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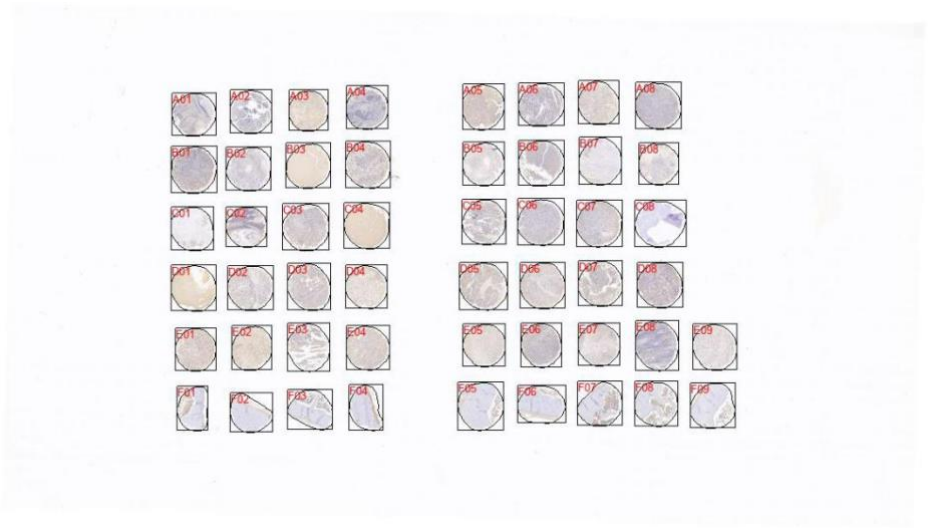
SUPPLEMENTAL DATA

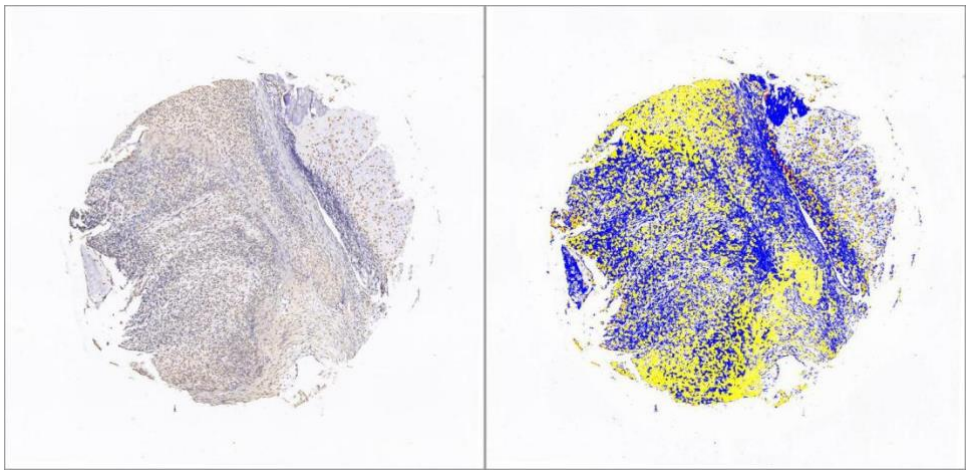
PLCE1 is a poor prognostic marker and may promote immune escape from osteosarcoma by the CD70-CD27 signaling pathway

1. Tissue chip immunohistochemistry

BON501 G022 PLCE1



BON501 G022 PLCE1-A01



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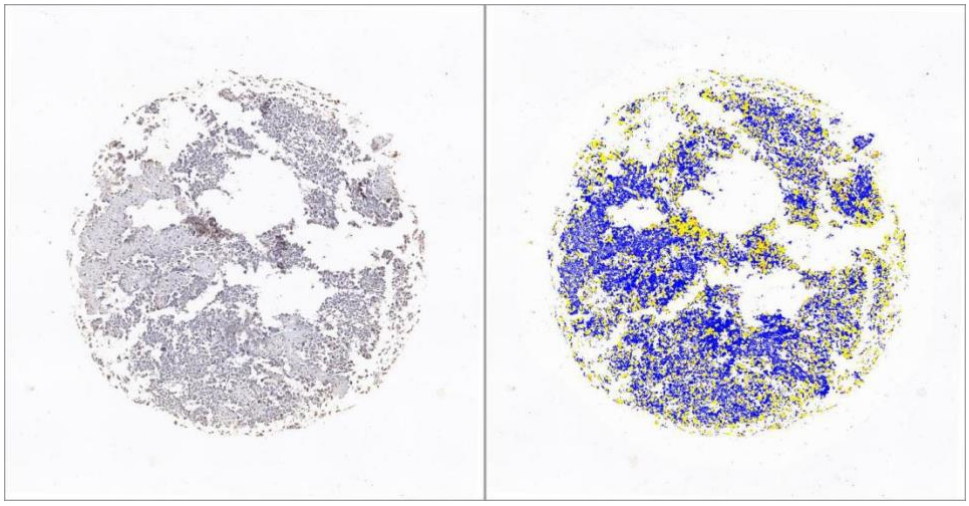

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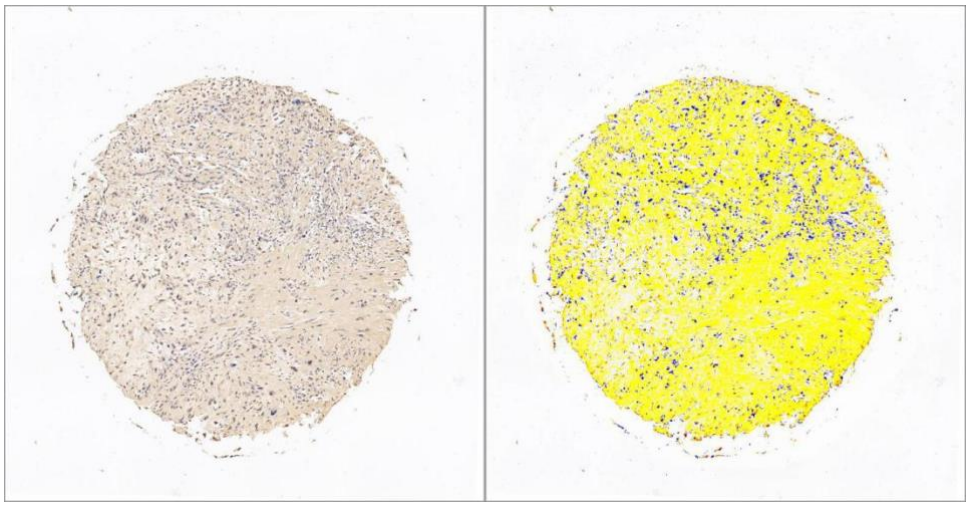

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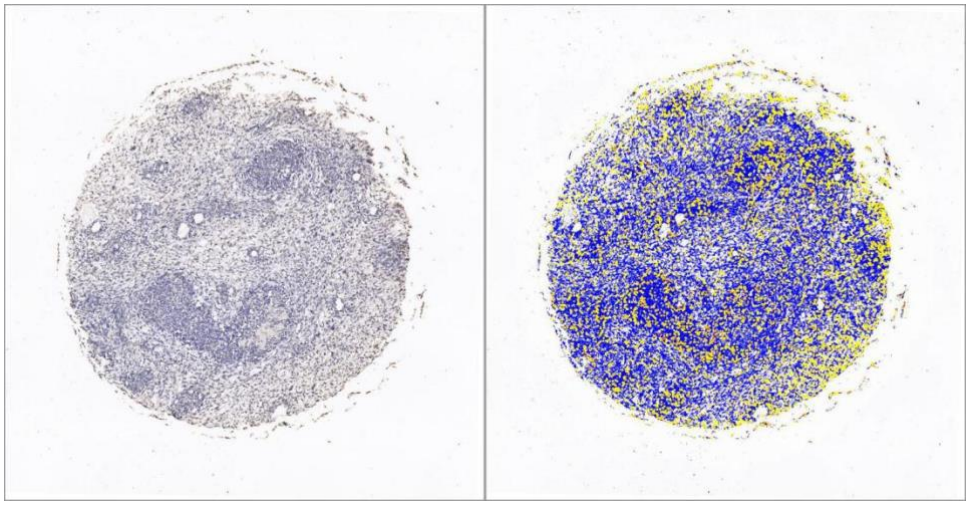

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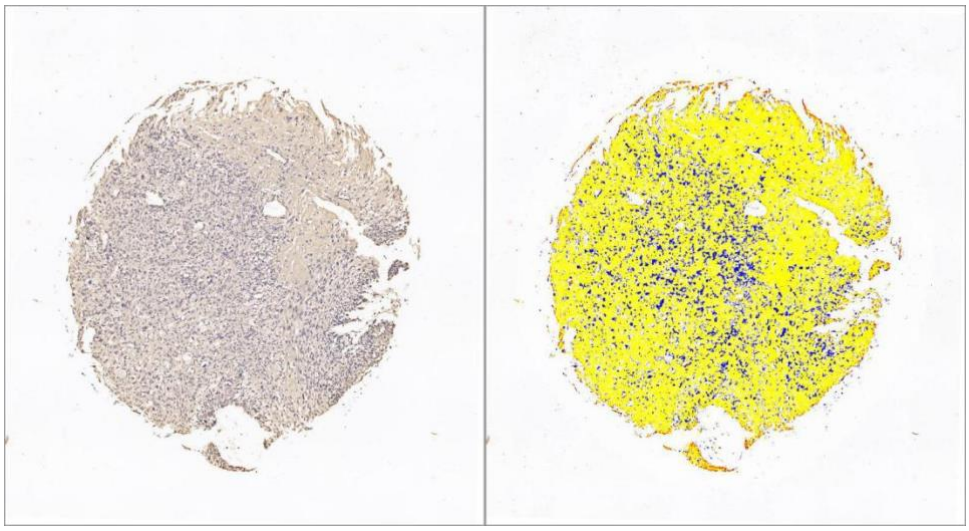

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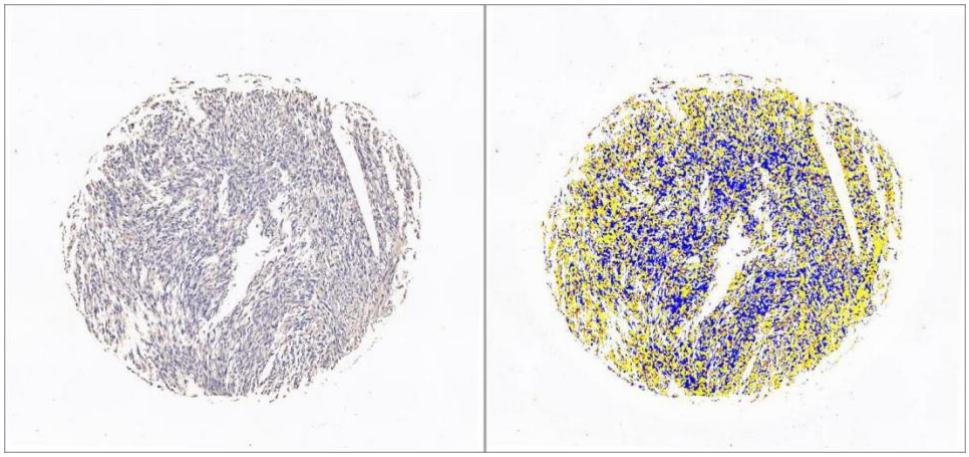

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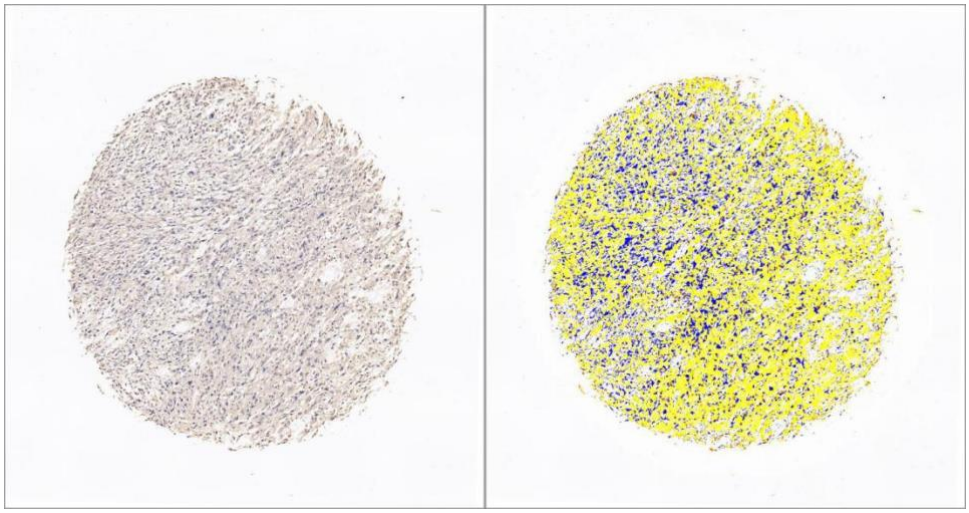

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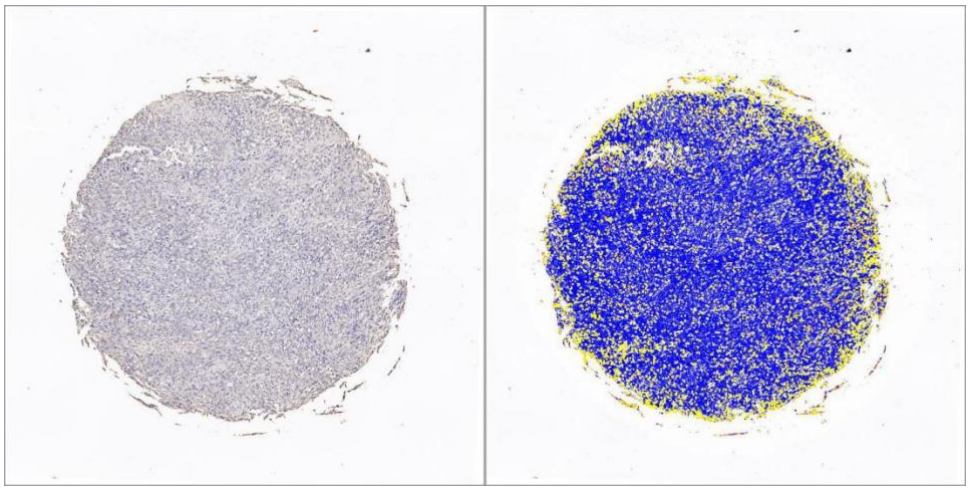

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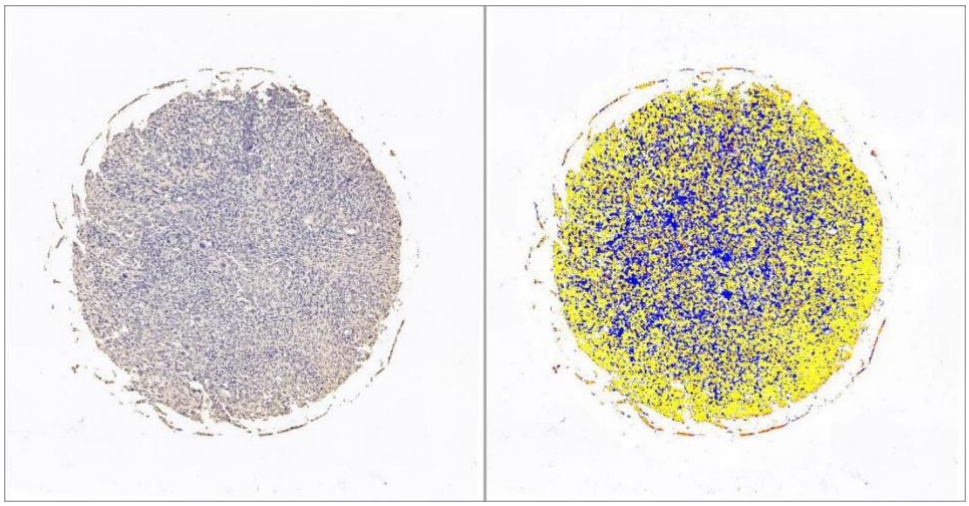

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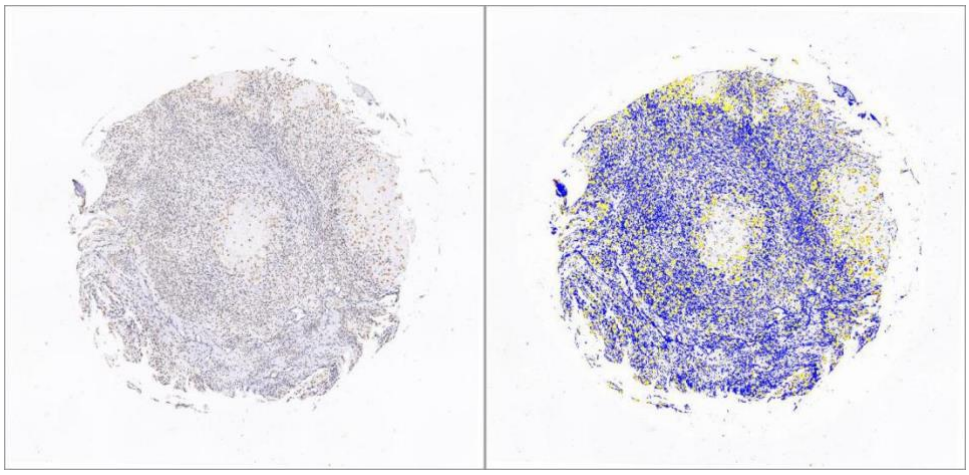

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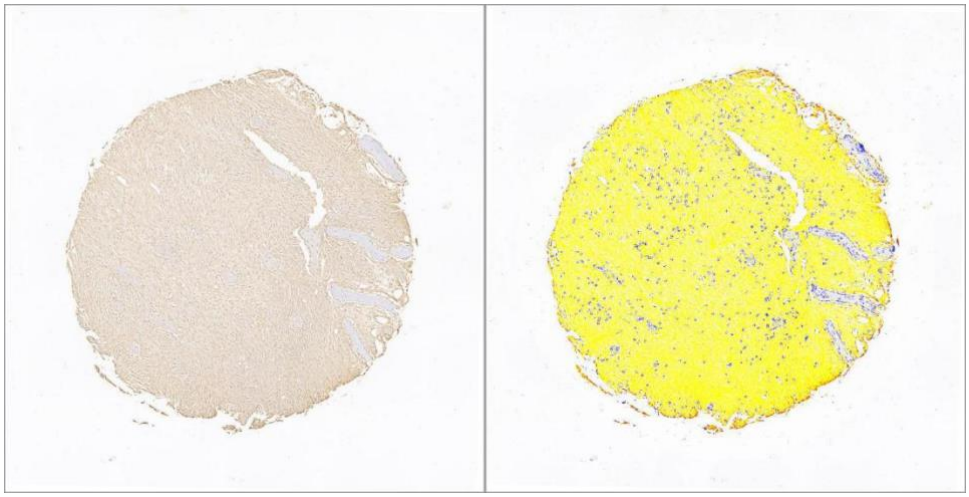

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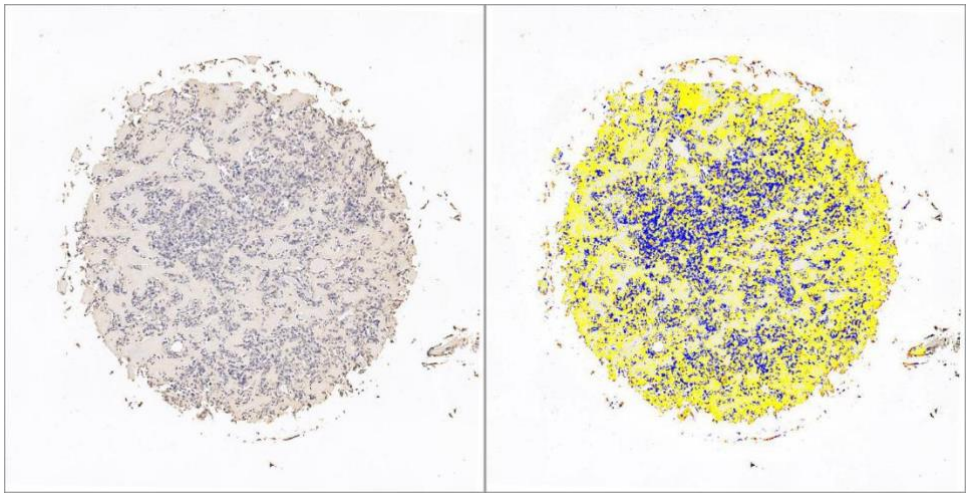

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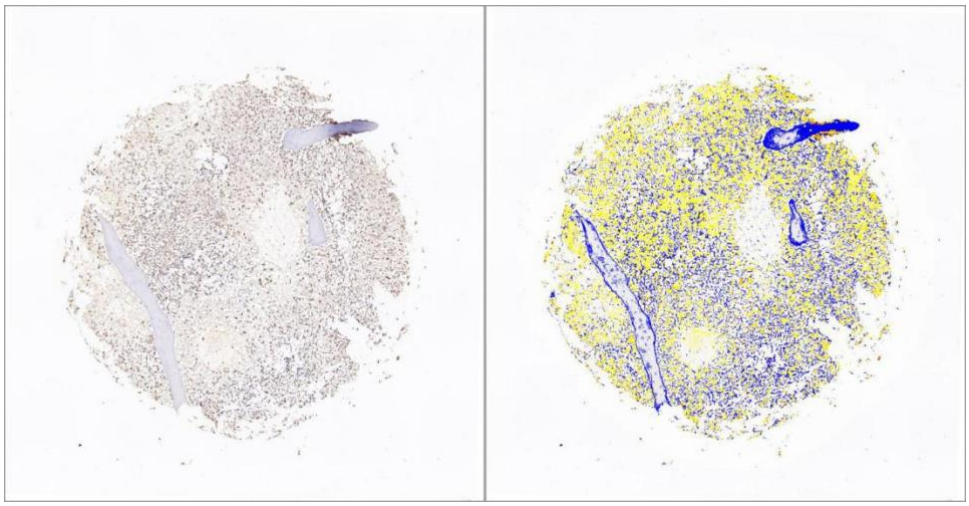

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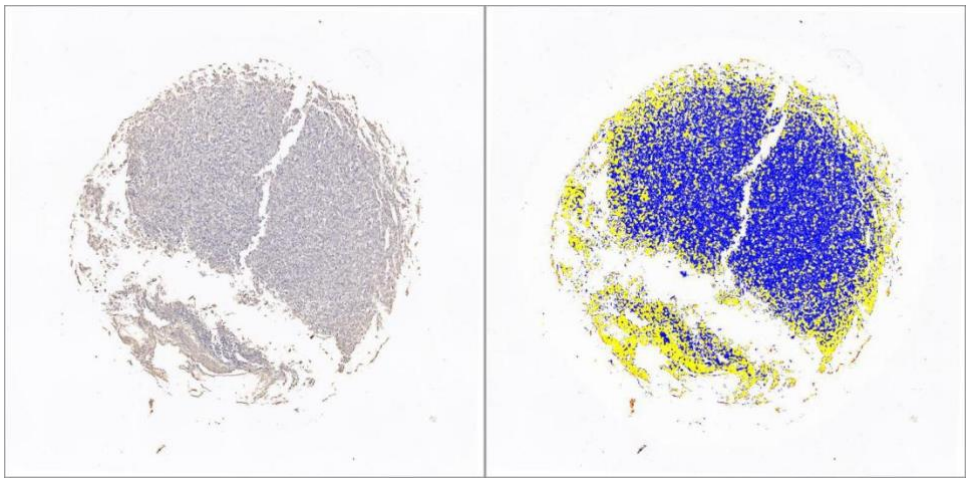

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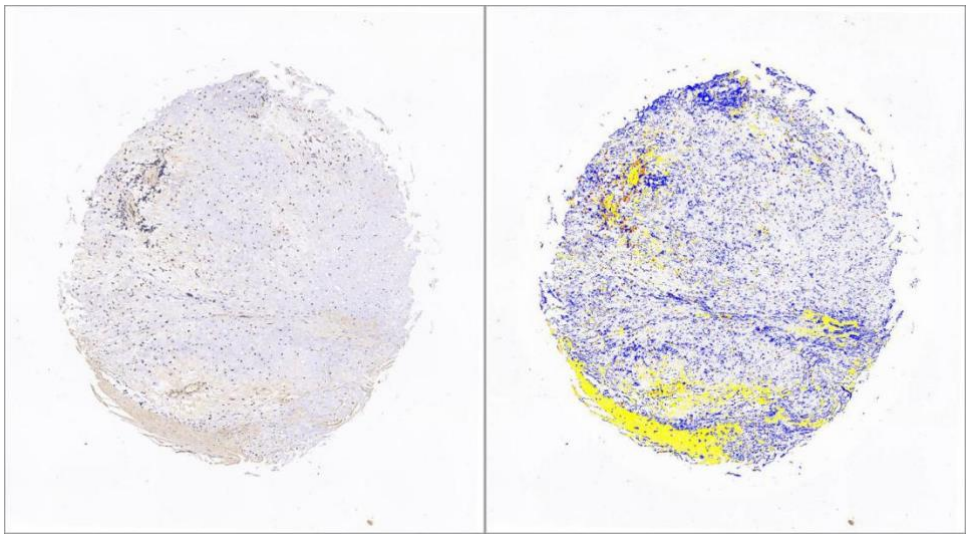

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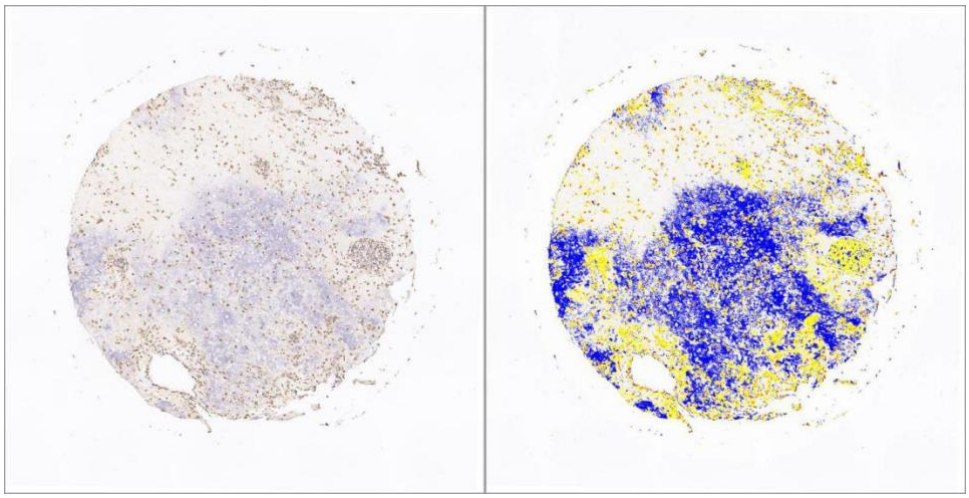

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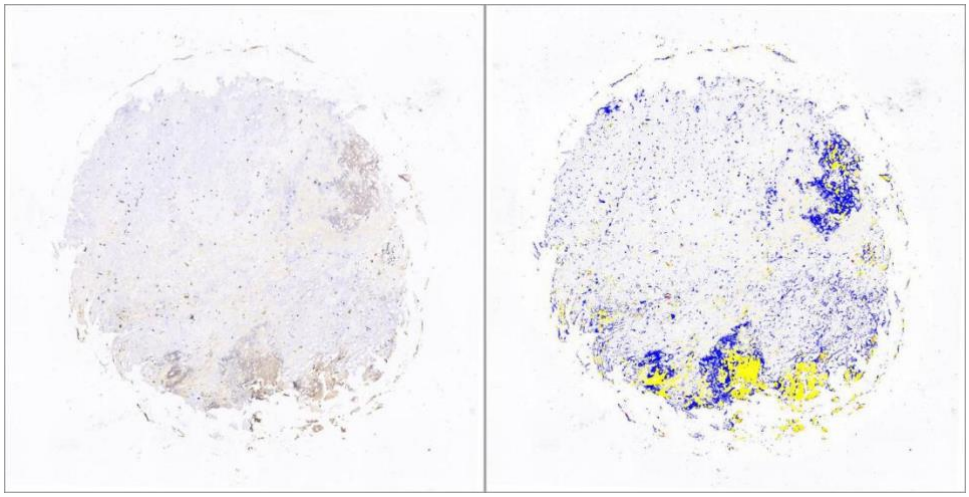

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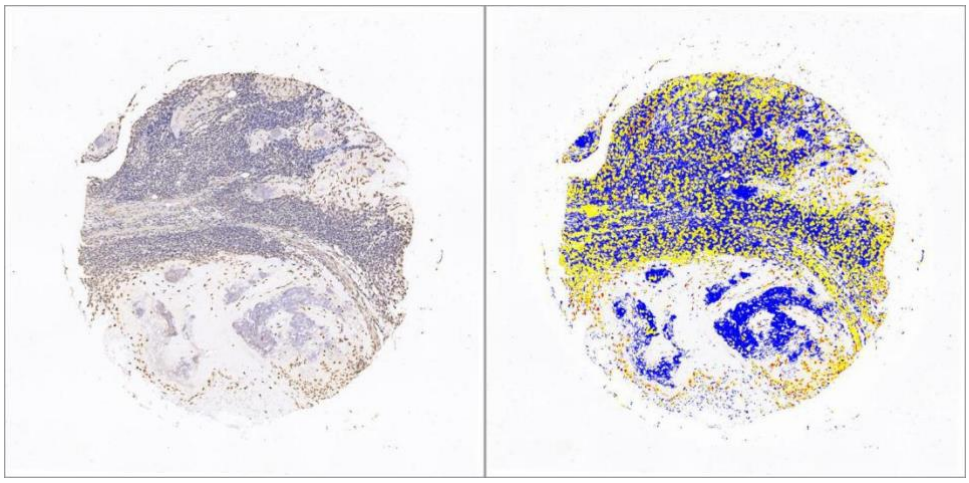

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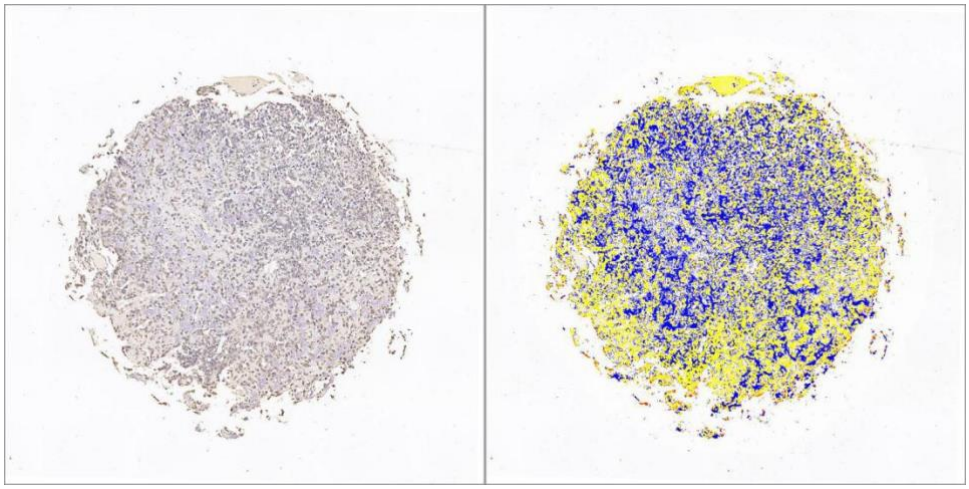

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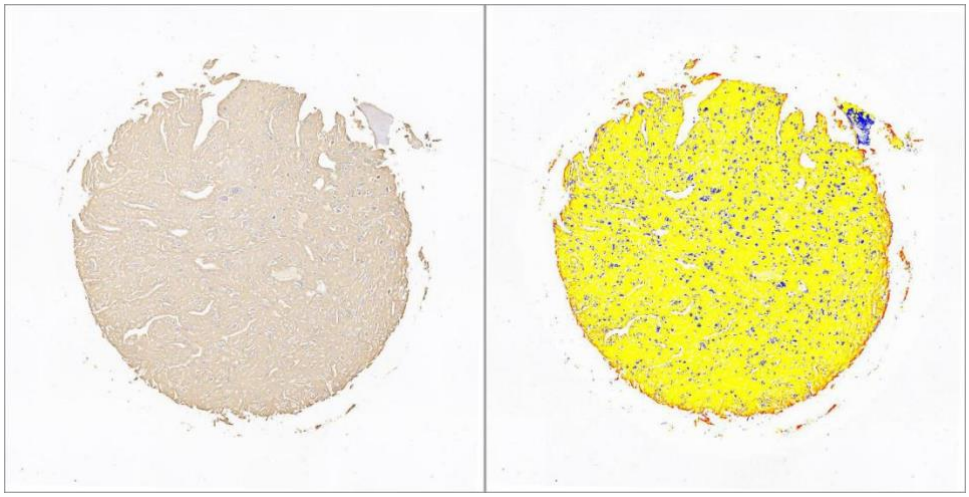

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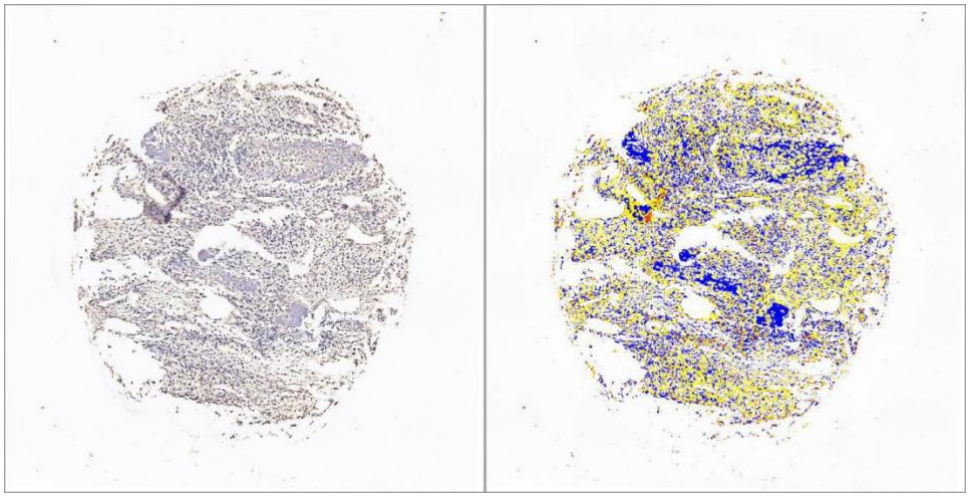

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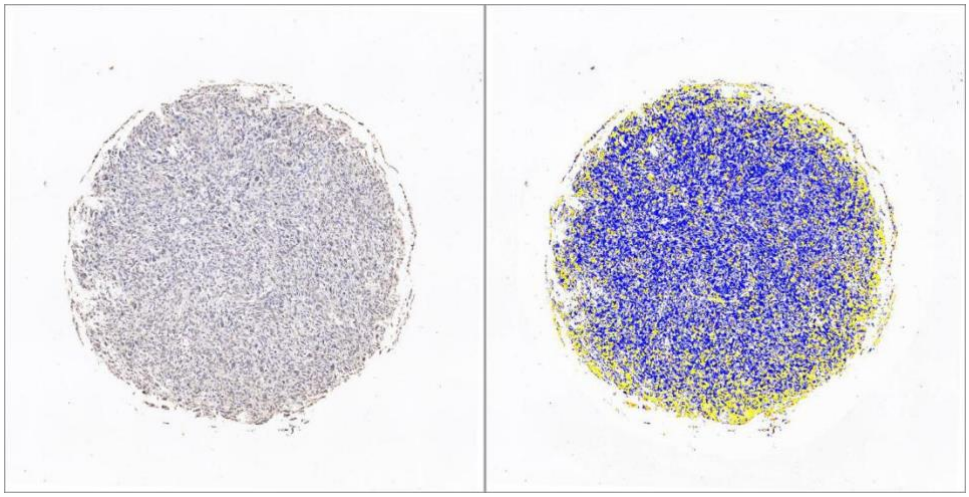

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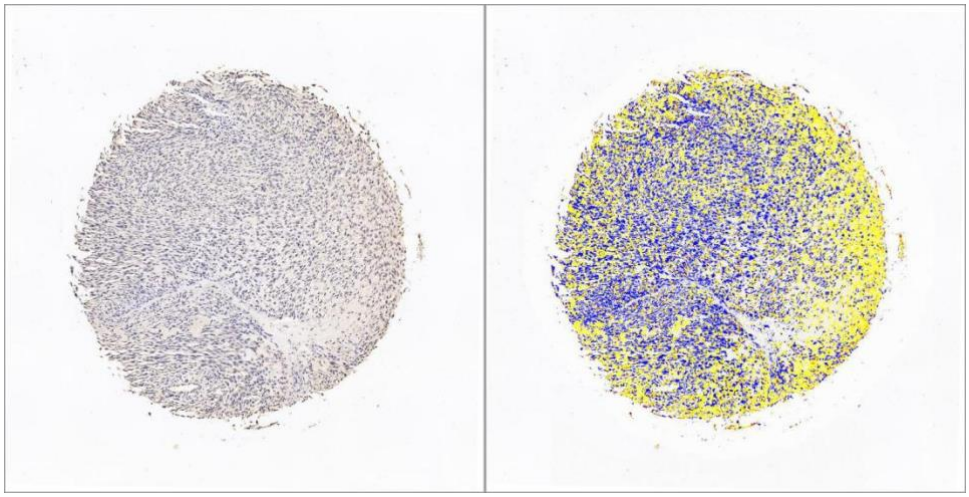

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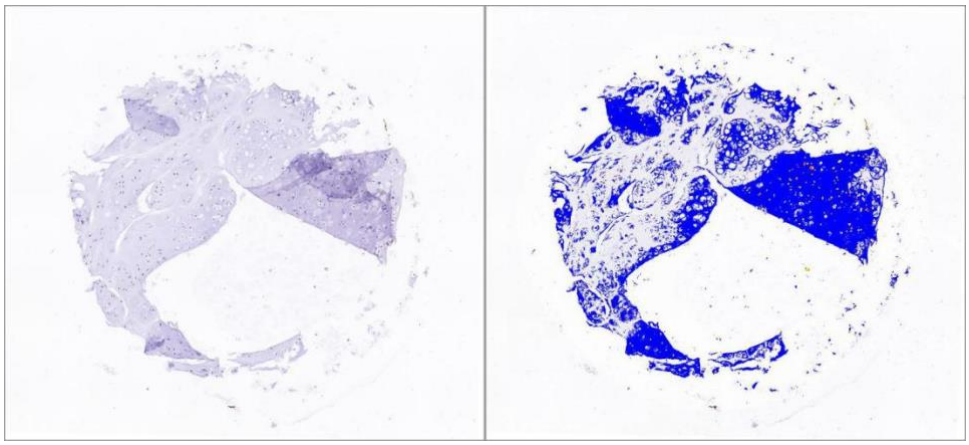

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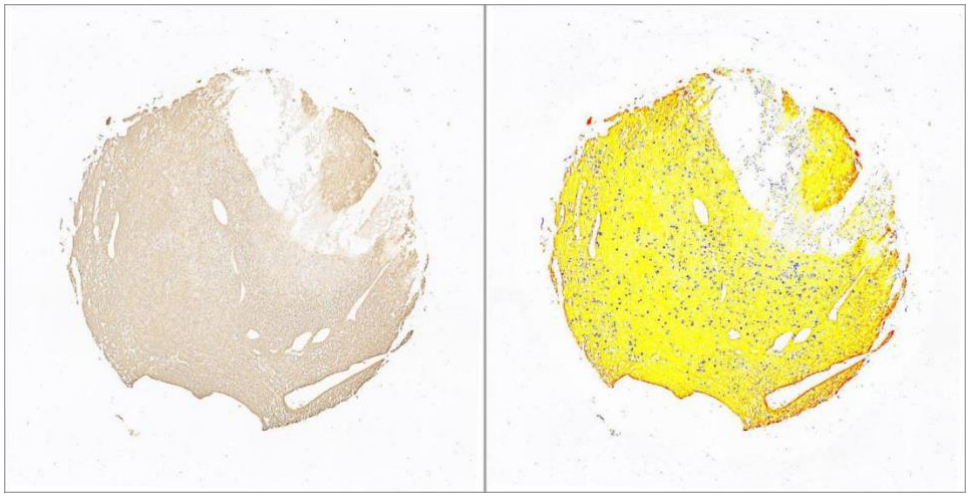

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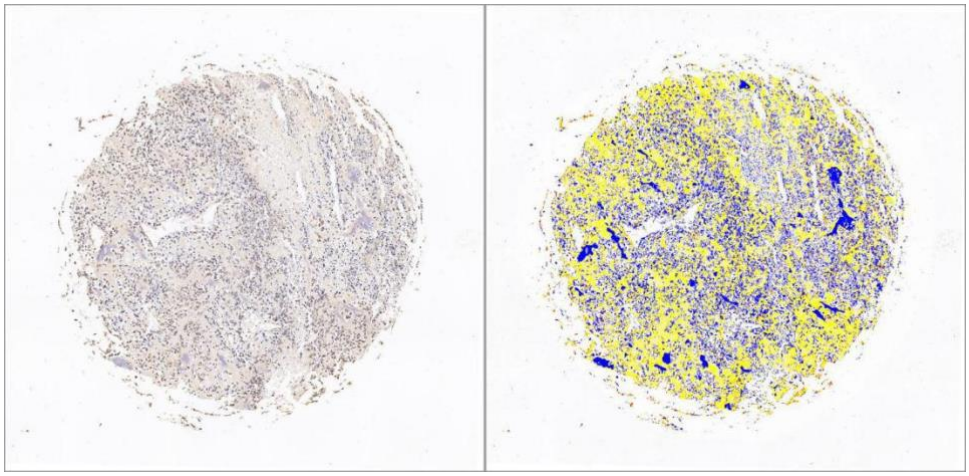

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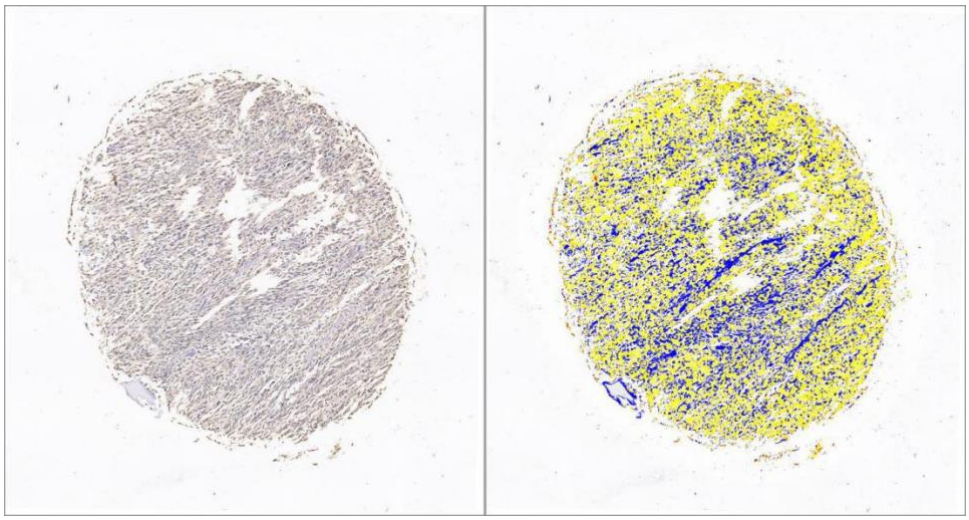

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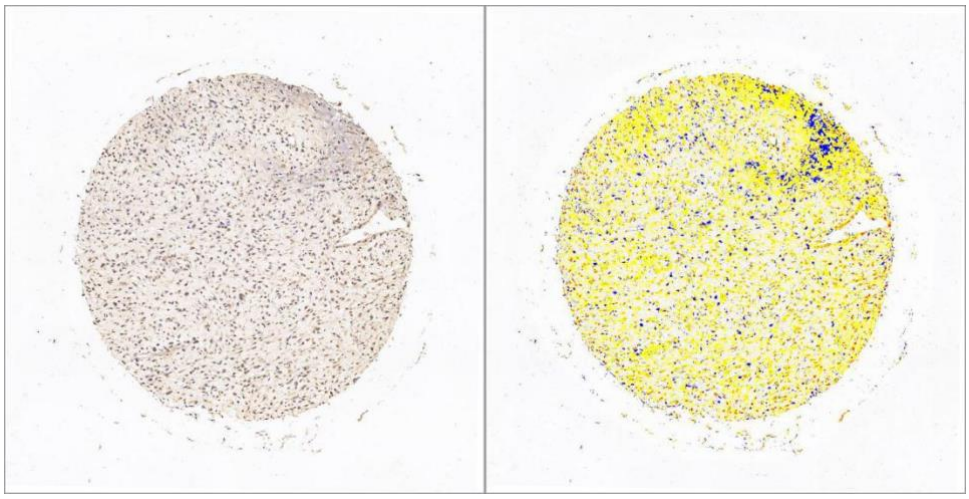

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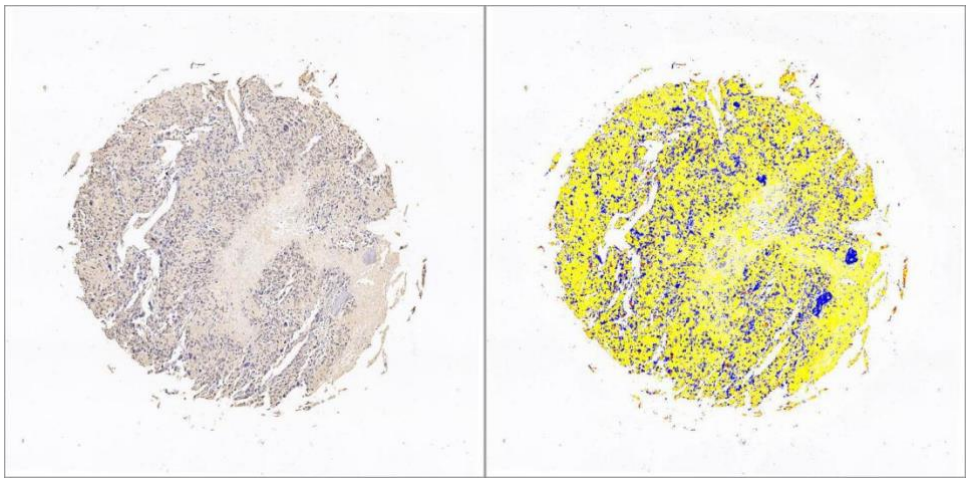

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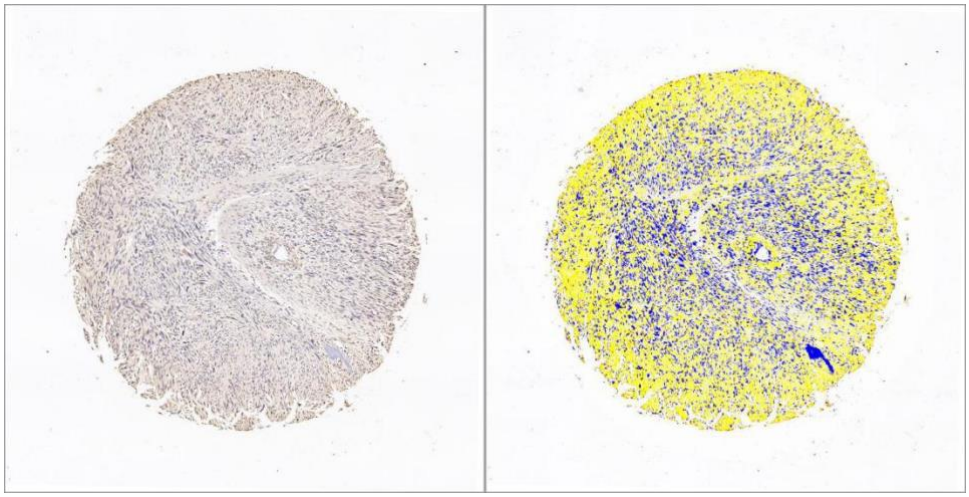

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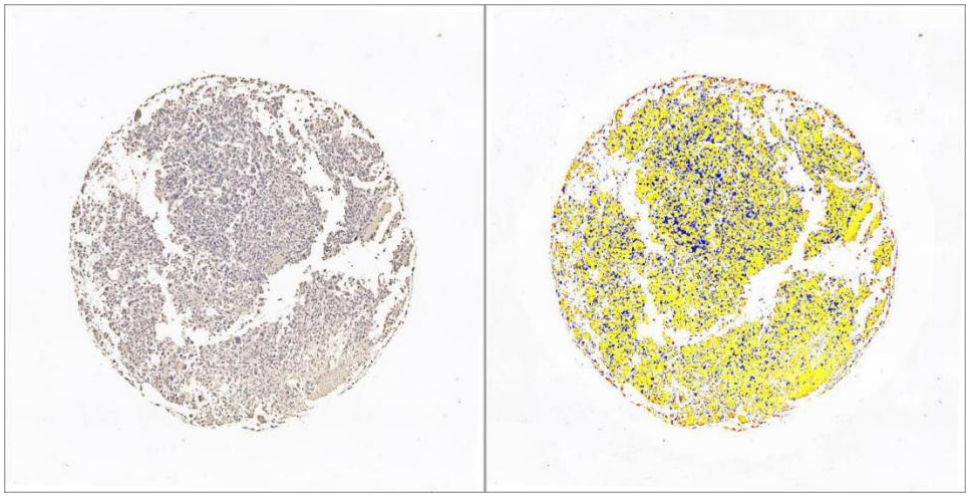

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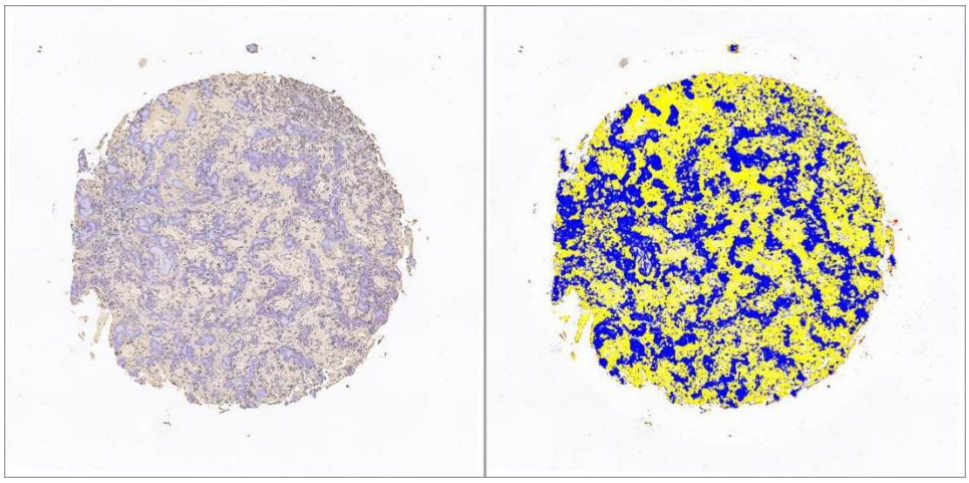

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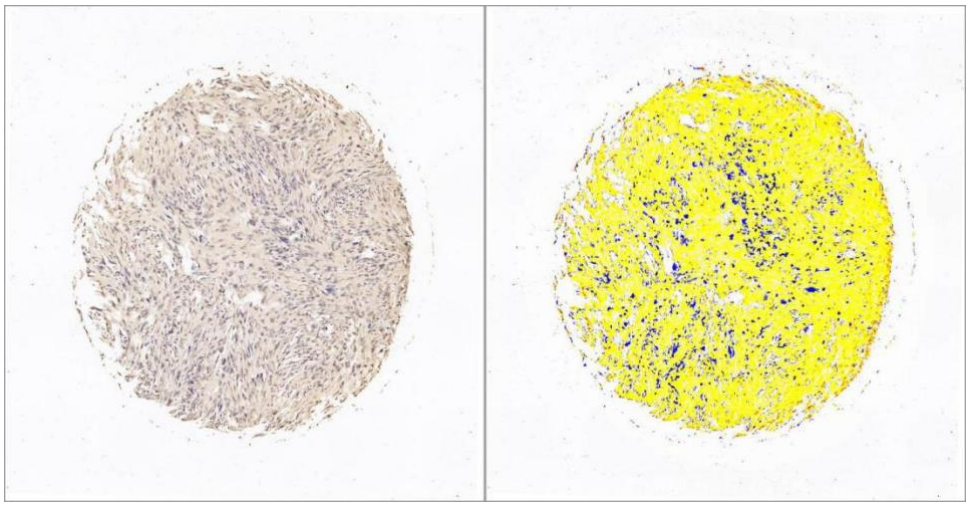

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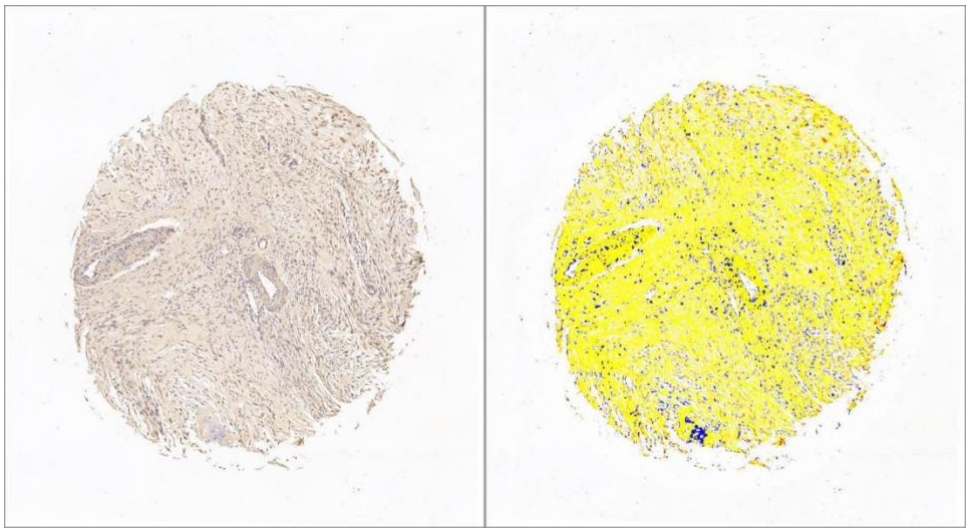

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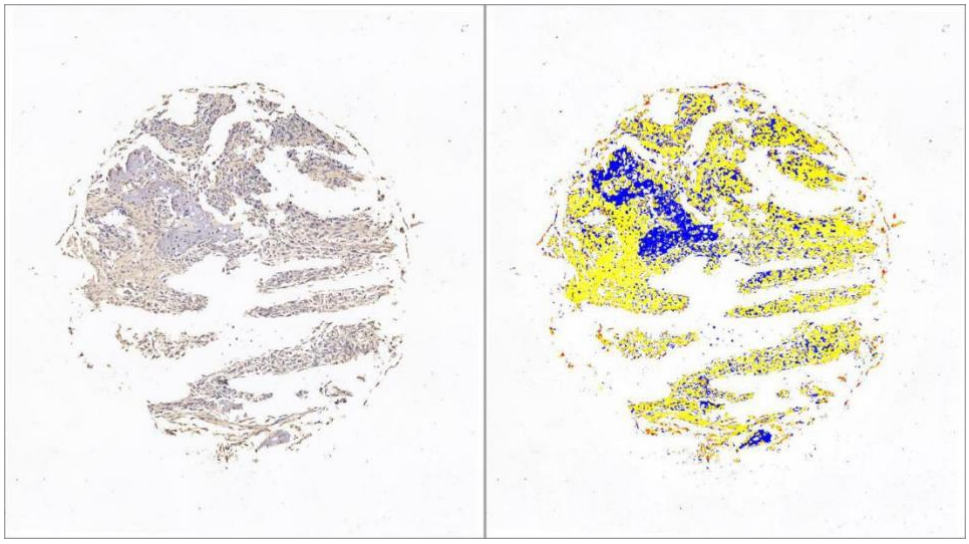

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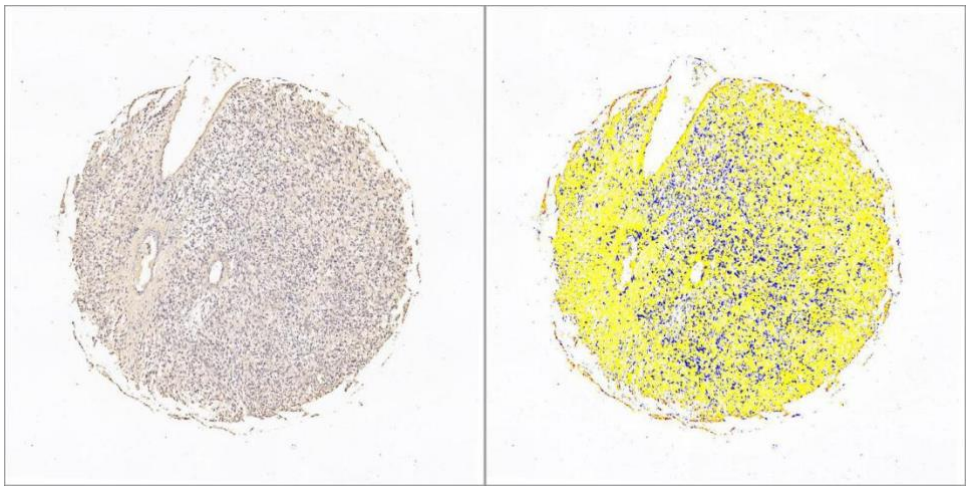

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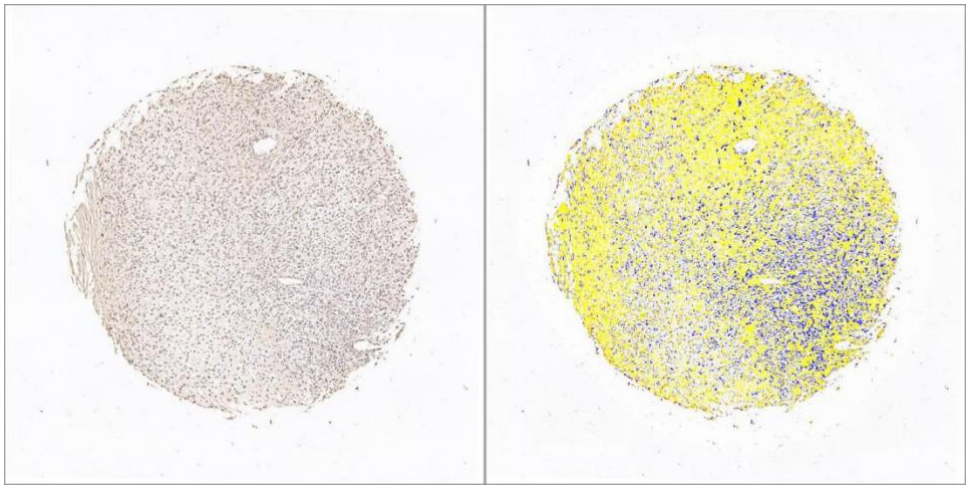

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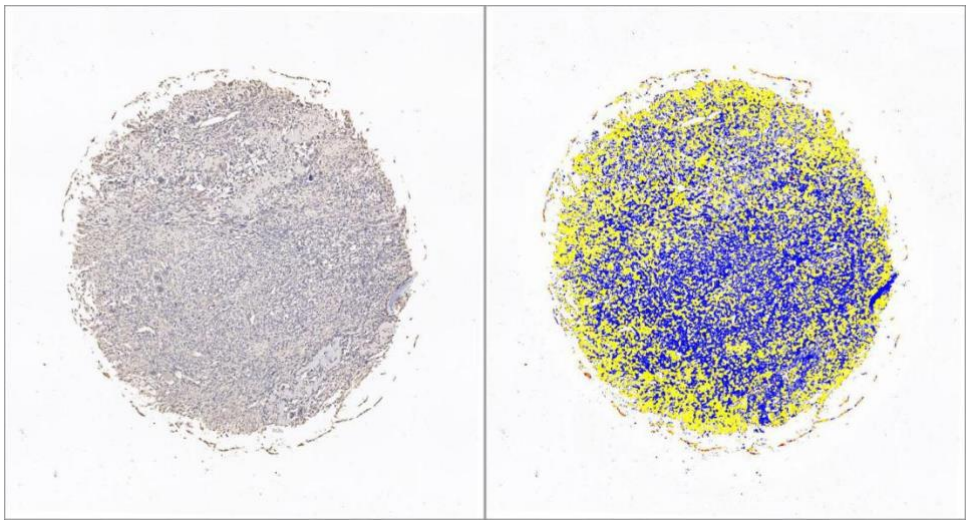

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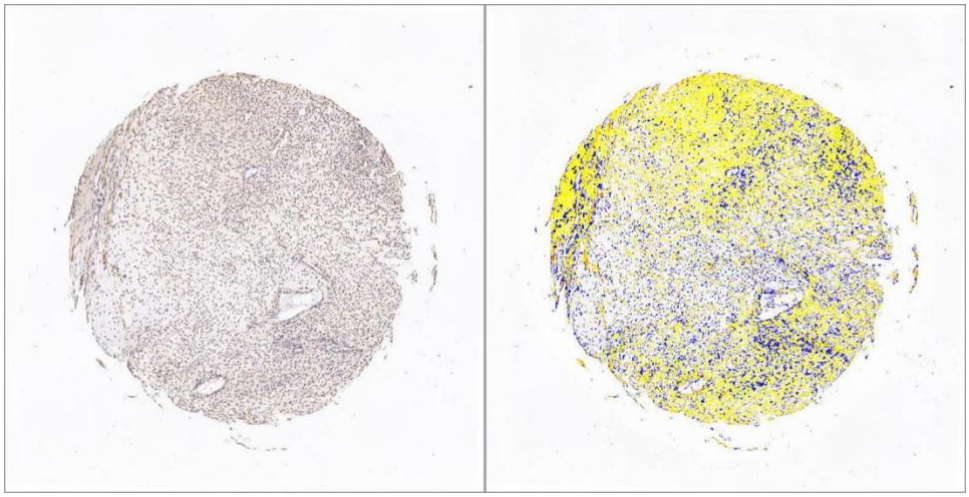

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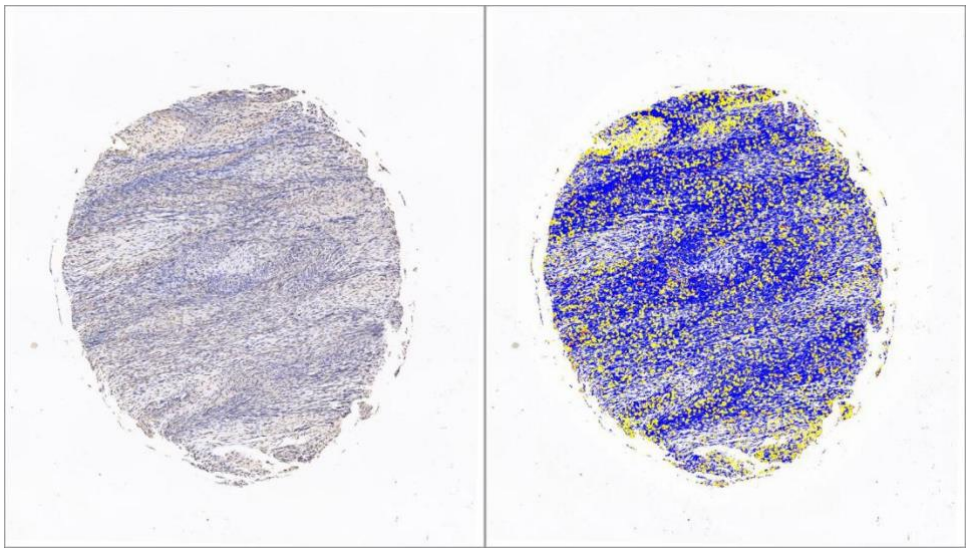

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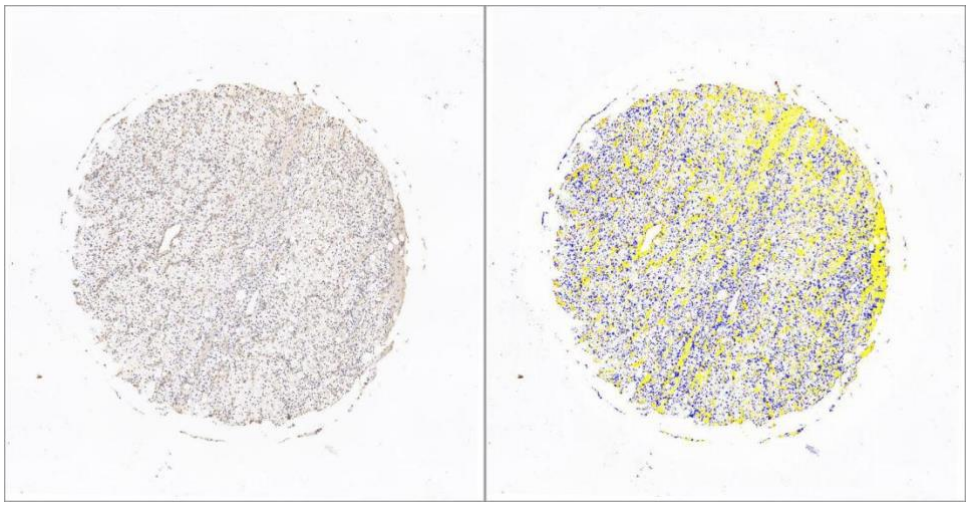

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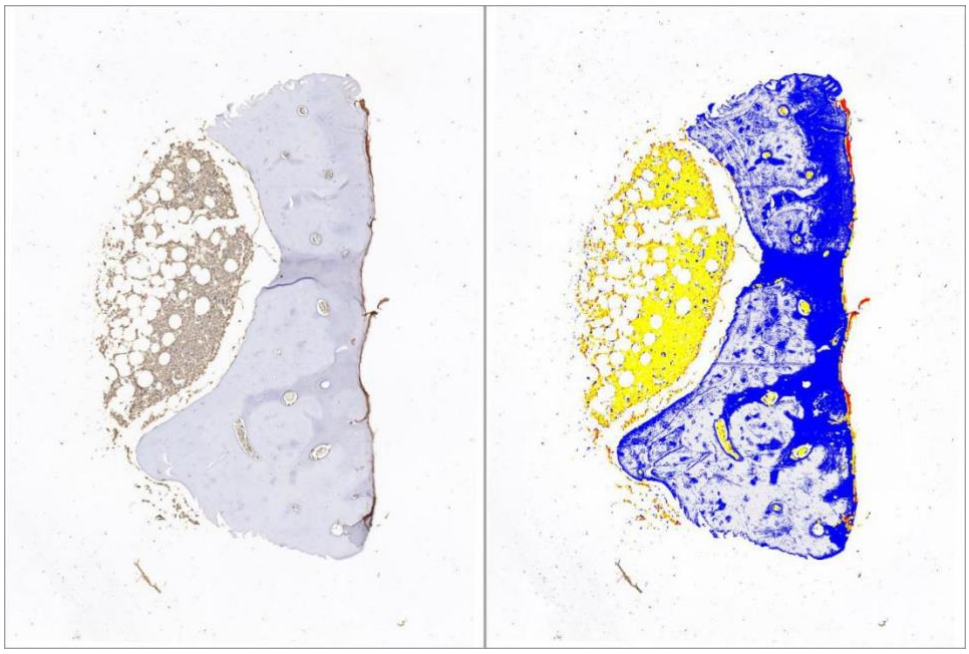

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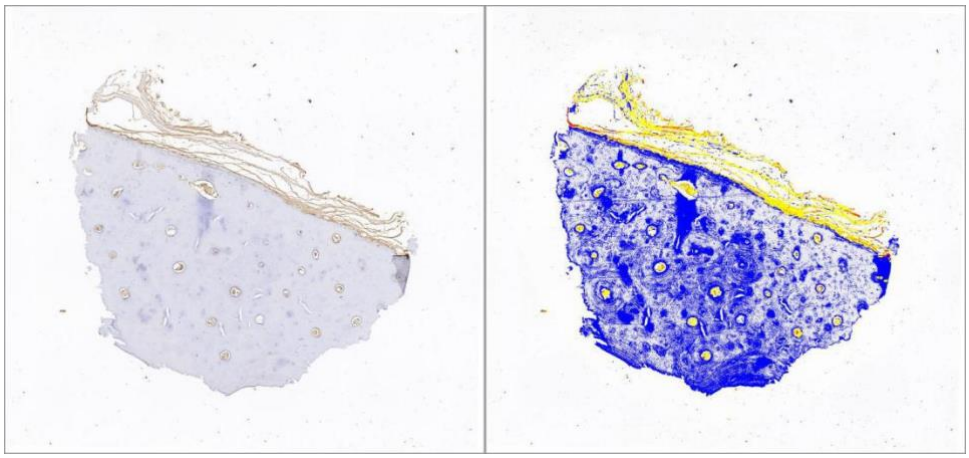

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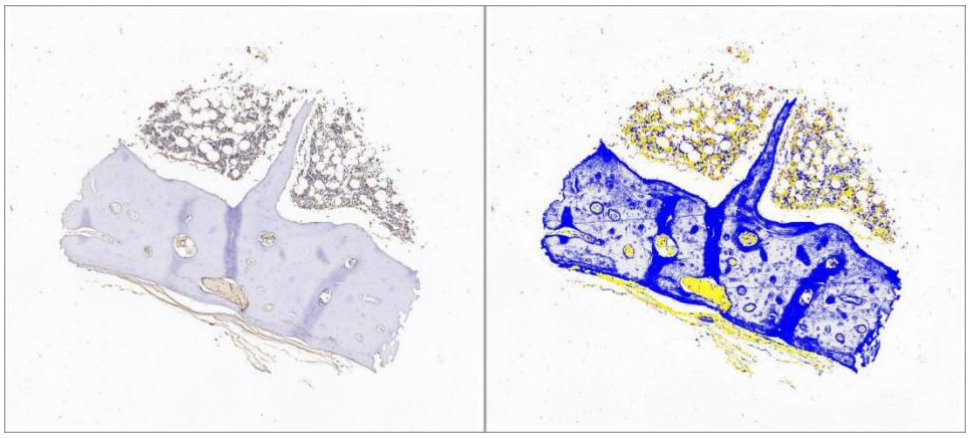

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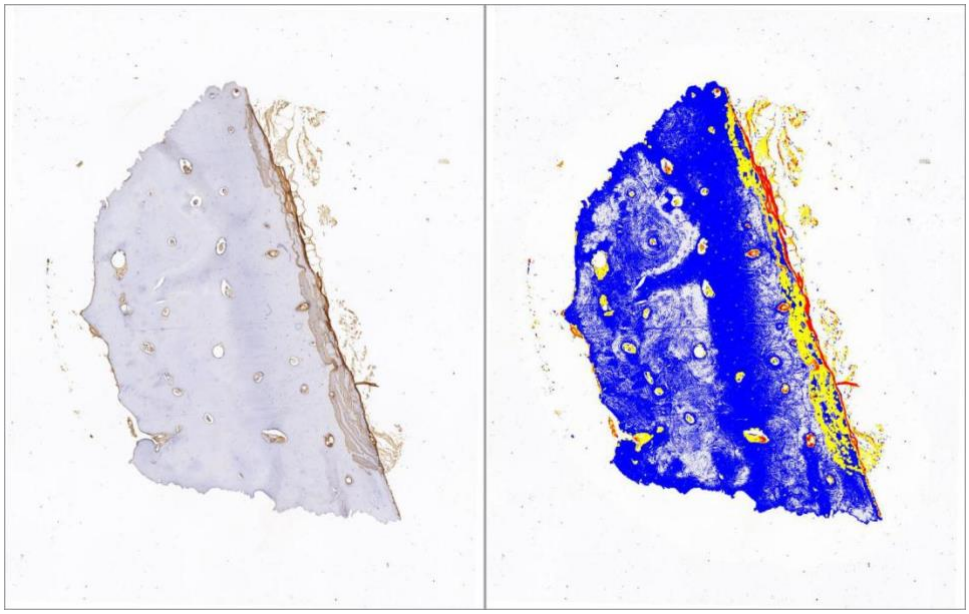

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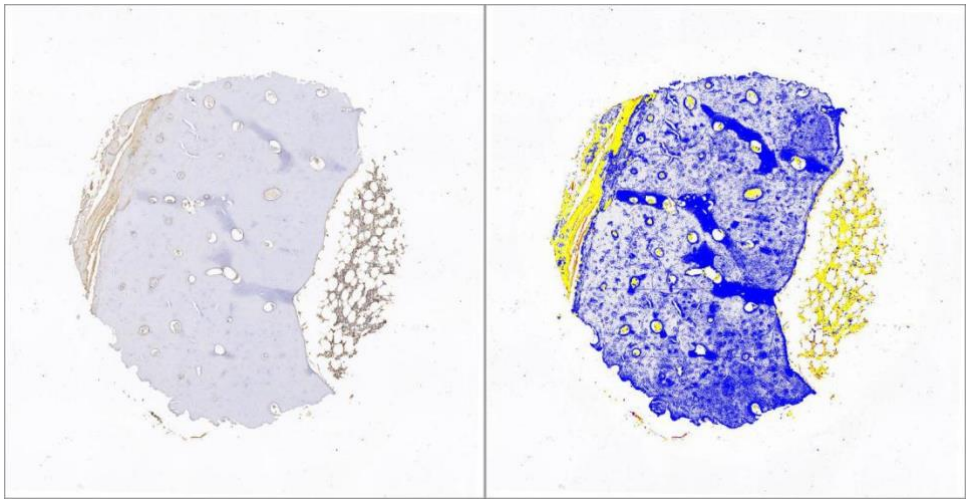

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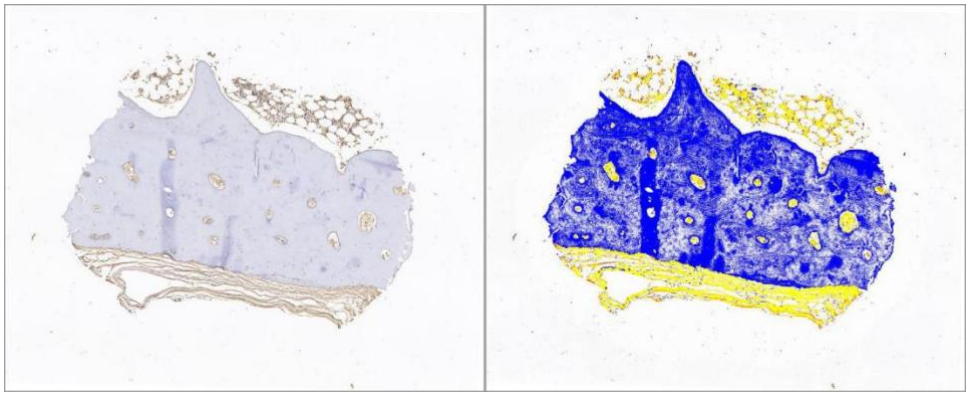

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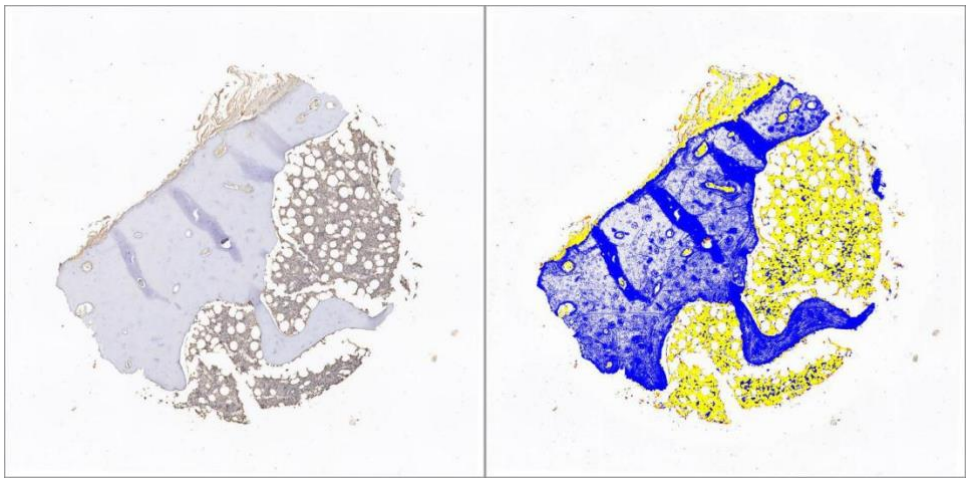

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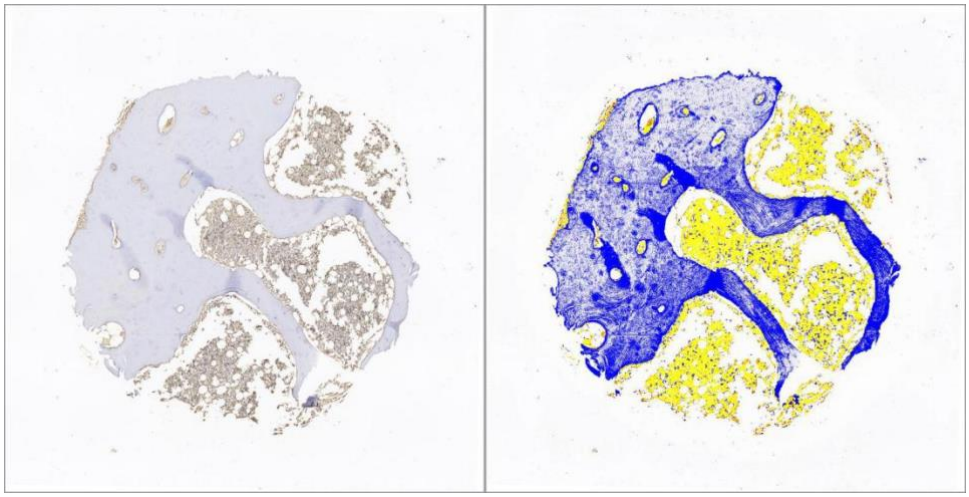

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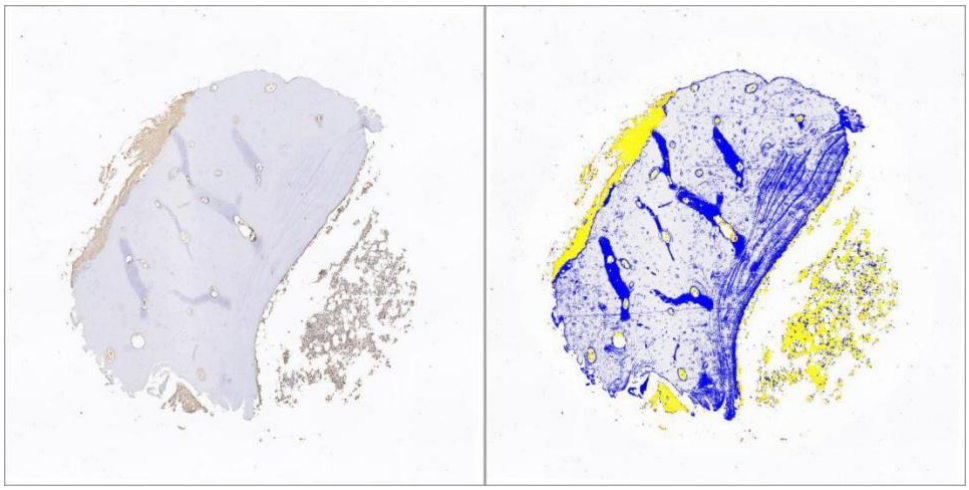

2. ORIGINAL BASIC DATA

Names of Images	Positive Area 1 weak (mm^2)	Positive Area 2 Moderate (mm^2)	Positive Area 3 Strong (mm^2)	Negative Area, (mm^2)	IOD	Positive Area, (mm^2)

BON501 G022 PLCE1-A01	0.529	0.025	0.013	0.691	114279	2.030
BON501 G022 PLCE1-A02	0.321	0.029	0.007	0.597	119099	1.350
BON501 G022 PLCE1-A03	1.039	0.044	0.018	0.083	135425	1.926
BON501 G022 PLCE1-A04	0.456	0.053	0.018	0.936	151783	2.091
BON501 G022 PLCE1-A05	1.261	0.045	0.022	0.207	215316	2.048
BON501 G022 PLCE1-A06	0.631	0.083	0.046	0.612	173964	1.892
BON501 G022 PLCE1-A07	0.832	0.028	0.015	0.293	135877	1.886
BON501 G022 PLCE1-A08	0.413	0.028	0.017	1.456	102769	2.425
BON501 G022 PLCE1-B01	1.054	0.098	0.037	0.669	256819	2.361
BON501 G022 PLCE1-B02	0.250	0.014	0.009	0.685	70985	2.144
BON501 G022 PLCE1-B03	1.190	0.063	0.013	0.057	108353	2.250
BON501 G022 PLCE1-B04	0.978	0.037	0.033	0.410	182832	2.327
BON501 G022 PLCE1-B05	0.394	0.021	0.011	0.325	82133	1.575
BON501 G022 PLCE1-B06	0.501	0.020	0.007	0.827	98717	1.784
BON501 G022 PLCE1-B07	0.201	0.010	0.012	0.360	37078	2.321
BON501 G022 PLCE1-B08	0.236	0.043	0.024	0.427	55757	1.953
BON501 G022 PLCE1-C01	0.109	0.004	0.002	0.197	15157	1.978
BON501 G022 PLCE1-C02	0.412	0.053	0.031	0.561	146925	1.949
BON501 G022 PLCE1-C03	0.704	0.040	0.029	0.672	191056	2.231
BON501 G022 PLCE1-C04	1.543	0.123	0.024	0.115	140229	2.296
BON501 G022 PLCE1-C05	0.442	0.041	0.048	0.466	178746	1.576
BON501 G022 PLCE1-C06	0.452	0.032	0.018	1.010	122492	2.266

BON501 G022 PLCE1-C07	0.669	0.034	0.034	0.614	194237	2.327
BON501 G022 PLCE1-C08	0.012	0.004	0.003	0.836	5335	1.836
BON501 G022 PLCE1-D01	1.097	0.201	0.033	0.074	113948	2.057
BON501 G022 PLCE1-D02	0.674	0.036	0.032	0.481	153825	2.134
BON501 G022 PLCE1-D03	0.812	0.030	0.014	0.478	181766	2.001
BON501 G022 PLCE1-D04	0.606	0.067	0.030	0.106	126904	2.020
BON501 G022 PLCE1-D05	1.186	0.057	0.036	0.356	218226	2.365
BON501 G022 PLCE1-D06	0.840	0.029	0.021	0.372	163667	2.297
BON501 G022 PLCE1-D07	0.765	0.080	0.045	0.288	229599	1.698
BON501 G022 PLCE1-D08	1.060	0.040	0.018	0.886	180011	2.472
BON501 G022 PLCE1-E01	1.183	0.046	0.012	0.141	164309	2.027
BON501 G022 PLCE1-E02	1.049	0.065	0.018	0.094	141719	2.067
BON501 G022 PLCE1-E03	0.529	0.042	0.027	0.270	135810	1.245
BON501 G022 PLCE1-E04	0.945	0.034	0.019	0.214	182152	2.036
BON501 G022 PLCE1-E05	0.660	0.046	0.015	0.206	128979	1.969
BON501 G022 PLCE1-E06	0.727	0.020	0.014	0.747	166010	2.016
BON501 G022 PLCE1-E07	0.564	0.031	0.015	0.266	138088	1.999
BON501 G022 PLCE1-E08	0.454	0.056	0.025	1.219	150368	2.498
BON501 G022 PLCE1-E09	0.400	0.022	0.022	0.303	108156	1.936
BON501 G022 PLCE1-F01	0.192	0.036	0.017	0.470	55355	1.300
BON501 G022 PLCE1-F02	0.091	0.021	0.007	0.603	21567	1.555
BON501 G022 PLCE1-F03	0.157	0.018	0.017	0.604	70166	1.519

BON501 G022 PLCE1-F04	0.113	0.025	0.029	0.901	34979	1.497
BON501 G022 PLCE1-F05	0.178	0.028	0.011	0.844	48707	2.327
BON501 G022 PLCE1-F06	0.191	0.034	0.011	0.870	50503	1.804
BON501 G022 PLCE1-F07	0.445	0.033	0.014	0.687	130820	1.814
BON501 G022 PLCE1-F08	0.380	0.038	0.010	0.584	98645	1.855
BON501 G022 PLCE1-F09	0.198	0.016	0.005	0.438	46495	1.978

3. Immunohistochemical positive area intensity analysis method

A tissue section digital scanner or imaging system was used to collect scanned files or images on immunohistochemical sections. An image analysis system was used to automatically read the tissue measurement area. The positive grades were first divided into: negative without staining, 0 point; weak positive yellow light, 1 point; medium positive brownish yellow, 2 points; strong positive brown, 3 points. Then, the weak, medium, and strong positive areas in the measurement area were analyzed and calculated respectively. The tissue area, the positive cumulative optical density IOD value, and the positive area were also analyzed and calculated. The results shown below were calculated to reflect the degree of positivity. The following indicators can be selected to evaluate the positive intensity according to the slide situation.

① Positive area ratio: positive area/tissue area. It reflects the amount of positive area [1].

② Average optical density value: positive cumulative optical density IOD value/positive area. It reflects the average depth of the positive signals [2].

③Positive surface density: positive cumulative optical density IOD value/tissue area. It reflects the average positive depth in the entire measurement area [2].

④H-score: abbreviation for Histochemistry score, an immunohistochemistry-related histological scoring method. The number of positive areas and staining intensity in each slide were converted into corresponding values to achieve the purpose of semi-quantitative tissue staining. H-Score (H-SCORE=∑(pi×i)=(percentage of weak intensity area ×1)+(percentage of moderate intensity area ×2)+(percentage of strong intensity area ×3), where pi represents the percentage of positive signal pixel area; and i represents positive grade. H-score has a value between 0 and 300; the larger the value, the stronger the comprehensive positive intensity [3][4].

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