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QUANTITATIVE PATHOLOGY

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

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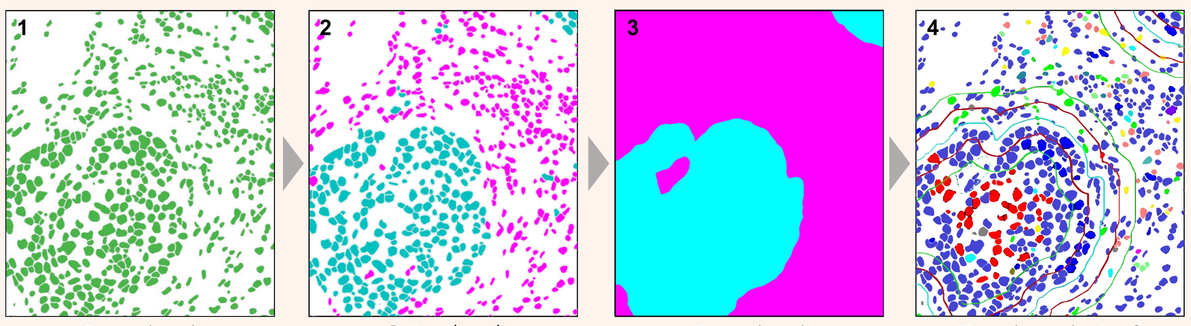
BACKGROUND

The tumor microenvironment (TME) is the seat of multiple cell interactions, including 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 the evaluation of the expression of ~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, CD45RO.

HALO® and HALO AI™ IMAGE ANALYSIS WORKFLOW

Image Analysis Workflow



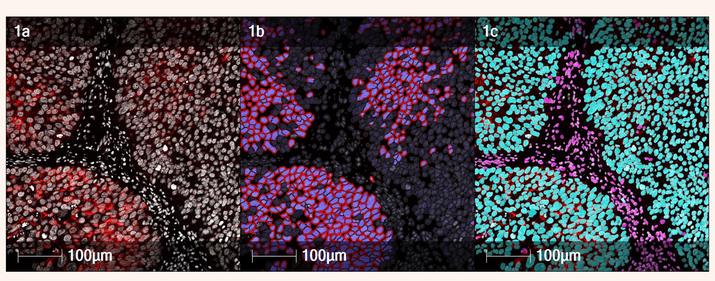
Building upon HALO AI's pretrained nuclear segmentation network, DNA channels were used to identify nuclei across multiple cancer types to create one effective segmentation network (1).

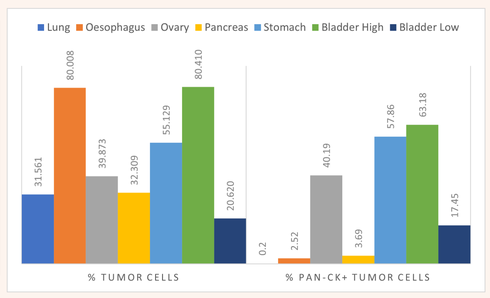
An AI nuclei phenotyper network was created to identify tumor cells based off the DNA channels in multiple cancer types (2). Relying on morphology as opposed to biomarkers allowed us to create a classifier which successfully identified tumor cells, irrespective of the differentially expressed markers.

An AI Tissue classifier was built to track the location of immune cells in relation to the tumor boundary (3).

Immune cells were identified with using positivity thresholding for various biomarkers in the HALO Highplex FL module. The coordinates of the single cells were used for subsequent spatial analysis to evaluate immune cell density and tumor infiltration (4).

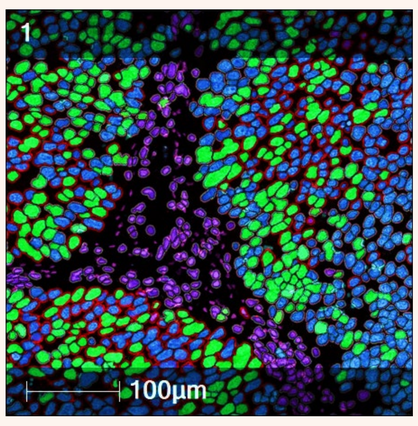
AI Phenotyping with DNA Channels

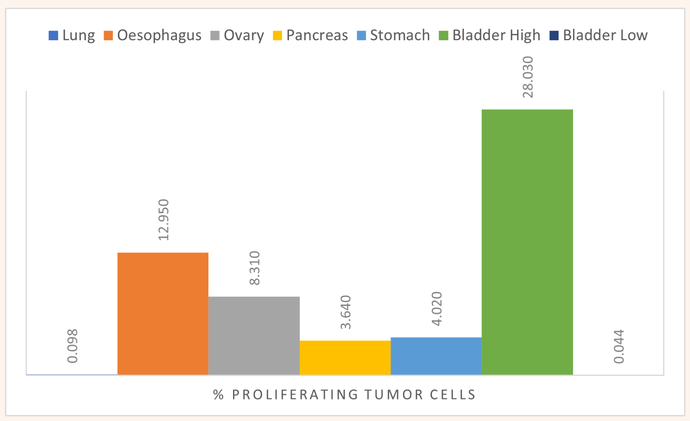




DNA in white, Ki67 in teal and Pan-CK in red are shown in high grade bladder urothelial carcinoma (1a), Highplex FL shows positivity for Pan-CK in red (1b), AI nuclei Phenotyper mark-up shows tumor cells in teal and stromal cells in magenta (1c). The percentage of tumor cells identified by the phenotyper was calculated from the total cell count across different cancer types. The histogram shows the AI phenotyper is able to identify tumor cells that cannot be quantified based off of variable Pan-CK staining.

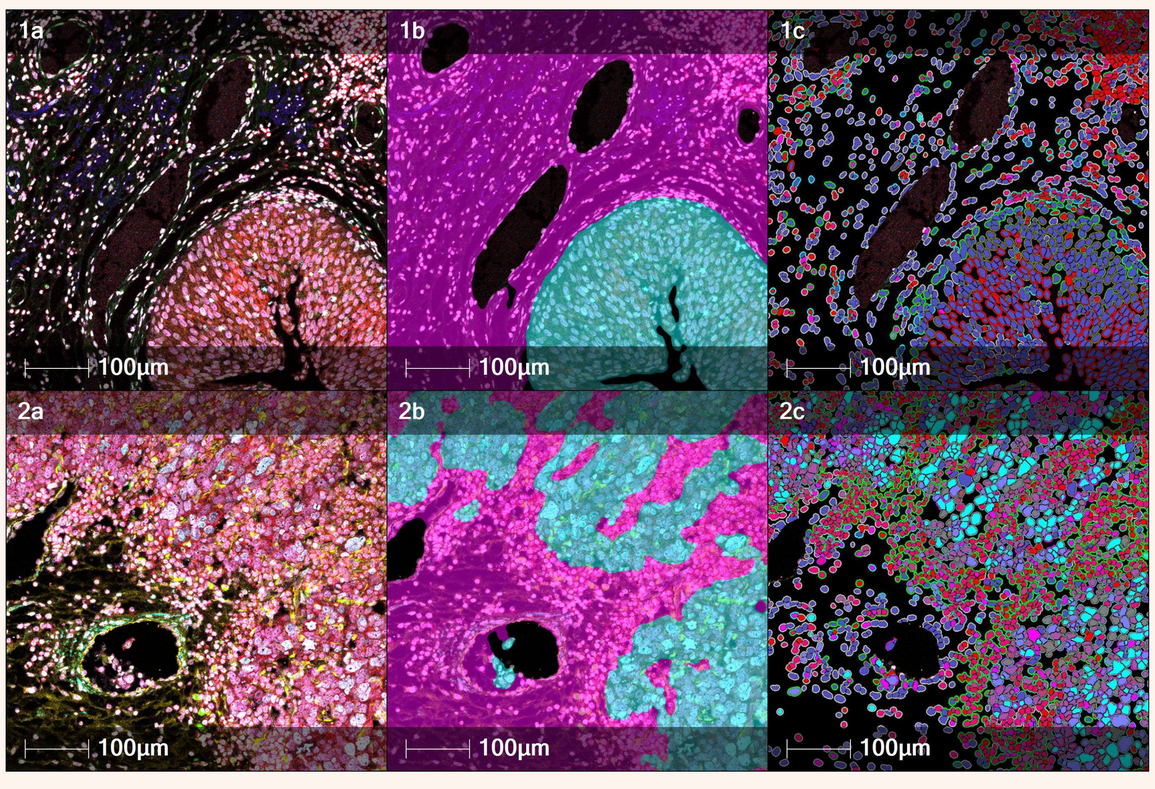
Biomarker Quantification in Tumor Cells



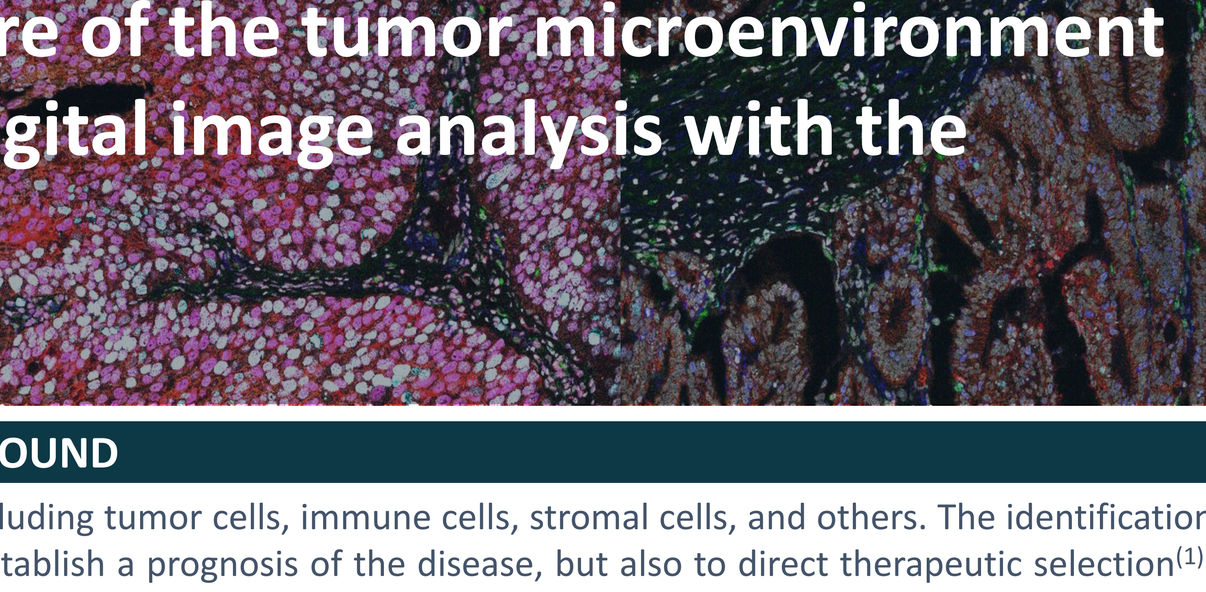


Highplex FL markup in High grade bladder urothelial carcinoma shows colocalization for tumor cells phenotyped with AI in blue, positivity for Pan-CK in red and Ki67 in green (1). Using AI phenotyping to identify tumor cells, along with Ki67 biomarker positivity, identifies proliferative tumor cells as shown in the histogram.

Immune Marker Quantification of the TME

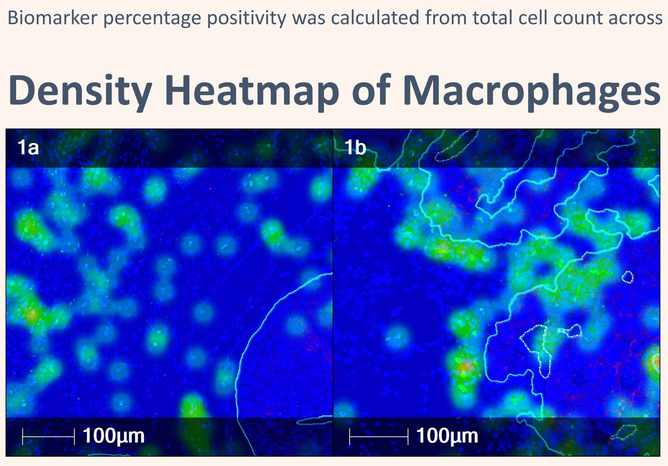


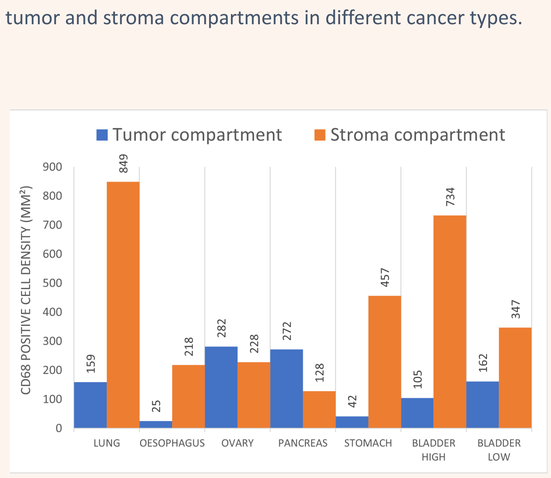
All channels are shown in low grade bladder urothelial carcinoma (1a) and ovarian adenocarcinoma (2a), tumor and stroma AI segmentation is shown in teal and magenta respectively (1b, 2b), Highplex FL colocalization shows positivity for 11 different biomarkers (1c, 2c).



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

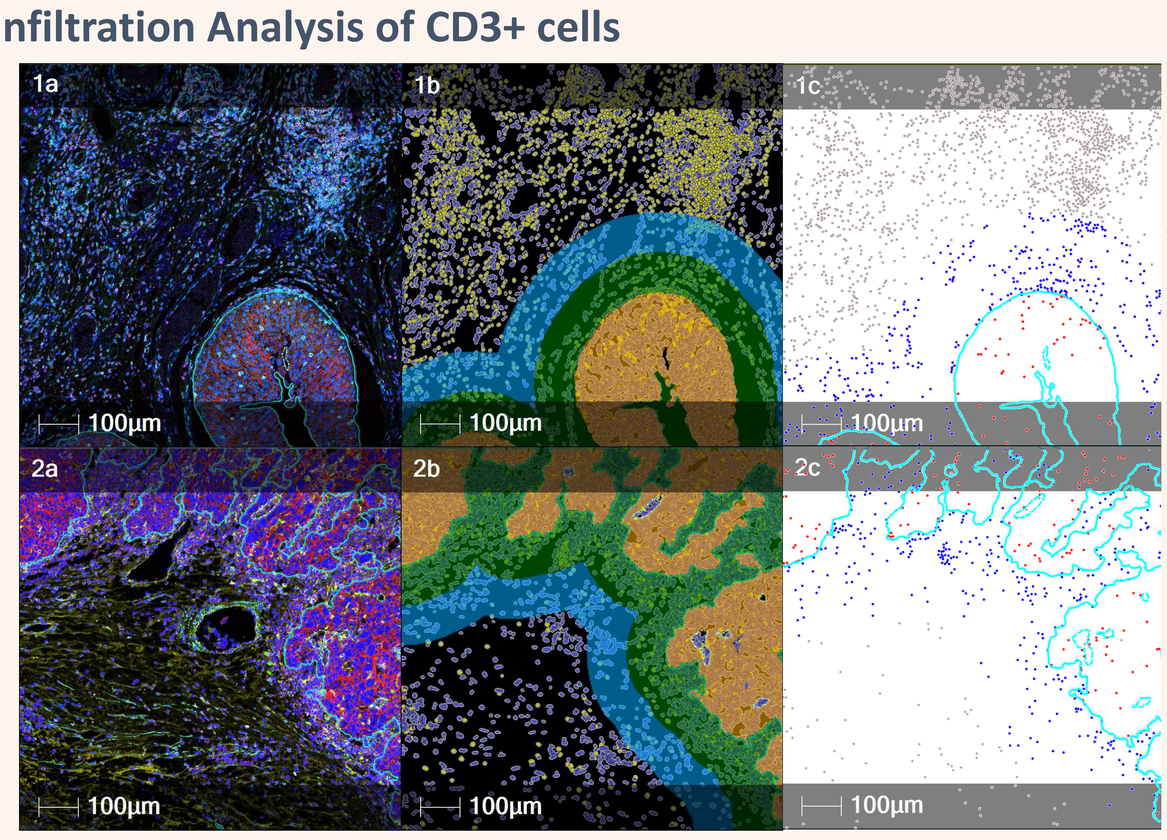
Density Heatmap of Macrophages



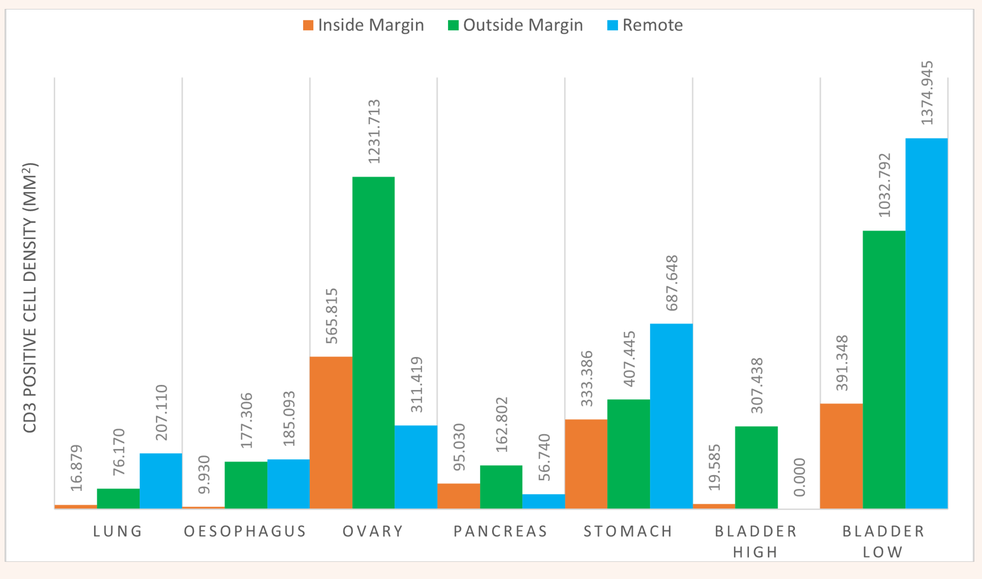


Using the cell data generated from Highplex FL with embedded AI networks, density heatmap analysis was performed within a 25 µm radius of every pixel within the image (1a,1b). Here we investigated the quantification and visualisation of CD68+ macrophages across multiple cancer types which can be used to evaluate prognosis and discover tumor associated macrophages. Hotspots of macrophages are localized to the stroma in low-grade bladder 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 bladder urothelial carcinoma (1a) and ovarian adenocarcinoma (2a) in teal annotations, generated by an AI tissue classifier. Highplex FL mark-up shows CD3 positivity in yellow, overlaid with the infiltration margin (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 orange, and 100µm and 200 µm outside the tumor boundary in green and blue 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, Zivanovic N, Jacobs A, Hattendorf B, 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).

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