A freely expandable 20-plex immuno-oncology antibody core panel for spatial biology experiments on human FFPE cancer sections: REAplex IO Core for MICS technology

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Introduction

Spatial biology is integral to understanding the interplay of immune cells, the tumor microenvironment (TME), and therapeutic responses in cancer research. To accelerate and standardize the assay development process, both community-sourced panels, such as OMAPs, or commercially available kits, such as the REAscreen Immuno-Oncology antibody kit, have been developed. Here, we introduce a pre-defined antibody panel for MACSima™ Imaging Cyclic Staining (MICS), staining 20 markers that provide key information about the TME, which is applicable to a wide range of FFPE samples. It can function as a stand-alone panel that allows the identification of the major tumor-infiltrating leukocytes with a focus on T-cells, and provides insight into the TME's architecture, neovascularization, and cellular states. It can also be combined and expanded with other antibodies, thereby functioning as a standardized base, that is then adopted to a research question. We demonstrate its performance and broad applicability, together with the analysis of the resulting datasets using the analysis templates for the MACS® iQ View analysis software that are part of the panel.

1 REAplex IO Core panel

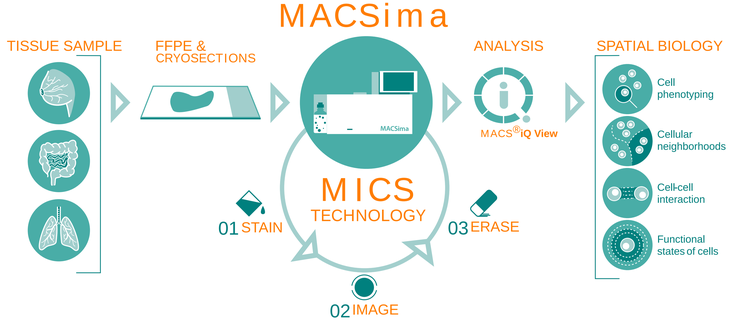
20 markers stained in this immuno-oncology panel for human FFPE sections and the rationale for staining these epitopes.



CELL TYPES		MARKERS
LYMPHOID	Immune cells	CD45
	T cells	CD3
	NK cells	CD56
	B cells	CD20
MYELOID	Dendritic cells	CD11c
	Macrophages	CD68
	M2-like macrophages	CD163
T CELL	Helper T cells	CD4
	Cytotoxic T cells	CD8a
	Regulatory T cells	FoxP3

TISSUE FEATURES	MARKERS
TUMOR CHARACTERIZATION	Cytokeratin 7
	Cytokeratin 20
	CD326 (EpCAM)
BLOOD VESSELS	CD31
TUMOR STROMA	Podoplanin
	Actin (Smooth muscle)
IMMUNOSUPPRESSION	CD274 (PD-L1)
	CD279 (PD1)
PROLIFERATION	Ki-67
APOPTOSIS	Cleaved PARP1

2 MACSima Imaging Cyclic Staining (MICS)

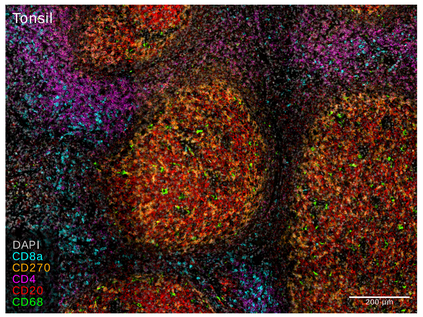


Cyclic immunostaining with MACSima Cyclic Staining (MICS) technology on the MACSima platform.

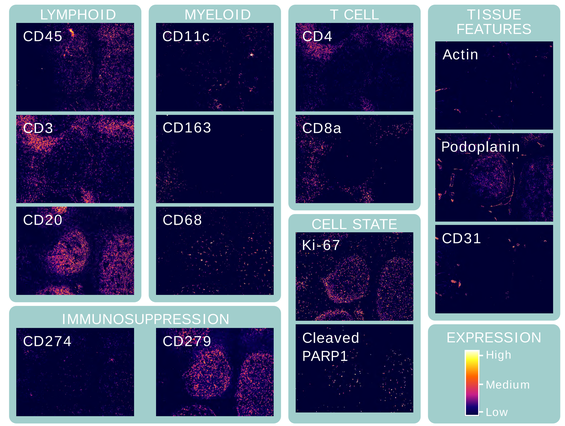
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3 Identification of key immune cell types & tissue structures

a) Multichannel immunofluorescence

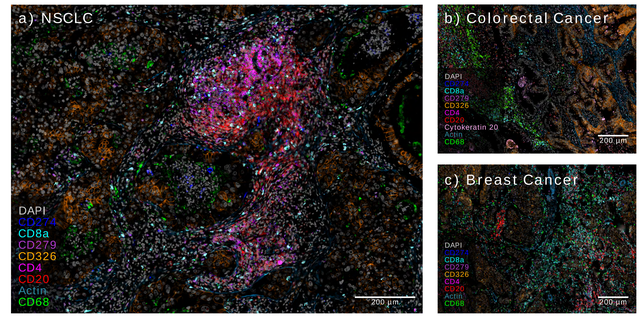


b) Individual channels



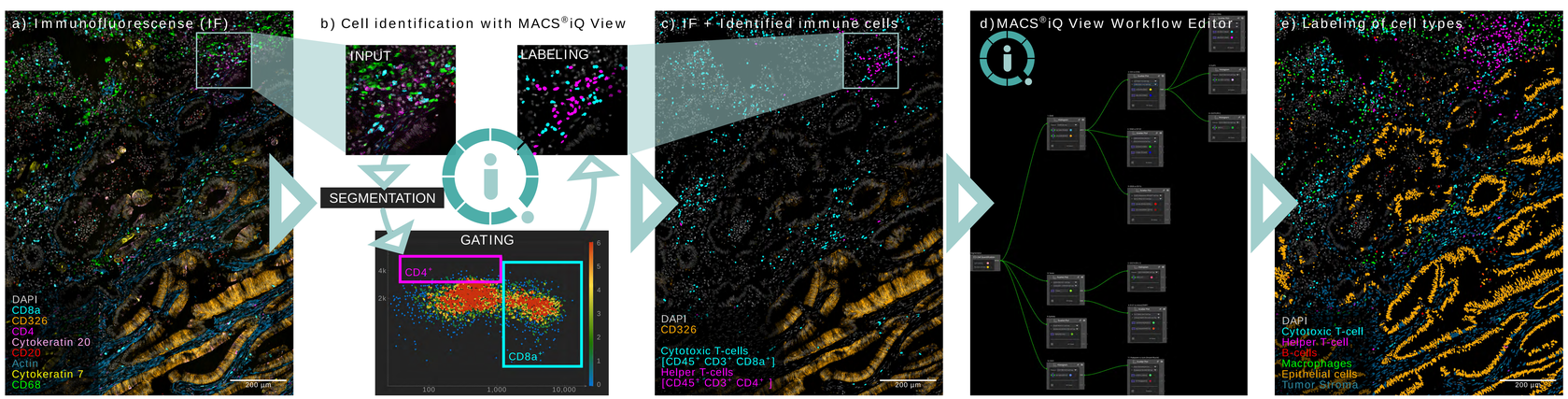
Whole ROI overview of a human palatine tonsil (FFPE) stained with the REAplex Immuno-oncology core panel. A germinal center is displayed in the center of the image, with additional germinal centers visible at the edges of the image. (a) Multichannel image with biomarkers as indicated. (b) Individual channels of the same region of interest, with intensity of the staining shown through a "thermal" look-up-table.

4 Universal applicability across cancer types



Application of the panel across multiple human tumor tissues. For all tissues shown, the pre-defined antibody panel, cycle arrangement, staining and imaging parameters supplied with the panel kit were used. (a) Image of lung tissue containing a non-small-cell lung carcinoma and a tertiary lymphoid structure in the upper middle of the image. (b) Colorectal adenocarcinoma with a prominent immune infiltrate on the left side of the image. (c) Breast tissue with a ductal adenocarcinoma.

5 Cell typing and characterization using MACS® iQ View Image analysis software



Workflow of data analysis to identify cells and tissue features using the MACS® iQ View Analysis Software with the REAplex Immuno-Oncology Core analysis template. (a) Image of colon tissue with an adenocarcinoma and immune cells. Multi-channel image showing part of the channels in the dataset / a subset of markers. After acquisition, images are pre-processed and then segmented. (b) Comparable to flow cytometry, cell subsets can be identified using scatterplots of cell markers. The exemplary scatterplot at the bottom shows CD4 vs CD8a expression of all CD45+ CD3+ cells in the dataset. The rectangular gates placed in the scatterplot show cytotoxic T-cells (cyan) and T-helper cells (magenta). (c) Cells selected with a gate are highlighted in the same color in the image to provide orientation in the dataset. (d) Overview of the analysis template provided with the kit. The leftmost square in this diagram represents the image segmentation, all other squares represent a plot (e.g. a scatterplot) to distinguish cells. A subpopulation identified within a gate in one node (i.e: CD3+ cells in the scatterplot CD56 vs CD3) can then be fed into the next plot (i.e. CD4 vs CD8a). These associations are shown with green lines. This identification via gating/thresholding was performed for all immune cell populations shown in the diagram. (e) Cell labelling: In this image, DAPI staining is shown in grey. The nuclei of identified cells were colored according to the population of cells they were grouped with during analysis.