

SEQUENTIAL IMMUNOHISTOCHEMISTRY AND VIRTUAL IMAGE RECONSTRUCTION USING A SINGLE SLIDE FOR QUANTITATIVE KI67 MEASUREMENT IN BREAST CANCER.

Supplementary table 1

Summary of the characteristics of the Ki67 APP.

Supplementary table 2

Summary of the TMAs used and evaluations performed by the three observers (OBS) on the study cohorts.

Supplementary table 3

Clinicopathological characteristics of the 13 cores where the difference between KiQuant and manual scoring (MS) Ki67 labelling index was outside the upper (19.51) and lower (-9.60) limits of agreement. N/A, not available.

Supplementary table 4

Agreement rates between KiQuant and MS across different Ki67 LI cut-offs. The 2.7% cut-off is used to determine cell cycle arrest in post-treatment biopsies after neo-adjuvant therapies; the 14% and 20% cut-offs have been proposed by the St. Gallen expert's panel. Additional incremental cut-offs (30%, 40%, and 50%) were also evaluated.

Supplementary Figure S1

Box-plots of the differences between the evaluations of MS and DIA for each of the histologies.

Supplementary Figure S2

Two-dimensional visualizations (scattered plots) of the digital image analysis Ki67 labelling index results obtained by KiQuant for the reproducibility studies. Comparison between A) two different runs and B) two different Ki67 antibodies.

Supplementary Figure S3

Kaplan-Meier overall survival curves of hormone receptor-positive (HR+), HER2-negative breast cancers according to Ki67 scores determined by three different observers (A, B and C) and KiQuant (D). Negative (black) and positive (red) lines correspond to patients having a Ki67 LI less or above the median Ki67 value, respectively. *P*-values are from the Log-rank test.

Image analysis algorithms:

KiQUANT analysis algorithms (tissue detection, cytokeratin mask, and Ki67 scoring) are made available upon request for download.

Supplementary table 1

Magnification	20X
Classification method	Cell classification
Classification feature	<p>Detection of Nuclei: Standard Positive Nuclei Sensitivity: 80% Size: 10um</p> <p>Separate Nucleus Type Standard Negative Nuclei Sensitivity:100% Size 9um</p>
Post processing	
<ul style="list-style-type: none"> • Change by area • Change by area • Change by intensity (Ki67 channel) <ul style="list-style-type: none"> • Change • Apply counting frame 	<ul style="list-style-type: none"> • Lbl: label002, Max: 10µm² • Lbl: positive, Max: 10µm² • Lbl: label002; intensity: -inf -> 195, % object 0%-70%, negative • Lbl: label002 to clear • Lbl: all, replace with clear
Output	<ul style="list-style-type: none"> • All tumour cells • %Ki67 positive tumour cells • Total area

Supplementary table 2

TMA	Cohort	BC subtype	Patients	Arrayed Cores	Excluded Cores	Scoring methodology		
						OBS1	OBS2	OBS3
TMA1	1	HER2	20	40	7	C	C	<i>Ns</i>
TMA2	1	HR+	23	69	8	C	C	E
TMA3	1	HR+	21	63	4	C	C	E
TMA4	1	TNBC	35	70	5	C	C	<i>Ns</i>
TMA5	2	HR+	87	87	0	C	C	E
Total			186	329	24			

Scoring methodology: C, counting; E, estimation. DIA, Digital image analysis. MS, Manual scoring. *Ns*, not scored.

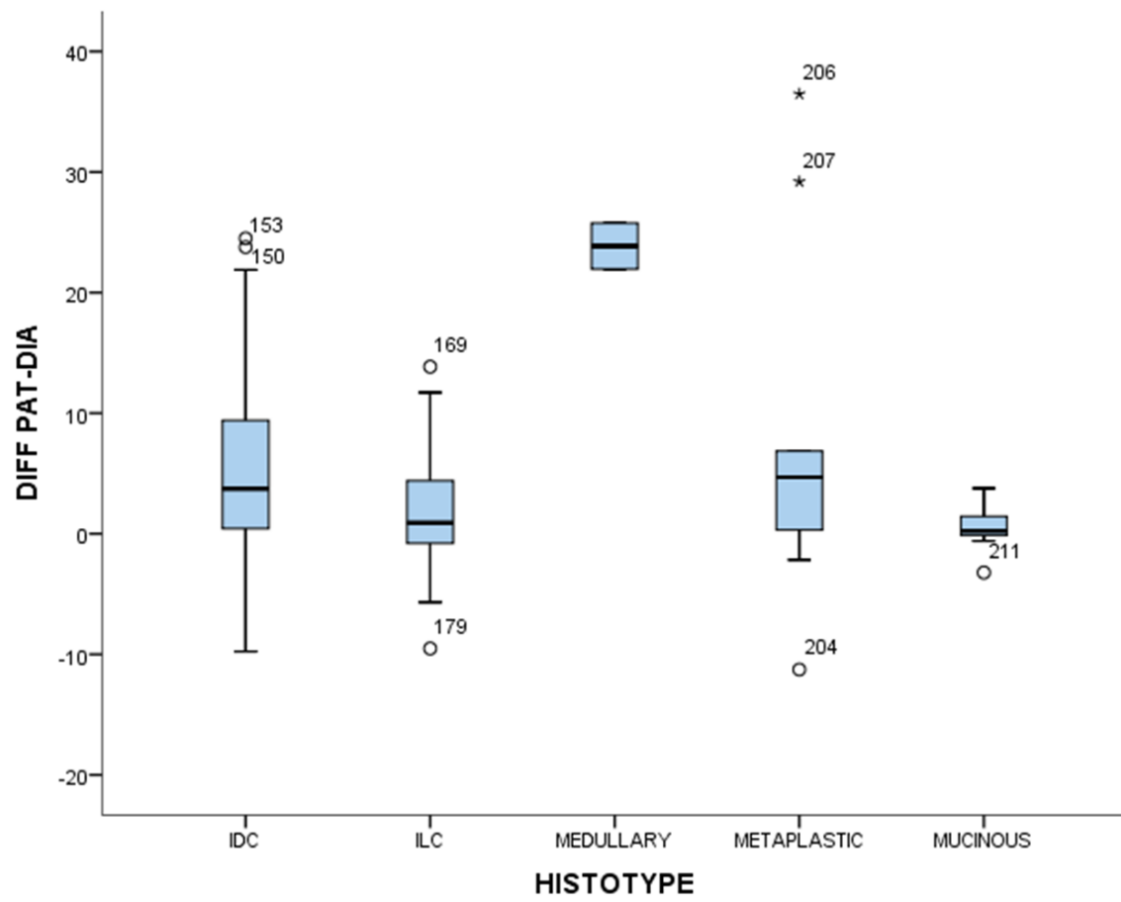
Supplementary table 3

Pat	MS	DIA	DIFF MS/DIA	HISTOLOGY	GRADE	STAGE	ER	PR	HER2	comments
1	69	46,6	21,9	INVASIVE DUCTAL CARCINOMA	N/A	N/A	+	+	0	post neo-adjuvant treatment
1	69	48,4	20,1							
2	63	38,0	24,5	INVASIVE DUCTAL CARCINOMA	III	pT1cN0	-	-	0	Highly cellular tumour with weak to moderate staining intensity in many cells; underestimation of the pathologist of negative cells
2	63	38,7	23,8							
3	85	59,2	25,8	MEDULLARY CARCINOMA	III	pT2N1	-	-	1+	Immune cells inside the tumour mask are counted as negative tumour cells
3	78	55,6	21,9							
4	78	41,0	36,5	METAPLASTIC CARCINOMA	III	pT3N0	-	-	0	mask counting negative dead cells in CK necrotic areas
4	55	25,8	29,2							
5	63	42,3	20,2	INVASIVE DUCTAL CARCINOMA	III	pT3N1	+	-	1+	mask counting negative dead cells in CK necrotic areas
6	47	27,2	19,8	INVASIVE DUCTAL CARCINOMA	III	pT2N0	-	-	0	mask counting negative dead cells in CK necrotic areas
7	49	28,8	19,7	INVASIVE DUCTAL CARCINOMA	III	pT3N1	-	-	3+	
8	31	42,3	-11,3	METAPLASTIC CARCINOMA	III	pT2N0	-	-	0	
9	17	26,3	-9,8	INVASIVE DUCTAL CARCINOMA	III	PT1N1	+	+	1+	

Supplementary table 4

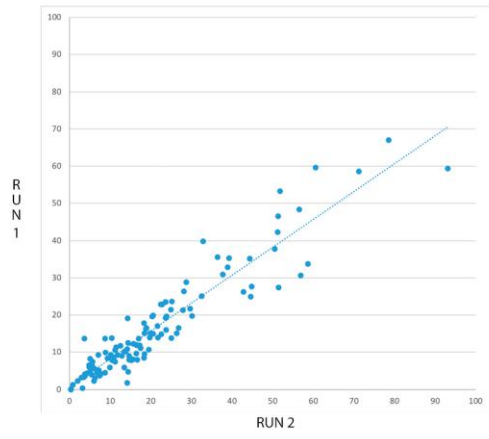
DIA cut-off	OBS1	OBS2	OBS3
2.7%	99	100	98
14%	85	72	81
20%	86	71	76
30%	84	90	74
40%	96	95	84
50%	100	99	95

Supplementary Figure S1

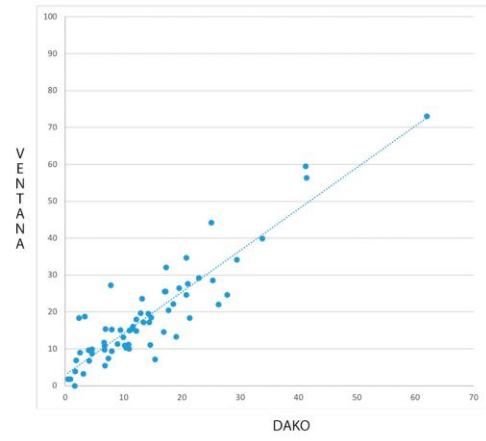


Supplementary Figure S2

A



B



Supplementary Figure S3

