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Exploring interplay between tumor intrinsic features and immune cell phenotypes in multi-tumor tissue microarrays by a single step 15-plex fluorescence immunohistochemistry and imaging on the Orion Platform

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STUDY SUMMARY

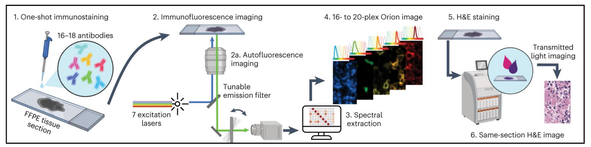
Purpose: Deeper understanding of the intricacies between tumor microenvironments and immune cell infiltrates is critical for creation of next generation therapies. However, current immunohistochemistry protocols in clinical practice are restricted to one to six markers (cell types) and rely on multiple rounds of antigen retrieval and sequential staining over two days. This current workflow has high risk for tissue and signal loss. Hence, we explored utility of a single step staining workflow using 15 distinct antibody-conjugated fluors (Argo Dye System) of formalin fixed paraffin embedded (FFPE) tumor microarrays and imaged on the Orion microscope (RareCyte). Fidelity of the staining was assessed by comparing data from the Orion platform to internally developed clinical grade assays. The implementation of higher plex imaging will enable greater insight into immune surveillance, mechanisms of resistance and patient stratification while minimizing patient tissue required for analysis.

Study Design: We utilized a custom panel developed by RareCyte (Hoescht, CD3e, CD4, CD8a, CD20, CD68, CD163, FOXP3, Ki67, Pan-CK, PD1, PDL1, CD45, Granzyme B, LAG3) to evaluate expression of immune markers on a pan-tumor and melanoma TMA including Hodgkin's lymphoma, ovarian cancer, esophageal carcinoma, hepatic cell carcinoma, head and neck cancer. Serial sections were stained with 6-plex clinical grade assays, and correlation scores were determined between the 15-plex Orion and 6-plex assays utilizing TSA-based amplification.

Results: In this study, the performance of Orion platform was assessed by quantitative and qualitative measures. Linear regression between the 6-plex clinical grade assays and 15-plex Orion workflow were calculated for a panel of biomarkers including CD3, CD4, CD8, CD68, CD163, CK, FOXP3, GZMB, Ki67, and PD1. Our results demonstrated that the Orion performed similarly to clinical grade assays both in terms of proportion of cells and fluorescence intensity (R^2 ~ 0.7-0.98, Slope ~ 0.7-1.1).

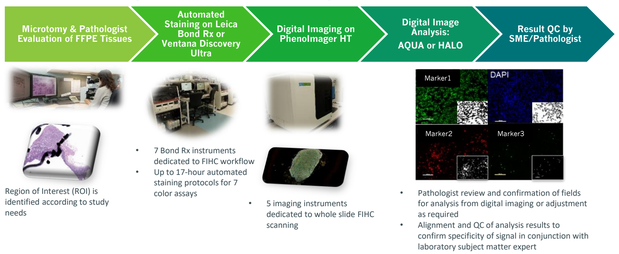
METHODS

Single Step ORION Multiplex IHC Workflow



Brief Description: Formalin fixed paraffin embedded tissues undergo a one-shot staining process with up to 18 AlexgFuor-conjugated antibodies. Images are scanned utilizing the Orion platform and the staining intensity for each biomarker is computed by spectrally unmixing the final multiplexed image. The same slide can then be processed for H&E. *Workflow Image Source: Lin et al. (2023)*

Multi Step OPAL-TSA Multiplex Workflow



Single Step staining performance on Head and Neck Carcinoma

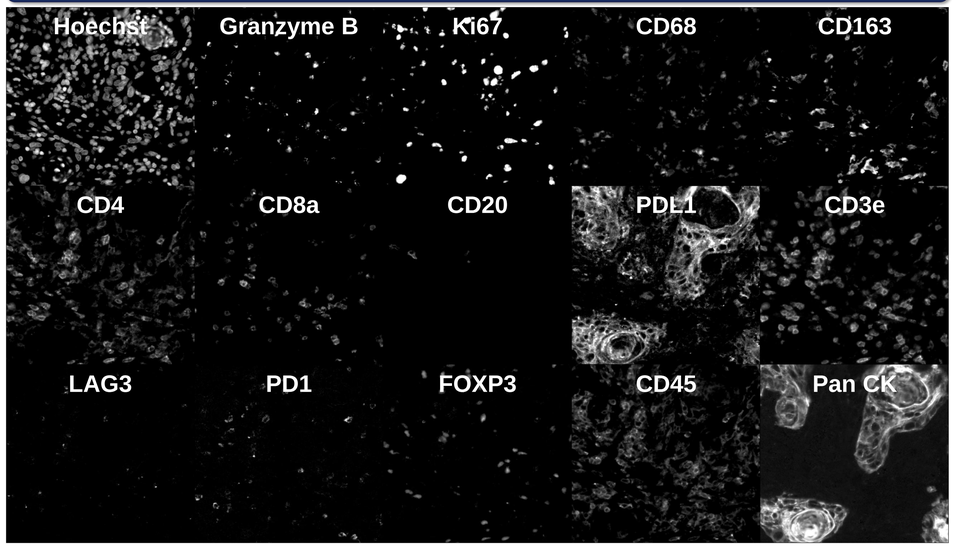
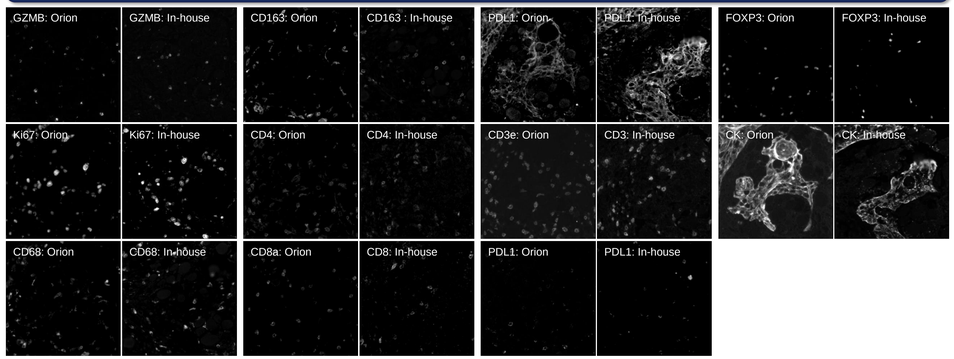


Figure 1: TMA panels were stained with RareCyte panel (Hoechst/Granzyme B/Ki67/CD68/CD163/CD4/CD8a/ CD20/PDL1/CD3e/LAG3/ PD1/FOXP3/ CD45/PanCK) and imaged using the Orion platform. Sample image from a head and neck carcinoma TMA core is shown above.

Single versus Multi Step Staining in Esophageal Carcinoma



	GZMB	Ki67	CD68	CD163	CD4	CD8	PDL1	CD3	PD1	FOXP3	CK
R^2	0.77	0.87	0.72	0.78	0.87	0.97	0.95	0.96	0.88	0.92	0.98
Slope	0.74	0.85	0.69	0.95	0.90	0.95	0.88	1.00	1.10	0.89	1.10

Figure 2:. The Orion panel was compared to in-house TSA-OPAL multiplex assays for eleven overlapping biomarkers. Fourteen cores were used to construct linear regression curves for the Orion versus in-house assay staining. The R^2 and slopes from the linear regression are summarized in the table above. Sample image from sequentially sectioned esophageal carcinoma are shown above.

Imaging of rare cell population on ORION Platform

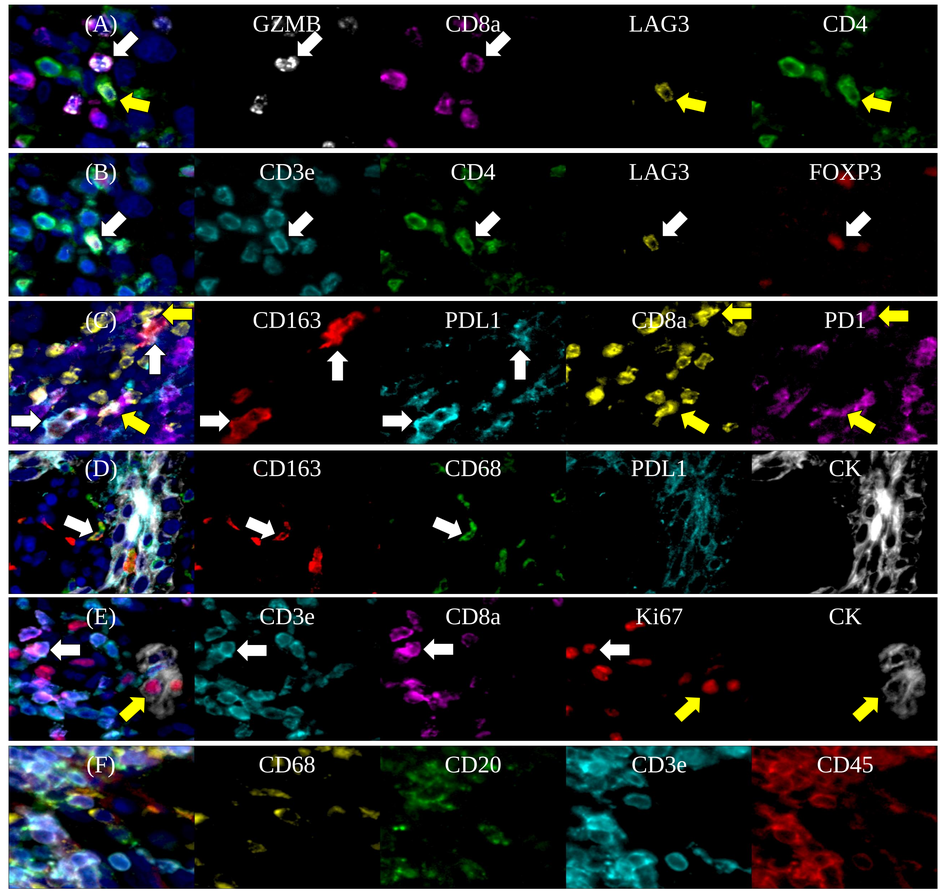


Figure 3: Orion platform was used to capture rare cell populations as shown above. **(A)** A cytotoxic T-cell (CD8a+GZMB+) is seen near an exhausted helper T-cell (CD4+LAG3+) in head and neck carcinoma. **(B)** An exhausted Treg (CD3e+CD4+LAG3+FOXP3+) is seen in head and neck carcinoma. **(C)** PDL1+ M2 macrophages are seen interacting with PD1+ cytotoxic T-cells in Non-Hodgkin lymphoma. **(D)** M2 macrophages (CD68+CD163+) are seen nearby PDL1+ tumor cells in head and neck carcinoma. **(E)** A proliferating cytotoxic T-cell (CD3+CD8a+Ki67+) are seen nearby proliferating tumor cells (CK+Ki67+) in head and neck carcinoma. **(F)** Broad immuno-landscape of macrophages (CD68+), B-cells (CD20+), T-cells (CD3e+) and general leucocytes (CD45+) in esophageal carcinoma.

CONCLUSIONS

Our results suggest that the Orion platform offers a reliable and efficient solution for comprehensive biomarker analysis. Our results demonstrate that this platform can successfully capture the expression of 15 biomarkers on a single FFPE slide which offers workflow advantages over traditional TSA-based fIHC multiplexing that is limited to detection of 6 biomarkers on a single slide. The ability to analyze a wider range of biomarkers through a single platform has the potential to enhance the efficiency of biomarker analysis, leading to improved diagnostic and therapeutic outcomes in clinical practice.

REFERENCES

1) Lin, JR., Chen, YA., Campton, D. *et al.* High-plex immunofluorescence imaging and traditional histology of the same tissue section for discovering image-based biomarkers. *Nat Cancer* **4**, 1036–1052 (2023). https://doi.org/10.1038/s43018-023-00576-1

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