3D spatial multiomics characterization of ovarian cancer tissue samples

Poster ID: 1225

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Introduction

High-grade serous ovarian cancer (HGSOC) exhibits pronounced spatial heterogeneity, where interactions between tumor, immune, and stromal compartments drive progression. Addressing this complexity demands integrating broad molecular profiling with detailed structural resolution. We present a novel workflow approach (Fig. 1) that utilizes MACSima™ Imaging Cyclic Staining (MICS), a cyclic staining strategy comprising iterative rounds of staining, imaging, and signal erasure. Within this framework, fast scanning using the High Speed Mode is employed for high-plex single-plane multiomic screening of thin sections to map the whole-tissue section landscape and identify key cellular niches via segmentation and clustering. These discoveries guide targeted volumetric analysis of sequential thick sections (30 µm) using High Contrast Mode for improved axial resolution. This approach enables flexible data evaluation through high-resolution Z-projections, plane-by-plane analysis, or full 3D reconstruction. By linking whole-tissue section discovery with targeted volumetric characterization, we achieve a robust representation of the 3D tumor architecture and the microenvironmental interactions driving HGSOC.

Methods and Results

1 Multiscale 3D spatial multiomics: a strategy for mapping ovarian cancer heterogeneity

Figure 1

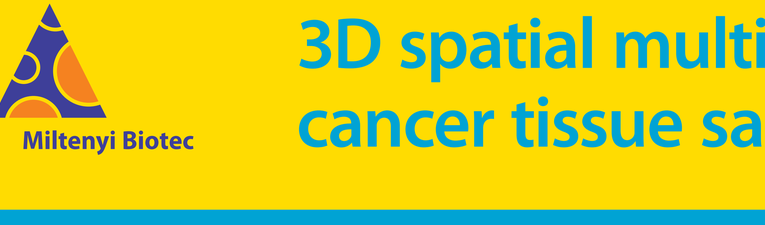
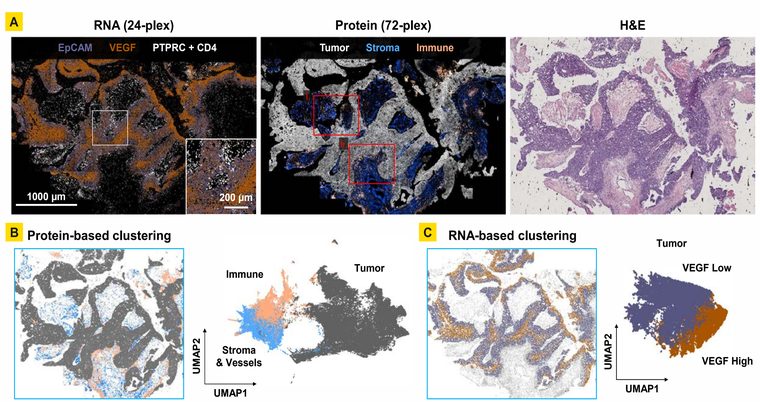


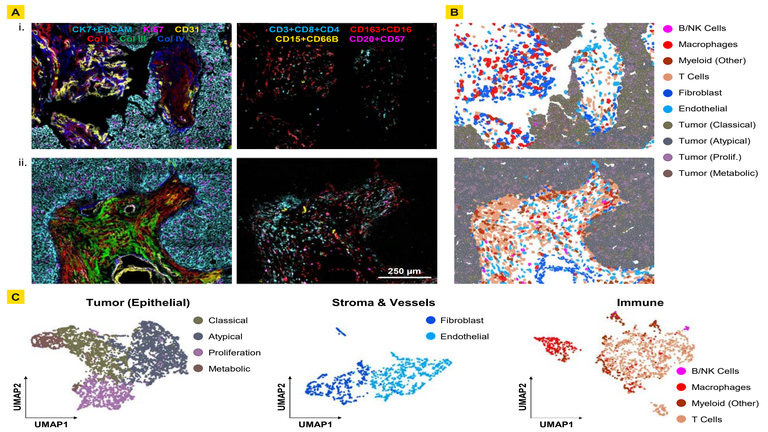
Figure 2



Whole-tissue section mapping (Fig. 2) captures the molecular and structural organization of the HGSOC landscape. High Speed Mode enables rapid, large-area acquisition, supporting RNAsky®-based high-plex transcriptomics to identify spatial gene expression patterns, and REAfinity® and REAlease™ based high-plex proteomics to delineate major cellular compartments.

2 2D profiling in distinct regions highlights intra-tumor diversity and identifies areas for targeted 3D analysis

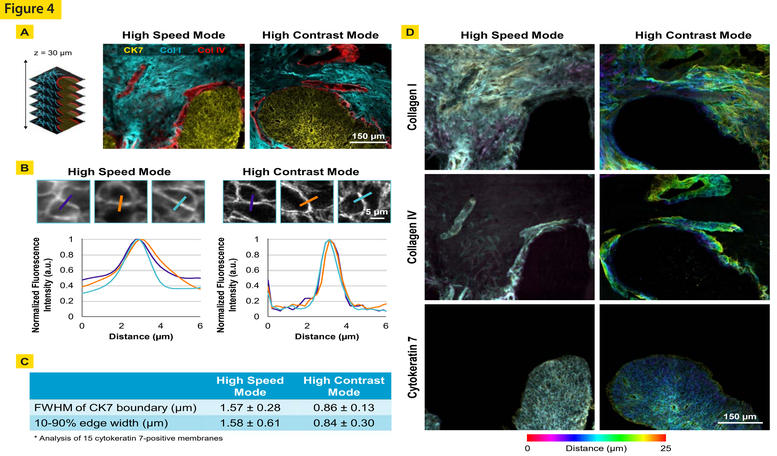
Figure 3



Multiplexed imaging of regions I and II in Fig. 2A resolves distinct architectural neighborhoods (Fig. 3). UMAP projections of the combined ROIs further delineate phenotypic diversity within major populations, providing the high-resolution characterization required to select strategic areas for subsequent 3D analysis. This workflow bridges whole-tissue section mapping with local interactions defining the HGSOC landscape.

3 High Contrast Mode imaging improves axial resolution

Figure 4



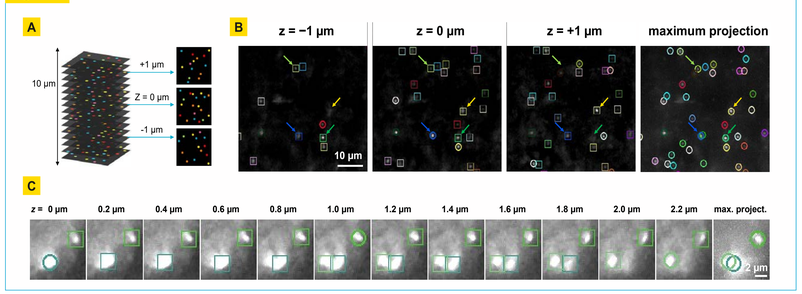
	High Speed Mode	High Contrast Mode
FWHM of CK7 boundary (µm)	1.57 ± 0.28	0.86 ± 0.13
10-90% edge width (µm)	1.58 ± 0.61	0.84 ± 0.30

* Analysis of 15 cytokeratin 7-positive membranes

3D acquisition of 30 µm stacks (Fig. 4, 6) provides high-resolution insights into tissue micro-architecture. High Contrast Mode resolves fine structural details, such as CK7 membrane boundaries, with sub-micron precision. Quantitative line-profile analysis confirms that High Contrast Mode significantly improves resolution (FWHM) and edge-width metrics within thick sections. Depth-coded reconstructions further visualize the spatial organization of epithelial and extracellular matrix components.

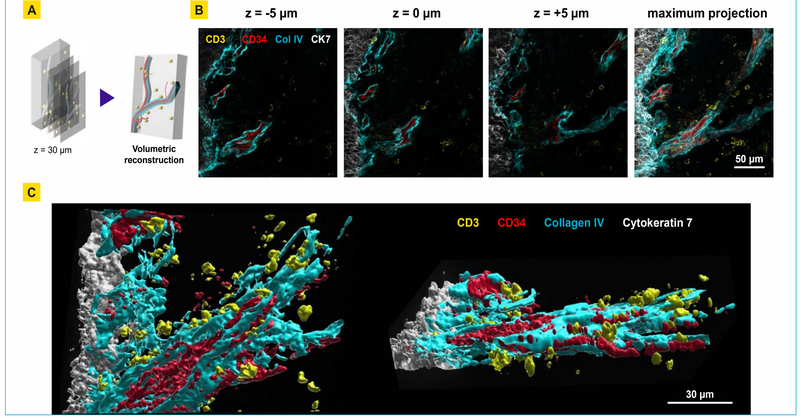
4 Volumetric analysis recovers biological information hidden in single-plane acquisitions

Figure 5



Multiplane acquisition and 3D analysis (Fig. 5) capture RNA rolonies often missed in 2D. This approach resolves overlapping signals in close XY proximity and detects features outside a single focal plane, ensuring a more comprehensive and accurate dataset.

Figure 6



Conclusion & Outlook

This study established a multiscale spatial workflow that linked whole-tissue section 2D multiomic mapping with targeted 3D volumetric analysis in HGSOC. High-plex 2D screening enabled the identification of spatially distinct microenvironmental niches, guiding the selection of regions for high-resolution 3D interrogation. Extending analysis into the z-dimension improved delineation of tumor–stroma interfaces and preserved spatial transcript information that is partially lost in single-plane projections, providing a more accurate representation of cellular organization within the tumor micro-environment. By focusing volumetric analysis on selected regions of interest, this approach remained scalable while increasing structural and molecular resolution (Fig. 6). This framework provided a foundation for future studies linking 3D tissue architecture with therapeutic response and resistance in ovarian cancer.

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