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An artificial intelligence-based framework for BLEACH&STAIN mfIHC facilitates automated prognosis marker assessment in breast cancer

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Introduction and Objectives

The assessment of prognostic markers in routine clinical practice of breast cancer is currently performed using multi gene RNA panels. However, the unknown proportion of normal breast tissue in relation to malignant breast tissue can reduce the predictive value of such tests. Multiplex fluorescence immunohistochemistry holds the potential for a better assessment of tumors because tumor cells can be separately analyzed.

Materials & Methods

To enable automated prognosis marker detection (i.e. TROP2, GATA3, androgen receptor [AR], progesterone receptor [PR], estrogen receptor [ER], HER2, PD-L1, Ki-67, TOP2A), we have developed and validated a framework for automated breast cancer characterization, which comprises three different artificial intelligence analysis steps and an algorithm for cell-distance analysis of 11+1 marker BLEACH&STAIN multiplex fluorescence immunohistochemistry staining in 1780 breast cancers in a TMA-format.

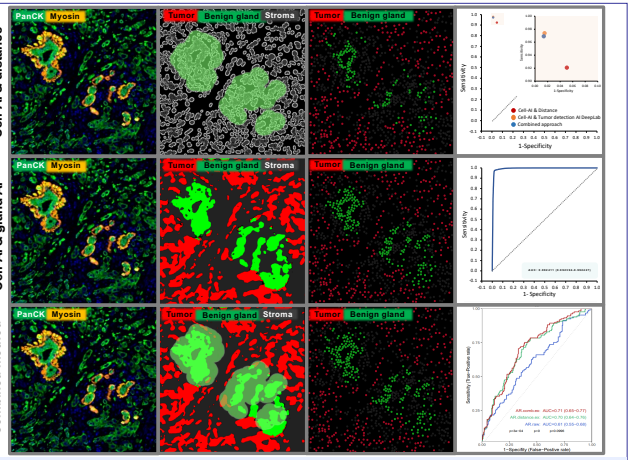
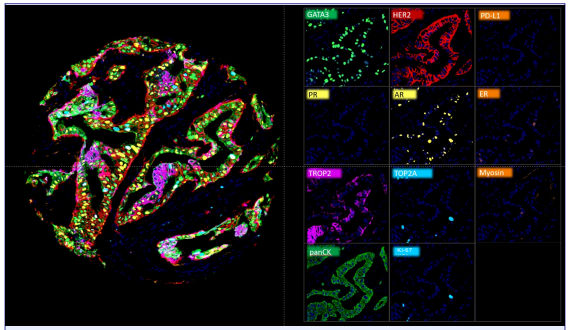
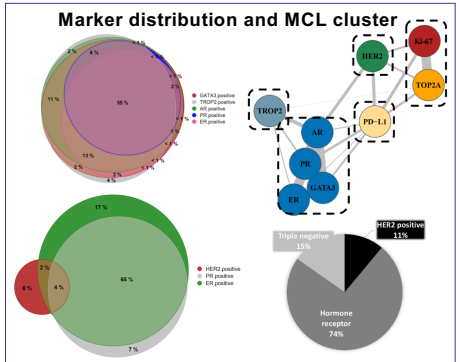


Image analysis was performed using a previously trained deep learning-based framework (U-Net) for cell detection followed by single cell intensity measurement of the fluorophores. Cell-to-cell distance analysis was then used to calculate the distance from epithelial cells to Myosin$^+$ basal cells. Epithelial cells close to Myosin$^+$ basal cells (< 25µm) were classified as benign epithelial cells and excluded from the study. This approach was combined with a deep-learning based framework (DeepLab3$^+$) for automated breast cancer detection resulting in an improved prognostic performance.

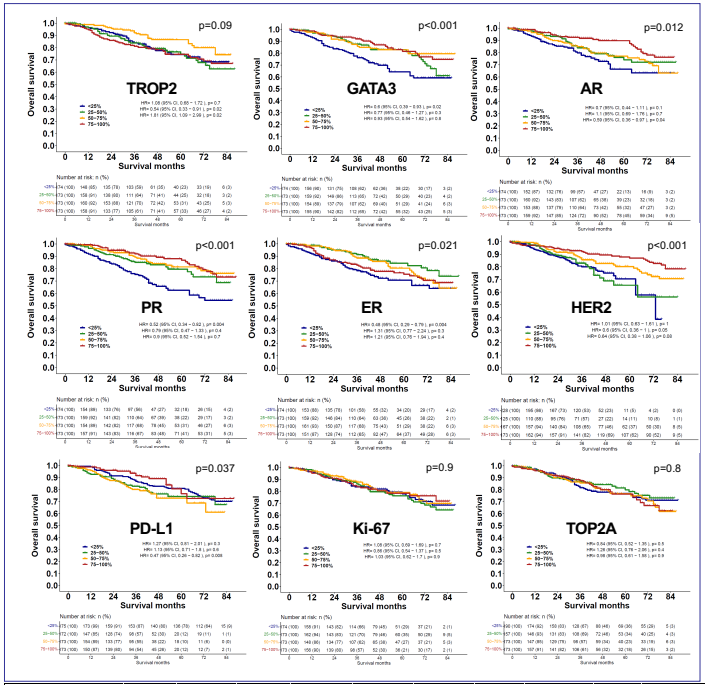


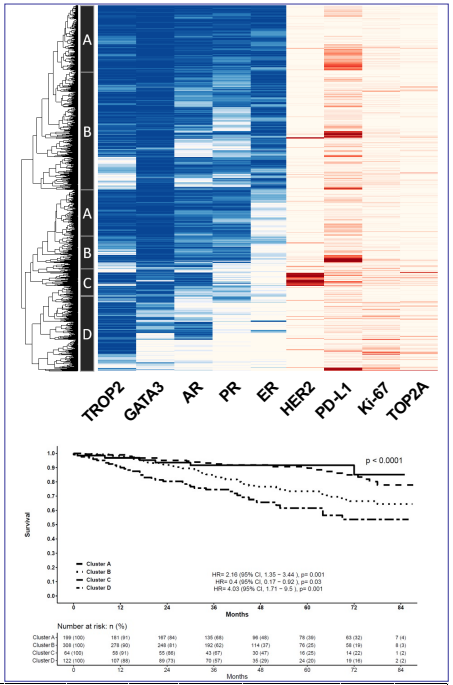
Representative picture of a TMA core using 11+1 BLEACH&STAIN multiplex fluorescence immunohistochemistry. The staining was conducted in four sequential staining and imaging rounds of three biomarkers at a time (two biomarkers within the last round) and a bleaching step between every cycle. Finally, the four sequential digital images were aligned and thus merged into a single 11+1 mfIHC image.



The analysis framework was validated by the concordance with well-characterized biological findings, such as the identification of 11% HER2$^+$, 74% PR$^+$/ER$^+$, and 15% triple negative cases in the study cohort.

RESULTS





Clinical parameter	n	Fraction of panCK$^+$TROP2$^+$ cells	p-value	Fraction of panCK$^+$GATA3$^+$ cells	p-value	Fraction of panCK$^+$AR$^+$ cells	p-value	Fraction of panCK$^+$PR$^+$ cells	p-value	Fraction of panCK$^+$ER$^+$ cells	p-value	Relative HER2 intensity	p-value	Relative PD-L1 intensity	p-value	Fraction of panCK$^+$Ki-67$^+$ cells	p-value	Fraction of panCK$^+$TOP2A$^+$ cells	p-value
pT			<0.001		<0.001		<0.001		<0.001		0.26		0.4		0.3		0.003		<0.001
pT1	812	82.8 (±25.2)		86.6 (±23.4)		71.6 (±29.6)		62.7 (±32.3)		50.0 (±38.7)		7.9 (±26.5)		18.8 (±24.1)		7.1 (±9.4)		1.2 (±2.7)	
pT2	740	77.1 (±29.9)		80.3 (±30.0)		63.9 (±33.6)		50.1 (±35.4)		51.5 (±40.6)		6.6 (±22.6)		17 (±21.8)		8.8 (±10.5)		1.8 (±3)	
pT3-4	172	73.6 (±29.9)		73.8 (±33.5)		56.5 (±33.4)		42.0 (±36.1)		46.0 (±41.4)		5.7 (±0.19.7)		17.2 (±35.2)		7.9 (±10.3)		2.1 (±3.7)	
pN			0.021		<0.001		<0.001		<0.001		0.036		1.0		0.001		0.088		<0.001
pN-	787	81.4 (±27.1)		85.1 (±25.6)		71.7 (±30.0)		60.4 (±34.2)		52.1 (±39.8)		7.6 (±23.9)		18.3 (±26.4)		7.0 (±9.4)		1.2 (±2.2)	
pN+	614	78.0 (±28.5)		79.3 (±29.5)		63.5 (±32.3)		48.8 (±34.5)		47.6 (±39.4)		7.6 (±26.2)		14.2 (±19.0)		7.9 (±9.7)		1.6 (±3.2)	
pM			0.043		<0.001		<0.001		<0.001		<0.001		0.09		0.4		0.002		<0.001
pM-	214	78.6 (±28.6)		87.5 (±21.5)		75.6 (±26.5)		67.8 (±30.9)		56.8 (±38.5)		10.3 (±24.8)		10.0 (±19.2)		6.1 (±8.0)		0.9 (±1.8)	
pM+	113	71.4 (±33.3)		71.1 (±34.1)		50.7 (±36.0)		32.1 (±33.7)		38.7 (±38.6)		5.9 (±16.9)		11.6 (±11.9)		9.6 (±12.1)		2.1 (±3.2)	
Grade			<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		0.003		<0.001		<0.001
1	193	88.4 (±19.8)		93.1 (±13.9)		78.1 (±22.9)		75.5 (±23.7)		49.4 (±36.3)		3.4 (±13.3)		18.0 (±18.7)		4.7 (±8.7)		0.4 (±1.0)	
2	963	80.7 (±26.7)		88.4 (±21.4)		70.8 (±29.0)		62.6 (±31.2)		57.2 (±38.1)		5.1 (±18.2)		16.1 (±20.4)		5.6 (±7.0)		1.0 (±2.0)	
3	612	73.9 (±31.5)		69.8 (±35.0)		55.1 (±36.8)		36.2 (±35.2)		38.7 (±41.0)		11.3 (±32.7)		20.4 (±30.7)		12.6 (±12.7)		2.7 (±4.1)	

Conclusions

- A deep learning-based framework for automated breast cancer identification using **BLEACH&STAIN** multiplex fluorescence IHC facilitates automated prognosis marker quantification in breast cancer.
- Automated tumor cell identification **improves prognostic performance** of prognosis marker quantification.

GATA3 HER2 PD-L1 PR AR ER TROP2 TOP2A Myosin panCK Ki-67

Conflicts of interest: The GATA3, PD-L1, PR, AR, ER, TROP2, TOP2A, Myosin, panCK, Ki-67 antibodies were provided by MS Validated Antibodies GmbH (owned by a family member of GS)

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