indica labs
QUANTITATIVE PATHOLOGY

Characterizing the cellular architecture of the tumor microenvironment using imaging mass cytometry and digital image analysis with the HALO® and HALO AI platforms

Natasha Carmell, Ben Dyer, Ghislaine Lioux
Indica Labs Inc., Albuquerque, New Mexico, United States

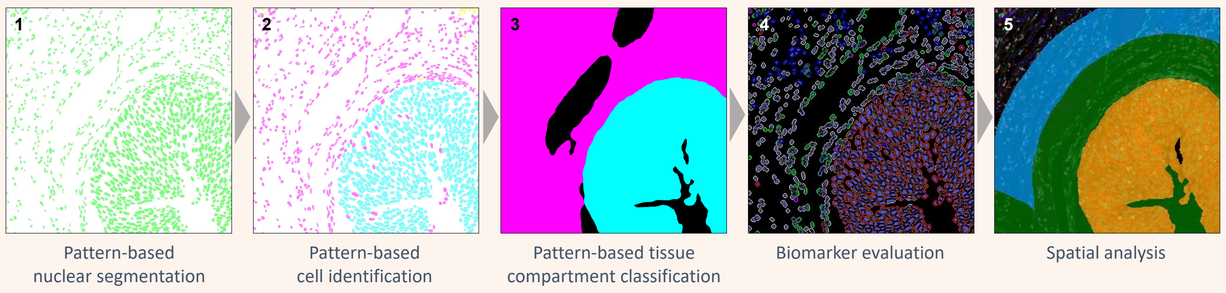
BACKGROUND

The tumor microenvironment (TME) is the seat of multiple cell interactions, including those between tumor cells, immune cells, stromal cells, and others. The identification of the markers expressed, and their spatial distribution can help not only to establish a prognosis of the disease, but also to direct therapeutic selection[(1)]. Imaging mass cytometry™ (IMC™) allows for the simultaneous evaluation of the expression of more than 40 protein biomarkers while investigating cellular and histological context[(2)]. In this study, we show that the HALO and HALO AI image analysis platforms provide a convenient workflow for analysis of the TME and highly multiplexed IMC images.

Panel includes alpha SMA, vimentin, pan-CK, PD-L1, FoxP3, CD4, E-cadherin, CD68, CD20, CD8a, PD-1, granzyme B, Ki67, collagen, CD3, PHH3, and CD45RO.

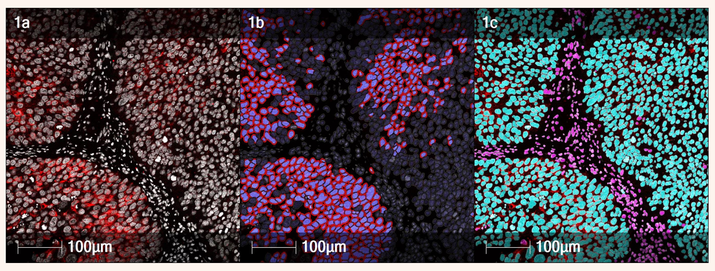
METHODS AND RESULTS

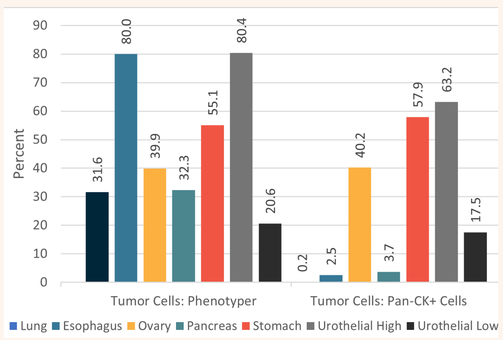
Image Analysis Workflow



Building upon HALO AI's pretrained nuclear segmentation network, DNA channels were used to identify nuclei across multiple cancer types to create one effective segmentation network (1). Next, an AI nuclei phenotyper network was created to identify tumor, stromal, and immune cells based off the DNA channels in multiple samples (2). Relying on morphology as opposed to biomarkers allowed us to create a classifier which identified cells irrespective of differentially expressed markers. An AI Tissue classifier was then built to track the location of immune cells in relation to the tumor boundary (3). The developed AI networks were embedded in the HALO Highplex FL module for various analyses included in this study and immune cells were identified within this module using positivity thresholding for various biomarkers (4). The coordinates of the single cells were used for subsequent spatial analysis to evaluate immune cell density and tumor infiltration (5).

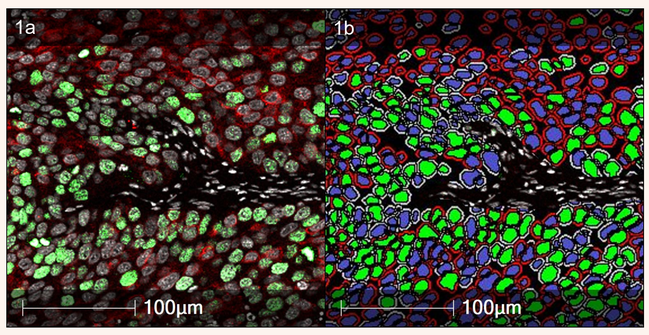
AI Phenotyping with DNA Channels

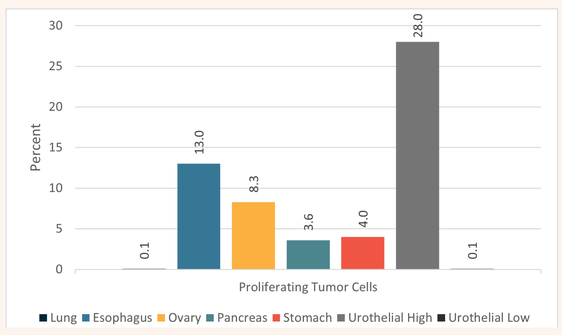




DNA in white, Ki67 in teal and pan-CK in red are shown in high-grade urothelial carcinoma (1a), Highplex FL shows positivity for pan-CK in red (1b), AI nuclei phenotyper mark-up shows tumor cells in teal and stromal cells in magenta (1c). The percentage of tumor cells identified by the phenotyper was calculated from the total cell count across different cancer types. The histogram shows the AI phenotyper identifies some cells as tumorous that are not quantified based on variable pan-CK staining.

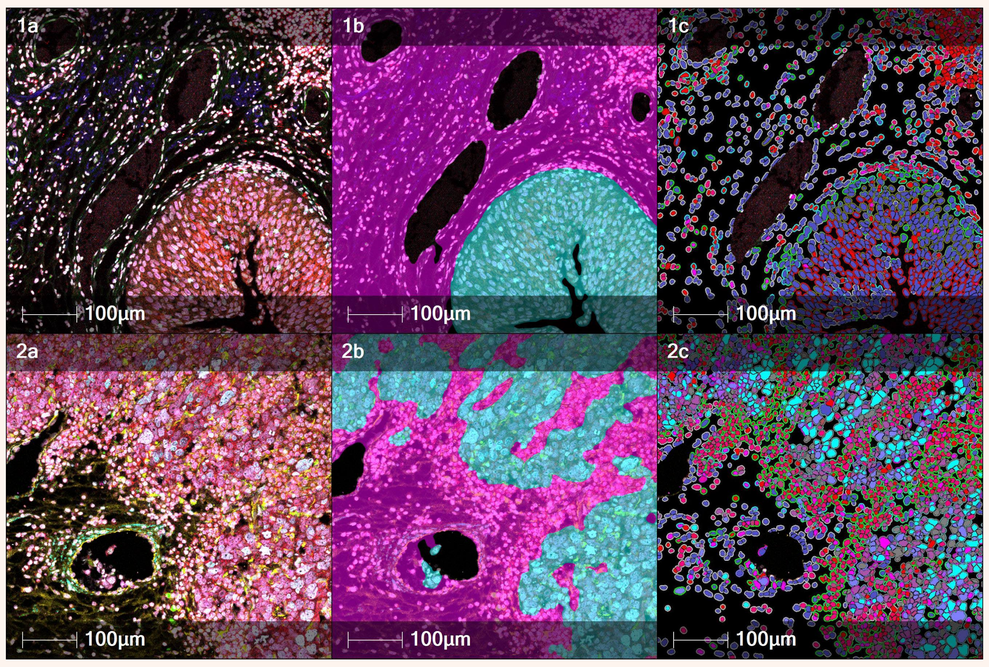
Biomarker Quantification in Tumor Cells



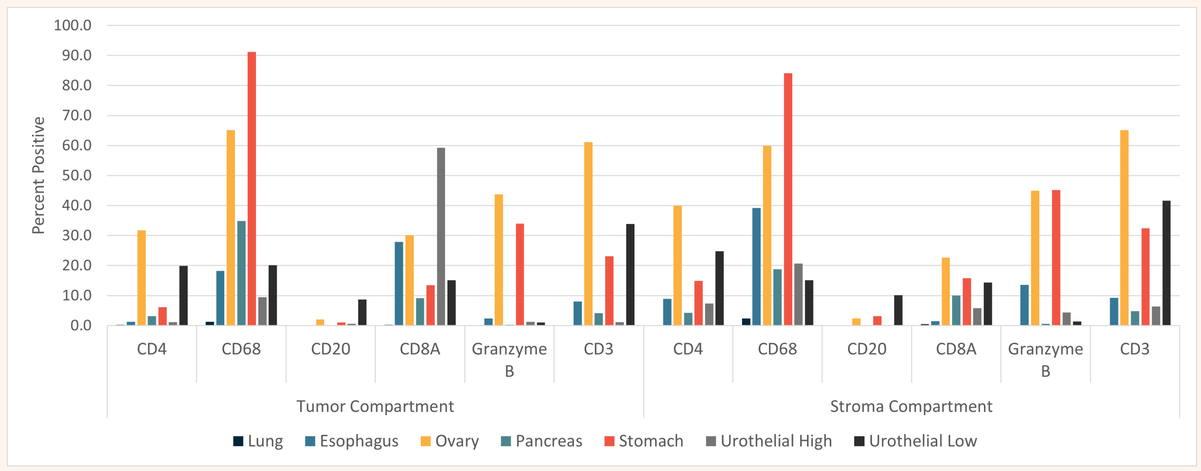


DNA in white, pan-CK in red, and Ki67 in green are shown in high-grade urothelial carcinoma (1a), Highplex FL shows colocalization for tumor cells phenotyped with AI in blue, cytoplasm positivity for pan-CK in red, and nuclear positivity for Ki67 in green (1b). Using AI phenotyping to identify tumor cells, along with Ki67 biomarker positivity, identifies proliferative tumor cells as shown in the histogram.

Immune Marker Quantification of the TME

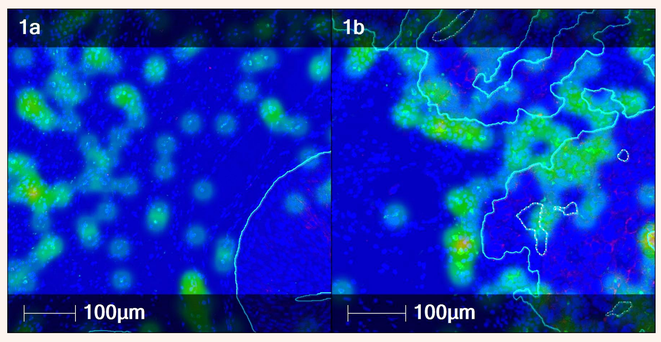


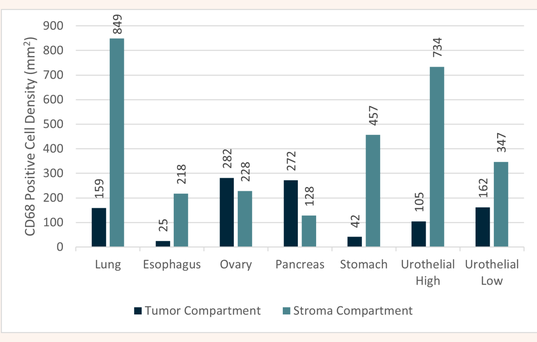
All channels are shown in low-grade urothelial carcinoma (1a) and ovarian adenocarcinoma (2a), tumor and stroma AI segmentation is shown in teal and magenta respectively (1b, 2b), Highplex FL colocalization analysis for 11 different biomarkers (1c, 2c).



Biomarker percentage positivity was calculated from total cell count across tumor and stroma compartments in different cancer types.

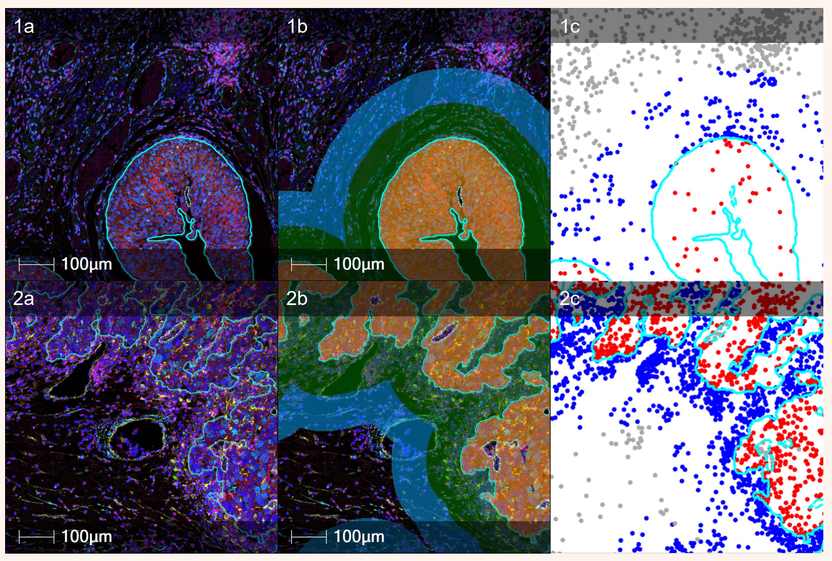
Density Heatmap of Macrophages



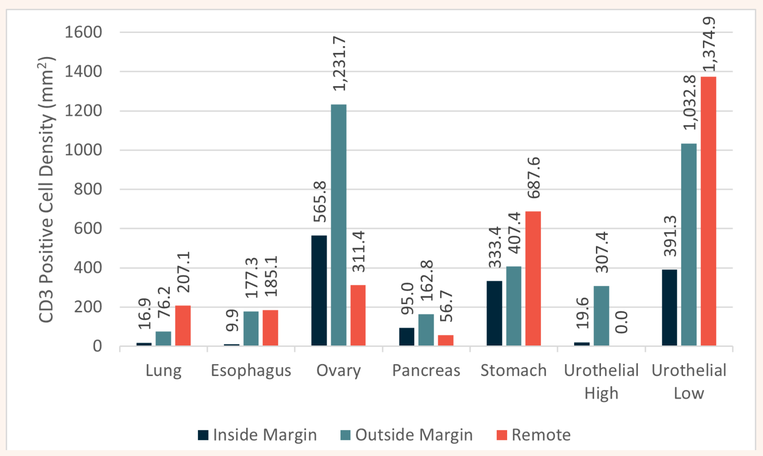


Using the cell data generated from Highplex FL with embedded AI networks, density heatmap analysis was performed within a 25 µm radius of every pixel within the image (1a,1b). We investigated the quantification and visualisation of CD68+ macrophages across multiple cancer types, which can be used to evaluate prognosis and discover tumor-associated macrophages. Hotspots of macrophages are localized to the stroma in low-grade urothelial carcinoma (1a) and distributed throughout the tumor in ovarian adenocarcinoma (1b). The quantification of macrophage densities in the tumor and stroma compartments are depicted in the histogram.

Infiltration Analysis of CD3+ cells



The tumor boundary is shown on low-grade urothelial carcinoma (1a) and ovarian adenocarcinoma (2a) in teal annotations, generated by an AI tissue classifier, while infiltration margin bands are shown in yellow, green, and blue (1b, 2b). Spatial map shows CD3 positive cells within the infiltration margin in red (inside margin) and blue (outside margin), and CD3 positive cells excluded from the analysis in grey (1c, 2c).



CD3 positive cell density was measured 100µm inside the tumor boundary in dark blue, and 100µm and 200 µm outside the tumor boundary in teal and orange respectively across multiple cancer types.

CONCLUSIONS

TME composition is an important component in a patient's response to treatment, and subsequently their prognosis[(3)]. Our analysis of hyperplex IMC images across multiple cancer types provides insights into immune marker expression, spatial distribution of macrophages, and immune cell tumor infiltration within the TME. The workflow presented here demonstrates how the combination of HALO and HALO AI image analysis platforms from Indica Labs results in an easy and straightforward workflow for streamlined, quantitative analysis of the TME in IMC images at the single-cell level across different tumor types.

REFERENCES

(1) Fridman, W., Zitvogel, L., Sautès–Fridman, C. *et al.* The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol* **14,** 717–734 (2017).
(2) Giesen C, Wang HA, Schapiro D, et al. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat Methods* (2014).
(3) Hanahan D and Weinberg RA. Hallmarks of Cancer: The Next generation. Cell 5 :144 (2011).

Correspondence: ncarmell@indicalab.com
info@indicalab.com