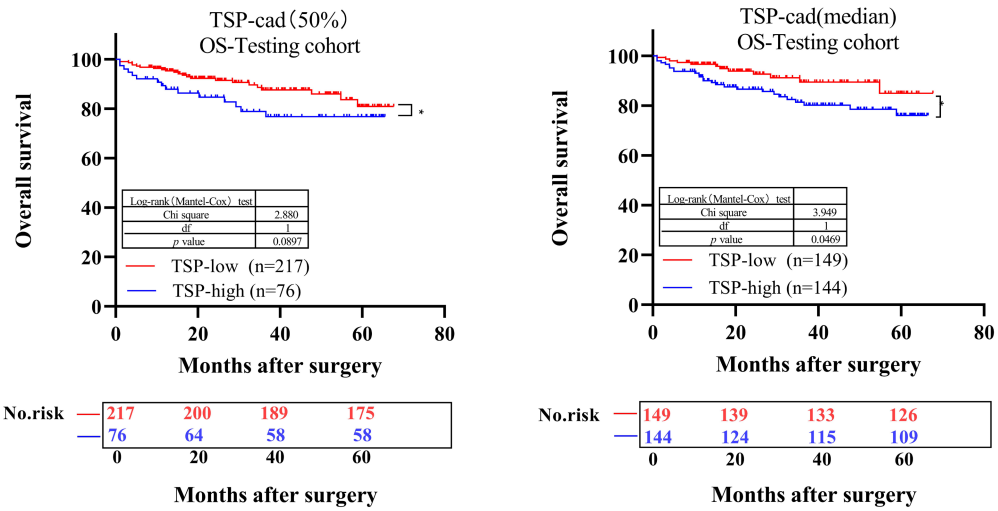

TSP-cad（50%）
OS-Training cohort
Overall survival
100
80
60
40
20
0
0
20
40
60
80
Months after surgery
Log-rank（Mantel-Cox） test
Chi square 4.683
df 1
p value 0.0305
TSP-low (n=494)
TSP-high (n=209)
*
No.risk
494 467 447 421
209 192 174 156
0 20 40 60
Months after surgery

D


TSP-cad(median)
OS-Training cohort
Overall survival
100
80
60
40
20
0
0
20
40
60
80
Months after surgery
Log-rank（Mantel-Cox） test
Chi square 5.743
df 1
p value 0.0165
TSP-low (n=349)
TSP-high (n=354)
*
No.risk
349 332 318 306
354 326 304 271
0 20 40 60
Months after surgery

E

F



Supplementary Figure 3. Kaplan-Meier survival analysis for overall survival of TSP-low verse TSP-high. Results for SGH cohort (A,B), Training cohort (C,D), Testing cohort (E,F), according to the TSP-cad(50%) and TSP-cad(medium) classifier stratified by clinicopathological risk factors. *P*-values were calculated by log-rank test.

Materials Table for

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Supplementary Table

Supplementary Table 1 Clinicopathological data for TMA cohort in relation to TSP-manual

Variable	TSP-manual[a]			
	N	TSP-low(%)	TSP-high(%)	p value
Age(years)				0.057
<65	55	37(59.7%)	18(40.9%)	
≥65	51	25(40.3%)	26(59.1%)	
Gender				0.947
Male	55	32(51.6%)	23(52.3%)	
Female	51	30(48.4%)	21(47.7%)	
TNM Stage				< 0.001*
I+II	51	41(66.1%)	10(22.7%)	
III+IV	55	21(33.9%)	34(77.3%)	
Depth of invasion				0.991
T1+T2	12	7(11.3%)	5(11.4%)	
T3+T4	94	55(88.7%0	39(88.6%)	
Lymph node metastasis				< 0.001*
N0	49	39(62.9%)	10(22.7%)	
N1+N2	57	23(37.1%)	34(77.3%)	
Nerve invasion				0.720
NO	79	47(75.8%)	32(73.7%)	
YES	27	15(24.2%)	12(27.3%)	
Vessel invasion				0.061
NO	66	34(54.8%)	32(72.7%)	
YES	40	28(45.2%)	12(27.3%)	

Differentiation				0.954
Well	19	11(17.7%)	8(18.2%)	
Moderate+poor	87	51(82.3%)	36(81.8%)	
Tumor size				0.323
<5cm	59	37(59.7%)	22(50.0%)	
≥5cm	47	25(40.3%)	22(50.0%)	
Tumor location				0.107
Right	41	20(32.3%)	21(47.7%)	
Left and rectal	65	42(67.7%)	23(52.3%)	

*P < 0.05

Supplementary Table 2 Clinicopathological data for TCGA cohort in relation to TSP-manual

Variable	TSP- manual[a]			
	N	TSP-low(%)	TSP-high(%)	p value
Age(years)				0.697
<65	200	160(46.2%)	40(44.0%)	
≥65	237	186(53.8%)	51(56.0%)	
Gender				0.647
Male	235	188(54.3%)	47(51.6%)	
Female	202	158(45.7%)	44(48.4%)	
TNM stage				0.024*
I+II	238	198(57.2%)	40(44.0%)	
III+IV	199	148(42.8%)	51(56.0%)	
Depth of invasion				0.021*
T1+T2	91	80(23.1%)	11(12.1%)	
T3+T4	346	266(76.9%)	80(87.9%)	
Lymph node metastasis				0.028*
N0	246	204(59.0%)	42(46.2%)	
N1+N2	191	142(41.0%)	49(53.8%)	
lymphatic invasion				0.237
NO	287	232(67.1%)	55(60.4%)	
YES	150	114(32.9%)	36(39.6%)	
Vessel invasion				0.61
NO	340	271(78.3%)	69(75.8%)	
YES	97	75(21.7%)	22(24.2%)	

Tumor location				0.662
Right	169	132(38.2%)	37(40.7%)	
Left and rectal	268	214(61.8%)	54(59.3%)	

*P < 0.05

Supplementary Table 3 Cross-tabulation of TSP-mannual versus TSP-cad after dichotomisation in the Training cohort

		TSP-manual		
		TSP-low	TSP-high	total
TSP-cad(50%)	TSP-low	444	50	494
K=0.827	TSP-high	4	205	209
	total	448	255	703
TSP-cad(median)	TSP-low	318	31	349
K=0.543	TSP-high	130	224	354
	total	448	255	703

Supplementary Table 4 Cross-tabulation of TSP-mannual versus TSP-cad after dichotomisation in the Testing cohort

		TSP-manual		
		TSP-low	TSP-high	total
TSP-cad(50%)	TSP-low	190	27	217
K=0.776	TSP-high	1	75	76
	total	191	102	293
TSP-cad(median)	TSP-low	139	10	149
K=0.575	TSP-high	52	92	144
	total	191	102	293

Supplementary Table 5 Predictive performance comparison among the different TSP in SGH cohort

SGH cohort	Linear trend $\chi 2$ score	LR test
Over survival		
TSP-manual	12.025	11.601
TSP-cad(50%)	10.953	10.3
TSP-cad(median)	13.445	13.673

LR, likelihood ratio;

Supplementary Table 6 Uni- and multivariate Cox regression analysis for overall survival in the Traning cohort

	Unvariate analysis		Multivariate analysis					
			TSP-manual		TSP-cad(50%)		TSP-cad(median)	
	HR	95%CI	HR	95%CI	HR	95%CI	HR	95%CI
Age(years)								
<65	1							
≥65	3.508	1.804-5.183						
Gender								
Male	1							
Female	0.594	0.368-0.956						
TNM Stage								

I+II	1		1		1		1	
III+IV	3.695	2.291-5.959*	1.818	0.563-5.869	1.822	0.571-5.811	1.755	0.569-5.411
Depth of invasion								
T1+T2	1		1		1		1	
T3+T4	2.966	1.364-6.452*	1.576	0.697-3.564	1.556	0.687-3.524	1.574	0.696-3.561
LN metastasis								
N0	1		1		1		1	
N1+N2	3.584	2.223-5.779*	1.497	0.464-4.830	1.494	0.469-4.759	1.516	0.494-4.653
Nerve invasion								
NO	1		1		1		1	
YES	2.165	1.359-3.450*	1.191	0.713-1.992	1.21	0.724-2.021	1.217	0.729-2.031
Vessel invasion								
NO	1		1		1		1	
YES	3.004	1.913-4.716*	1.981	1.190-3.298*	1.98	1.189-3.295*	1.965	1.182-3.267*
Differentiation status								
Well	1							
Moderate+poor	1.511	0.610-3.743						
Tumor size								
<5cm	1							
≥5cm	1.059	0.670-1.675						
Tumor location								
Right	1		1		1		1	
Left and rectal	0.564	0.360-0.884*	0.581	0.366-0.992*	0.575	0.363-0.913*	0.567	0.358-0.898*
TSP-manual								
TSP-low	1		1					
TSP-high	1.61	1.030-2.517*	1.376	0.876-2.161*				
TSP-cad(50%)								
TSP-low	1				1			
TSP-high	1.638	1.042-2.573*			1.413	0.908-2.256*		
TSP-cad(median)								
TSP-low	1						1	
TSP-high	1.759	1.101-2.809*					1.539	0.960-2.468*

Abbreviations: HR: hazard ratio; CI: confidence interval; LN metastasis:Lymph node metastasis

*P < 0.05

Supplementary Table 7 Uni- and multivariate Cox regression analysis for overall survival in the Testing cohort

	Unvariate nalysis		Multivariate analysis					
			TSP-manual		TSP-cad(50%)		TSP-cad(median)	
	HR	95%CI	HR	95%CI	HR	95%CI	HR	95%CI
Age(years)								
<65	1							
≥65	2.368	1.646-3.407						
Gender								
Male	1							
Female	0.613	0.413-0.909						

TNM Stage								
I+II	1		1		1		1	
III+IV	3.54	2.404-5.212*	1.171	0.443-3.096	1.197	0.46-3.114	1.214	0.486-3.169
Depth of invasion								
T1+T2	1		1		1		1	
T3+T4	2.878	1.504-5.506*	1.343	0.681-2.65	1.343	0.68-2.651	1.315	0.666-2.597
LN metastasis								
N0	1		1		1		1	
N1+N2	3.724	2.516-5.512*	2.334	0.869-6.268	2.305	0.872-6.092	2.186	0.841-5.678
Nerve invasion								
NO	1		1		1		1	
YES	2.298	1.575-3.353*	1.38	0.96-2.103	1.41	0.927-2.145	1.398	0.919-2.126
Vessel invasion								
NO	1		1		1		1	
YES	2.684	1.858-3.875*	1.608	1.055-2.451*	1.593	1.045-2.427*	1.593	1.046-2.425*
Differentiation status								
Well	1		1		1		1	
Moderate+poor	2.574	1.050-6.312*	2.613	0.874-5.352	2.19	0.884-5.423	2.198	0.888-5.443
Tumor size								
<5cm	1							
≥5cm	1.287	0.889-1.862						
Tumor location								
Right	1		1		1		1	
Left and rectal	0.502	0.348-0.723*	0.467	0.320-0.682*	0.461	0.316-0.673*	0.456	0.312-0.664*
TSP-manual								
TSP-low	1		1					
TSP-high	1.75	1.214-2.523*	1.512	1.045-2.187*				
TSP-cad(50%)								
TSP-low	1				1			
TSP-high	1.659	1.145-2.405*			1.443	0.993-2.097		
TSP-cad(median)								
TSP-low	1						1	
TSP-high	1.823	1.239-2.683*					1.632	1.105-2.410*

Abbreviations: HR: hazard ratio; CI: confidence interval; LN metastasis:Lymph node metastasis

*P < 0.05