

# Validating of Applied Spectral Imaging for Ki-67

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## Context

- Whole slide imaging (WSI) has been used for various clinical purposes.
- Some WSI systems also provide automatic intelligent toolsets to assist pathologists with scoring immunostains on digital images.
- The Ki-67 index is critical in many malignancies' grading that correlates to prognoses and therapeutics.
- However, the assessment is not standardized.
- Therefore, we conducted a study to validate the reliability of a digital image analysis system, HiPath Pro™, by Applied Spectral Imaging (ASI) to automate Ki-67 scoring.

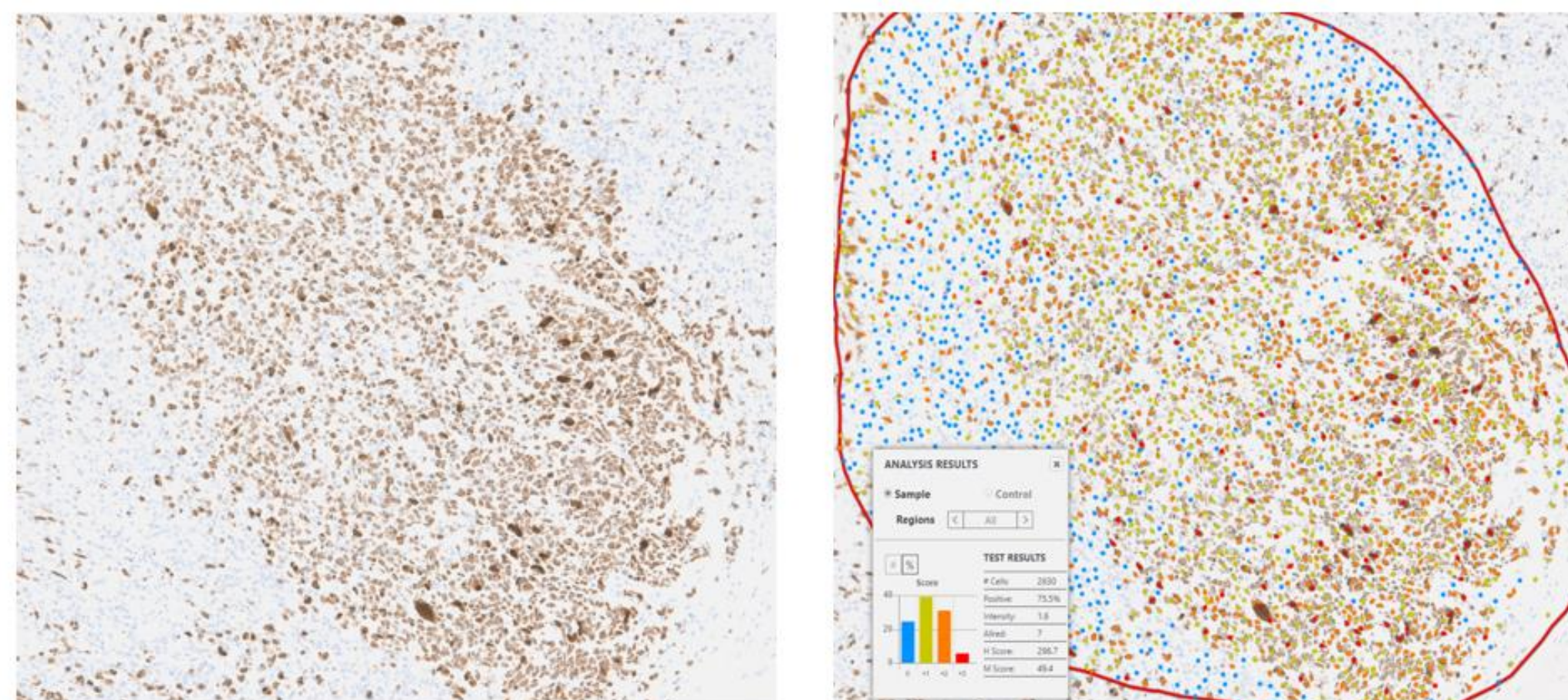
## Design

- Pathologists assessed Ki-67 index in 63 cases using manual analysis on a glass slide (MAGS), manual analysis on a digital image (MADI) and using ASI analysis on a digital image (AADI).
- Thirty-one cases had digital assessment first, while thirty-two had microscopic assessment first.
- Cases were reviewed in a random order with a minimum 2-week washout period between each assessment method, and results were compared.
- Concordance was defined as < 10% difference in Ki-67 scoring between the methods.

Table: Assessed Ki-67 Index By MAGS, MADI and AADI

Serial #	Manual Analysis on Glass Slide (MAGS)	Manual Analysis on Digital Image (MADI)	ASI Analysis on Digital Image (AADI)	MAGS-MADI Difference	MADI-AADI Difference	MAGS-AADI Difference
1	2.0	<5	2.1	0.0	0.0	-0.1
2	>95	>90	97.3	0.0	0.0	0.0
3	40.0	40.0	42.3	0.0	2.3	-2.3
4	60-70	60-70	70.6	0.0	0.6	0.6
5	5.0	5.0	6.2	0.0	1.2	-1.2
6	>90	>90	97.8	0.0	0.0	0.0
7	>90	>90	99.7	0.0	0.0	0.0
8	70.0	70.0	70.2	0.0	0.2	-0.2
9	70.0	50-60	68.8	10.0	8.8	1.2
10	>90	>90	96.4	0.0	0.0	0.0
11	90.0	90.0	91.8	0.0	1.8	-1.8
12	50-60	50-60	53.3	0.0	0.0	0.0
13	90.0	90.0	93.0	0.0	3.0	-3.0
14	5-10	10.0	10.7	0.0	0.7	0.7
15	5.0	5.0	8.2	0.0	3.2	-3.2
16	60-70	60-70	62.0	0.0	0.0	0.0
17	20.0	20.0	21.3	0.0	1.3	-1.3
18	90.0	90.0	94.6	0.0	4.6	-4.6
19	<2	1.0	0.4	0.0	-0.6	0.0
20	<5	5.0	3.4	0.0	-1.6	0.0
21	80.0	80.0	81.3	0.0	1.3	-1.3
22	70-80	70.0	73.2	0.0	3.2	0.0
23	50-60	60.0	53.2	0.0	-6.8	0.0
24	>90	90-100	100.0	0.0	0.0	0.0
25	70-80	80.0	77.7	0.0	-2.3	0.0
26	80.0	80.0	76.5	0.0	-3.5	3.5
27	<5	<5	1.9	0.0	0.0	0.0
28	30.0	20-30	22.7	0.0	0.0	7.3
29	5-10	10.0	10.5	0.0	0.5	0.5
30	60.0	60.0	64.9	0.0	4.9	-4.9
31	<10	10.0	12.0	0.0	2.0	2.0
32	50-60	60-70	61.4	0.0	0.0	1.4
33	80-90	80.0	82.6	0.0	2.6	0.0
34	10.0	10-20	15.7	0.0	0.0	-5.7
35	5.0	5-10	5.3	0.0	0.0	-0.3
36	80-90	80-90	89.5	0.0	0.0	0.0
37	<5	<5	2.3	0.0	0.0	0.0
38	20.0	20.0	18.3	0.0	-1.7	1.7
39	40-50	40-50	40.6	0.0	0.0	0.0
40	50-60	50-60	57.3	0.0	0.0	0.0
41	90.0	90.0	87.0	0.0	-3.0	3.0
42	5.0	5.0	5.4	0.0	0.4	-0.4
43	30-40	30.0	36.3	0.0	6.3	0.0
44	80.0	80.0	79.1	0.0	-0.9	0.9
45	>90	90.0	93.5	0.0	3.5	0.0
46	40-50	40-50	42.0	0.0	0.0	0.0
47	30-40	30.0	30.5	0.0	0.5	0.0
48	30-40	30-40	49.8	0.0	9.8	9.8
49	60-70	70-80	70.2	10.0	0.0	0.2
50	80-90	80.0	88.1	0.0	8.1	0.0
51	90.0	90.0	89.2	0.0	-0.8	0.8
52	50.0	50.0	46.5	0.0	-3.5	3.5
53	20.0	20.0	23.9	0.0	3.9	-3.9
54	>90	>90	96.9	0.0	0.0	0.0
55	20-30	30.0	22.6	0.0	-7.4	0.0
56	70.0	70.0	70.7	0.0	0.7	-0.7
57	>90	>90	98.1	0.0	0.0	0.0
58	80-90	80.0	82.5	0.0	2.5	0.0
59	50-60	50-60	61.8	0.0	1.8	1.8
60	>90	>90	98.5	0.0	0.0	0.0
61	5.0	5.0	4.9	0.0	-0.1	0.1
62	10.0	10.0	13.3	0.0	3.3	-3.3
63	90.0	90.0	90.9	0.0	0.9	-0.9

Figure: Manual and HiPath Pro™ Analysis of Ki-67 on Digital Image (MADI and AADI)



Left: A scanned Ki-67 immunostain slide showing a high proliferative index of 70-80% by MADI  
Right: HiPath Pro™ analyzes nucleated cells and provides an estimated proliferation index of 75.5% by AADI

## Results

- Two of the 63 cases showed a  $\geq 10\%$  Ki-67 index difference between MAGS and MADI (96.8% concordant).
- When comparing between MADI and AADI, none of the 63 cases showed a difference  $\geq 10\%$  (100% concordant), and six cases showed a difference of  $>5\%$  (90.5% concordant).
- Comparisons between MAGS and AADI demonstrated that none of the 63 cases had a  $\geq 10\%$  Ki-67 index difference (100% concordant), and only three of the cases showed a  $>5\%$  Ki-67 index difference (95.2% concordant).

## Conclusions

- ASI WSI, combined with computer-assisted analysis by ASI's HiPath Pro™ system, is reliable in assisting pathologists with scoring the Ki-67 index.

## References

- [1] Lu H, Papathomas TG. Automated Selection of Hotspots (ASH): enhanced automated segmentation and adaptive step finding for Ki67 hotspot detection in adrenal cortical cancer. *Diagn Pathol.* 2014 Nov 25;9:216. doi: 10.1186/s13000-014-0216-6. PMID: 25421287; PMCID: PMC4261753.
- [2] Yamazaki Y. Comparison of the methods for measuring the Ki-67 labeling index in adrenocortical carcinoma: manual versus digital image analysis. *Hum Pathol.* 2016 Jul;53:41-50. doi: 10.1016/j.humpath.2015.10.017. Epub 2016 Mar 7. PMID: 26980031.