

Background

- Immunotherapy has transformed melanoma treatment, but identifying which patients are most likely to benefit remains challenging¹.
- Immunohistochemistry is commonly used to assess predictive biomarkers; however, manual scoring can be time-consuming and subject to inter-observer variability².
- Automated quantitative image analysis may provide a faster and more reproducible approach to biomarker assessment, but its clinical reliability remains uncertain³.

Objectives

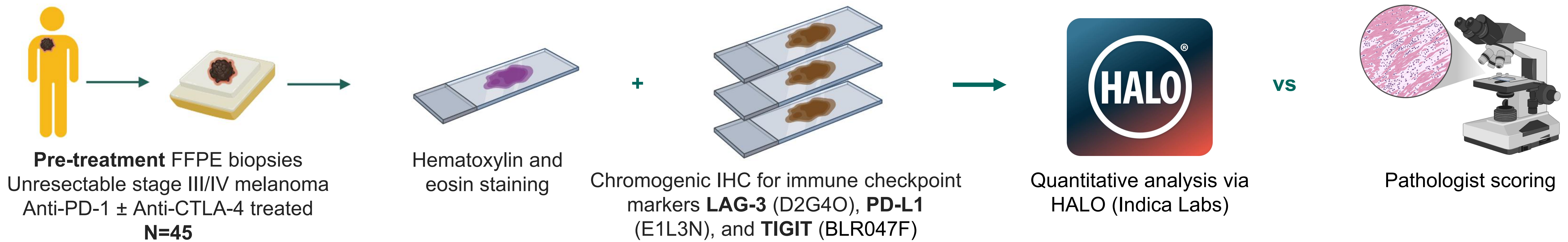
- To determine whether quantitative pathology using the HALO platform is comparable to gold-standard pathologist scoring
- To identify factors associated with discordance between quantitative scoring and pathologist scoring

Methods

Patient Cohort

Immunohistochemical staining

Analysis



Results

Immunohistochemical staining of immune checkpoint markers

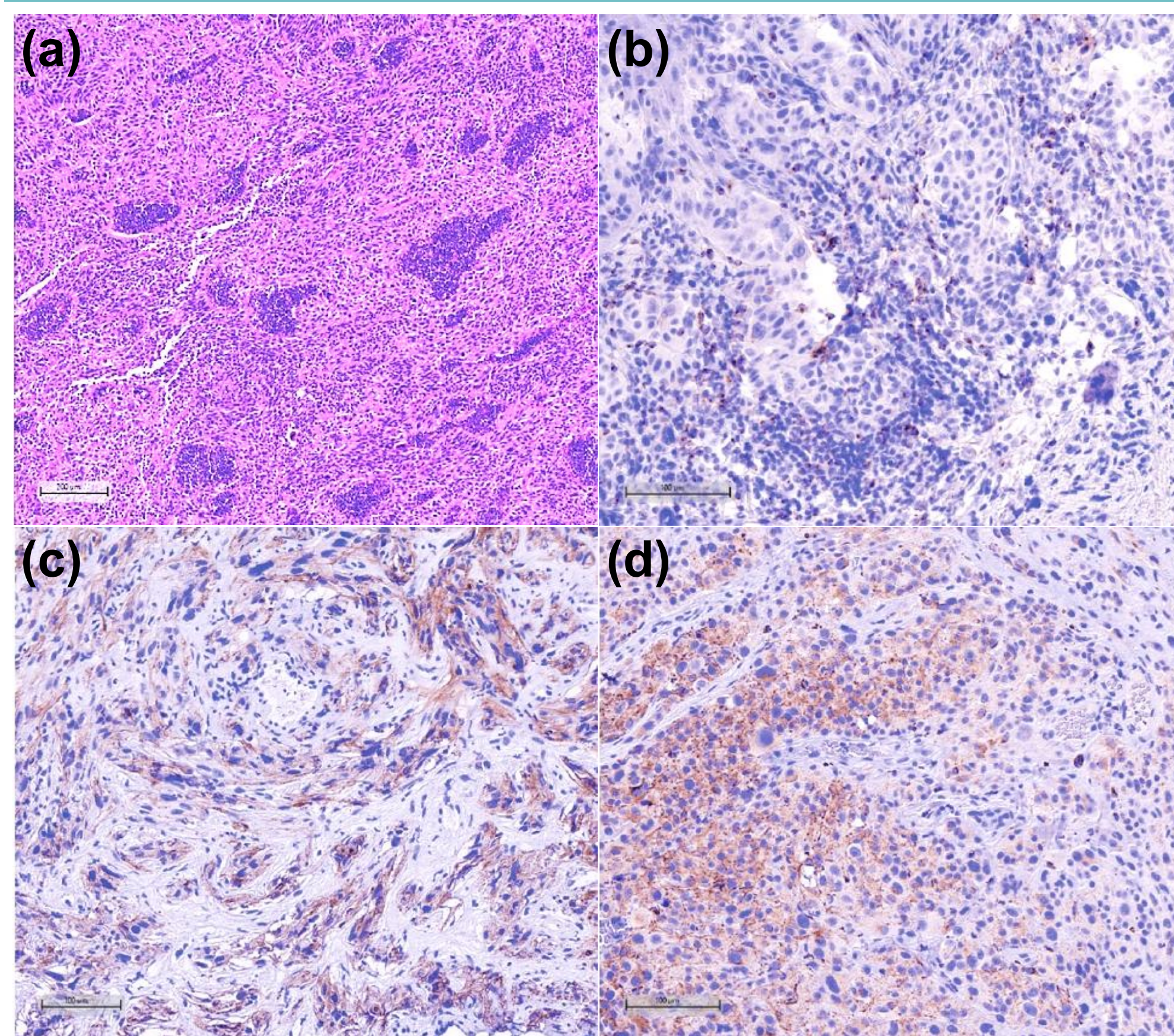


Figure 1: Representative image of (a) a hematoxylin and eosin-stained sample with TIL grade 3 (moderate or marked diffuse TIL infiltration) and TIL pattern 2 (perivascular TIL distribution). Representative images of IHC drug target staining for (b) LAG-3, (c) PD-L1, and (d) TIGIT.

Factors contributing to scoring discrepancies between the two methods

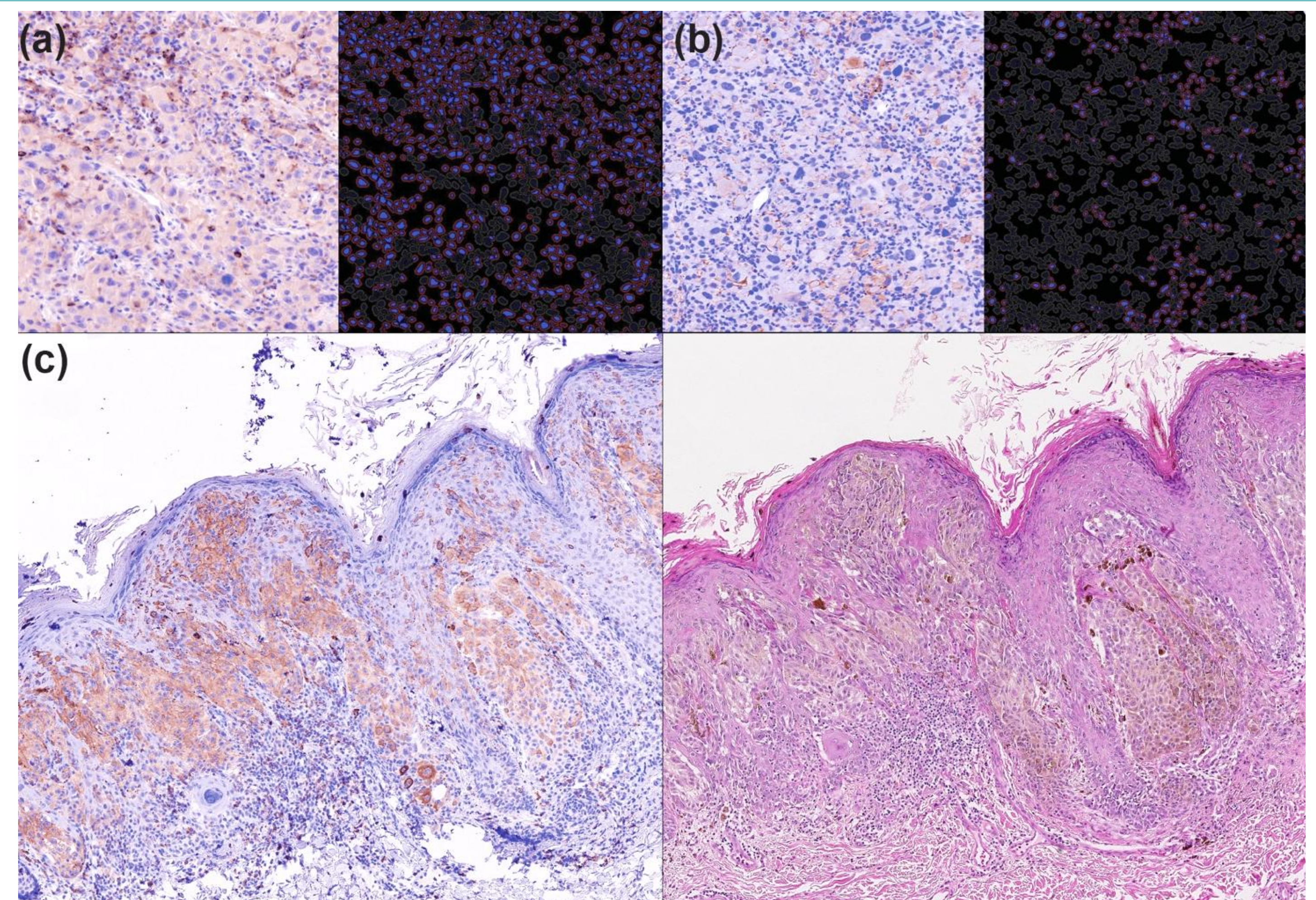


Figure 5: Factors associated with incorrect quantitative pathology scoring. (a) High background marker staining leading to over-reporting of positive cells; (b) incorrect nuclear segmentation of tumour cells causing misaligned marker positivity; and (c) exclusion of pigmented areas from analysis despite high marker positivity.

IHC scores for all 3 markers strongly correlate between the two methods

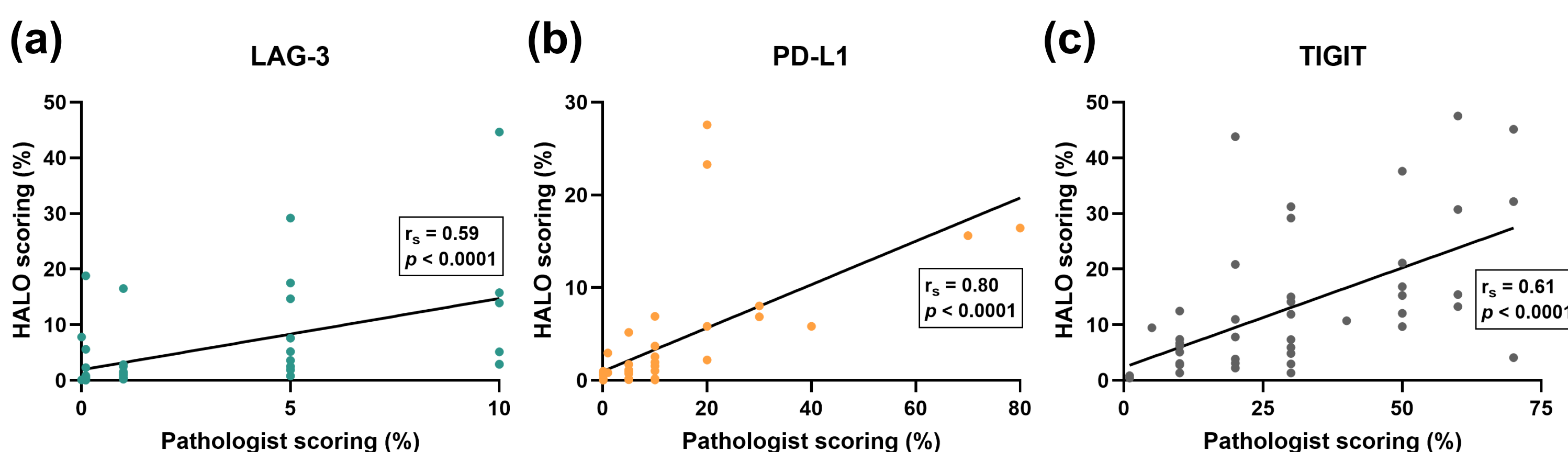


Figure 2: Scatter plots showing the relationship between pathologist scoring and quantitative pathology (HALO) for (a) LAG-3, (b) PD-L1, and (c) TIGIT. r_s – Spearman's rank correlation coefficient.

Multiplex immunofluorescence staining of immune checkpoints

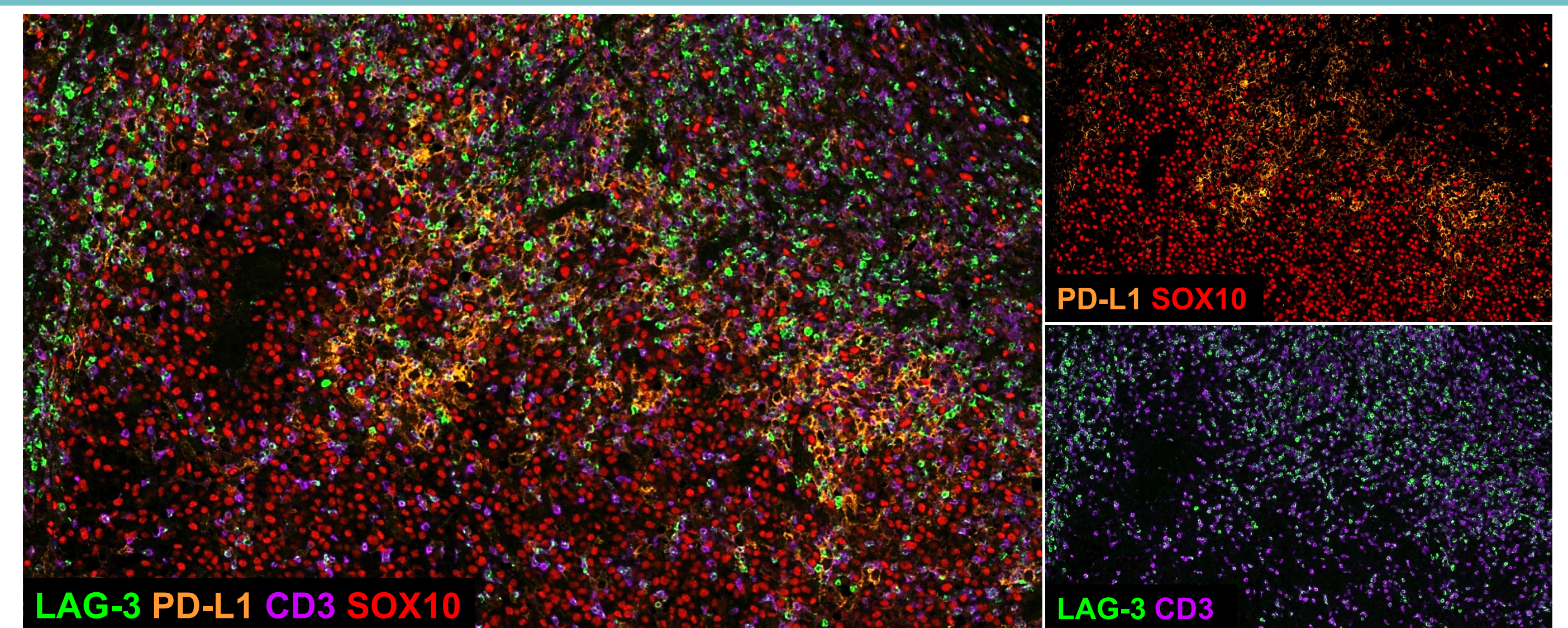


Figure 6: Representative multiplex immunofluorescence images showing staining of the LAG-3 and PD-L1 immune checkpoint markers, the CD3 pan T-cell marker, and the SOX10 melanoma marker.

Quantitative pathology reports lower PD-L1 and TIGIT scores

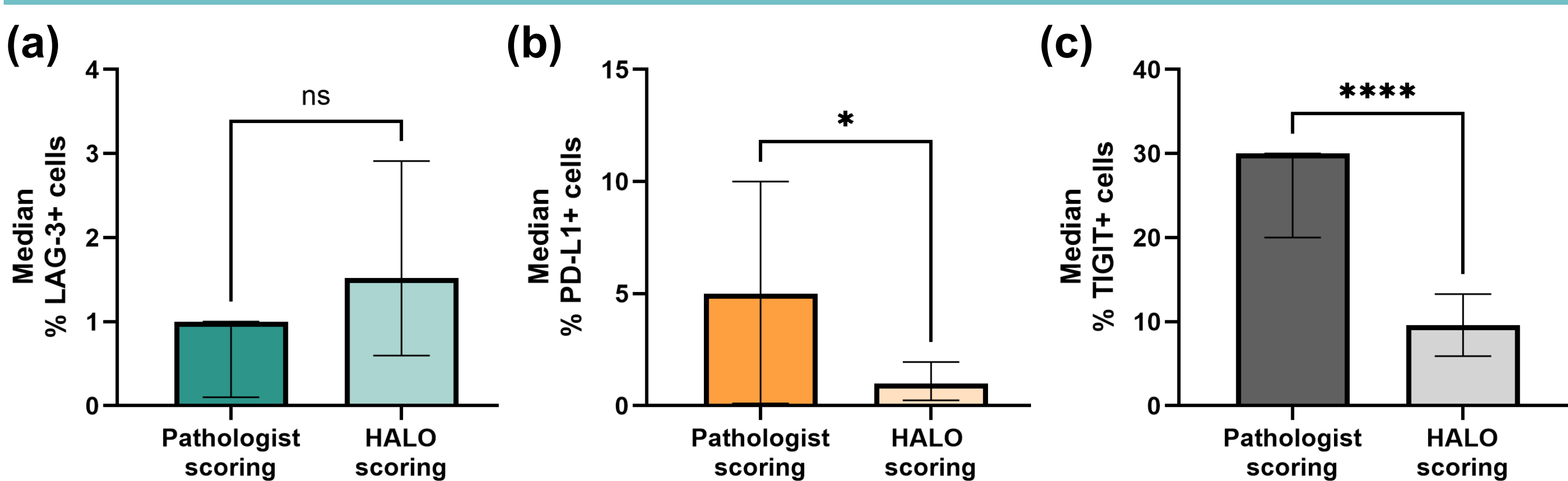


Figure 3: Bar graphs comparing median immune marker scoring between pathologist evaluation and quantitative pathology (HALO) for (a) LAG-3, (b) PD-L1, and (c) TIGIT. * $p < 0.05$, **** $p < 0.0001$, ns – non-significant. Error bars represent median with 95% CI.

TIGIT shows the largest scoring differences between methods

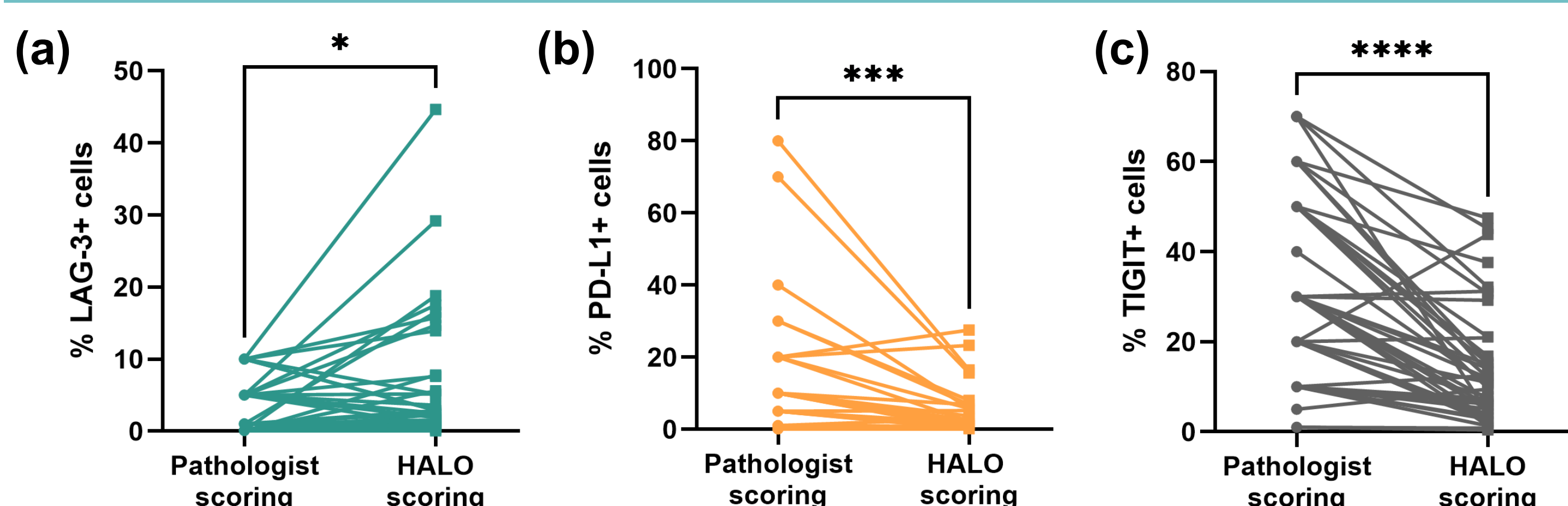


Figure 4: Comparison of immune checkpoint marker expression assessed by pathologist scoring and quantitative image analysis (HALO) in matched melanoma samples for (a) LAG-3, (b) PD-L1, and (c) TIGIT. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

Conclusions

- Strong correlations were observed between quantitative image analysis and pathologist scoring for LAG-3, PD-L1, and TIGIT.
- Scoring discrepancies were primarily associated with high background staining, inaccurate nuclear segmentation, and tumour pigmentation.
- Quantitative pathology shows promise as a scalable and reproducible approach for immune biomarker assessment in melanoma, with further optimisation needed to improve accuracy in challenging specimens.

Future Directions

Preliminary multiplex immunofluorescence demonstrates the feasibility of simultaneously visualising tumour cells, T cells, and immune checkpoint markers. Future studies will evaluate whether this approach can improve cell identification, segmentation, and biomarker quantification.

Acknowledgements

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References

- Pavlick, A. C., et al. *J Immunother Cancer* 2023;11:e006947
- Darmon-Novello, M., et al. *Histopathology* 2022;80:1091-1101
- Koelzer V. H., et al. *Histopathology* 2018;73:397-406

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