# Enabling single-cell multiomics to unravel complex data from human lung cancer tissue preserved in a novel long-term storage buffer

Poster# 596

**Cynthia Sakofsky**[1], Manish Thakran[1], Samuel Shi[2], Hye-Won Song[2], Tamiyo Kobayashi[3], Aruna Ayer[1]

[1]BD Biosciences, 155 N. McCarthy Blvd, CA 95035, [2]BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121, [3]BD Biosciences, National Cancer Hospital East Division of Cancer Immunology, Kashiwanoha Chiba, Japan

## Abstract

**Background:** Tumor tissues are inherently heterogeneous, composed of diverse immune and non-immune cell types that shape the tumor microenvironment (TME). High-resolution single-cell profiling is essential for understanding disease mechanisms, identifying biomarkers, and guiding targeted therapies. However, maintaining cellular integrity from collection through analysis remains a major challenge due to necrosis, degradation, and state-altering stress.

**Challenge:** Conventional preservation methods often rely on cross-linking fixatives, which can damage nucleic acids and proteins, ultimately compromising single-cell multiomic data quality.

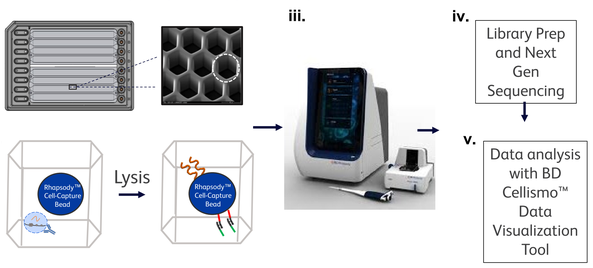
**Approach:** This study highlights a novel long-term cryopreservation buffer free of cross-linking agents, evaluated using dissociated human lung cancer cells. Tumor tissue was collected in BD® OMICS-Guard, held for 24 h at 4 °C, then processed into single cells. Cells were immediately frozen in the new cryopreservation buffer. Frozen samples were shipped for single-cell multiomic analysis profiling mRNA and surface proteins.

**Results:** Distinct cellular clusters were identified through unsupervised clustering, encompassing tumor-infiltrating lymphocytes (TILs), various immune populations, and non-immune tumor cells. Differential gene and protein expression revealed functionally distinct macrophage subpopulations. T-cell activation, exhaustion, and naïve gene signatures highlighted functional heterogeneity among TILs.

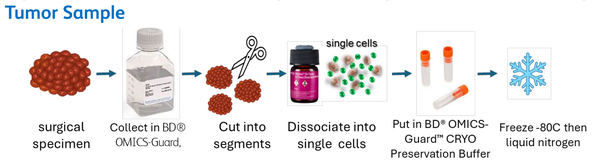
**Conclusion:** The novel cryopreservation buffer enables flexible sample handling, extended processing windows, and high-quality single-cell multiomic data. This approach offers a robust alternative to fixative-based preservation and supports reliable profiling of complex tumor microenvironments.

## Approach

### Figure 1A. BD Rhapsody™ Single Cell System

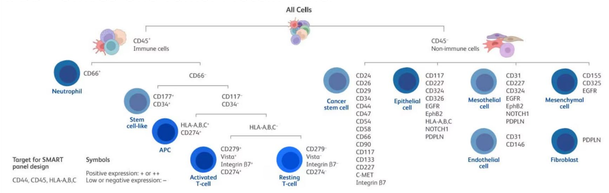



### Figure 1B. Workflow for collecting and freezing cells from Human Lung Tumor Sample




### Figure 1C. Lyophilized Oligo-tagged Antibody Panels for Cite-Seq

**i. BD® OMICS-One Tumor Protein Panel**



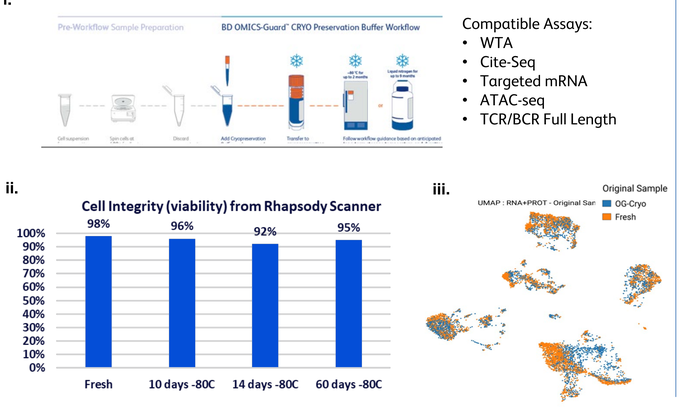

**ii. BD® OMICS-One Immune Profiler Protein Panel**

| Specificity | Clone | Specificity | Clone | Specificity |
|---|---|---|---|---|
| **CD3** | UCHT1 | **CD45RA** | HI100 | **CD196 (CCR6)** |
| **CD4** | SK3 | **CD56** | NCAM16 | **CD197 (CCR7)** |
| **CD8** | SK1 | **CD62L** | DREG-56 | **CD272** |
| **CD11c** | B-Ly6 | **CD127** | HIL-7R-M21 | **CD278** |
| **CD14** | MΦP9 | **CD134** | ACT35 | **CD279** |
| **CD19** | 3G8 | **CD137** | 4B4-1 | **CD279** |
| **CD25** | SJ25C1 | **CD161** | HP-3G10 | **CD357 (GITR)** |
| **CD27** | 2A3 | **CD183 (CXCR3)** | 1C6/CXCR3 | **CD366 (TIM-3)** |
| **CD27** | M-T271 | **CD185 (CXCR5)** | RF8B2 | HLA-DR |
| **CD28** | L293 | **CD186 (CXCR6)** | RF8B2 | IgD |
| **CD28** | L293 | **CD186 (CXCR6)** | 13B 1E5 | IA6-2 |

**Figure 1.** (A) The BD Rhapsody™ Single Cell System enables single-cell multiomic assays by partitioning cells into microwells where they settle gently by gravity. Samples were processed for single-cell whole-transcriptome RNA sequencing and protein analysis (CITE-seq). Cells were paired with barcoded magnetic beads, lysed, and mRNA and protein targets were captured for cDNA synthesis and library preparation prior to sequencing. (B) Workflow for collecting, processing, and cryopreserving cells from bulk tissue using BD® OMICS-Guard™ CRYO Preservation Buffer. Human lung tumor samples were frozen. After thawing, cells were prepared for downstream analysis and stained with oligo-tagged antibodies as shown in (C). (C) Cells were stained using two BD® OMICS-One Protein Panels, which are pre-formulated, lyophilized antibody panels designed for streamlined protein analysis

## Introduction to novel cryopreservation buffer BD® OMICS-Guard™ CRYO Preservation Buffer

### Figure 2A. PBMCs




Compatible Assays:
- WTA
- Cite-Seq
- Targeted mRNA
- ATAC-seq
- TCR/BCR Full Length

### Figure 2B. Whole Blood

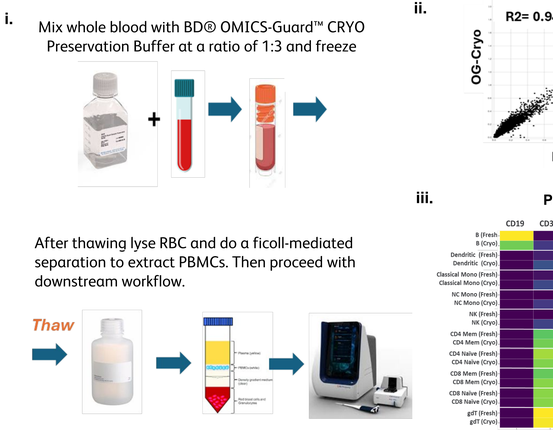



**Figure 2.** The BD® OMICS-Guard™ CRYO Preservation Buffer workflow is simple and compatible with multiple multiomic assays. The buffer supports cryopreservation of single-cell suspensions (A) and whole blood for long-term storage (B). Recommended storage conditions are up to 3 months at –80 °C or up to 9 months in liquid nitrogen. Comparison of frozen and fresh samples demonstrates high cell integrity, minimal batch effects, and preservation of mRNA expression, cell surface protein epitopes, and cell type distribution following cryopreservation.

## Cryopreserved human lung tumor–derived cells generate high-quality single-cell sequencing data

### Figure 3. Sequencing and Data Quality

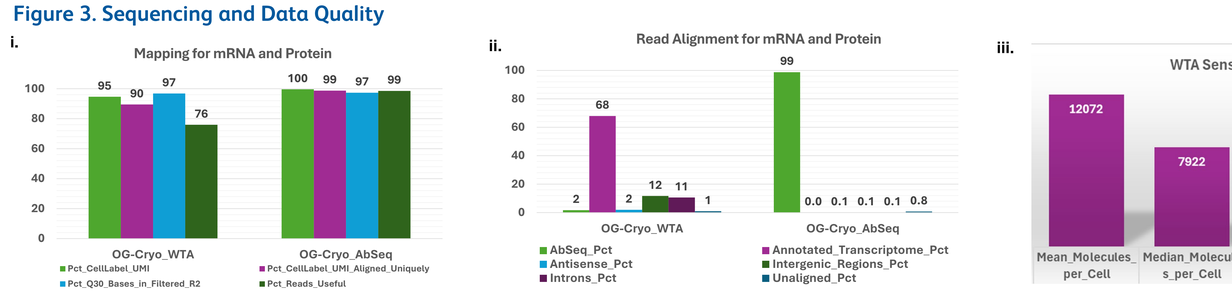
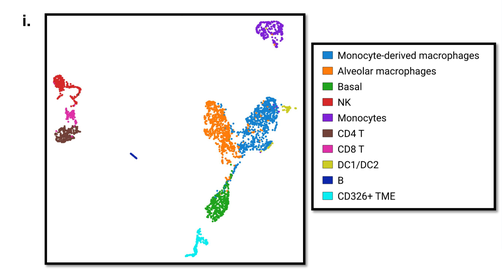


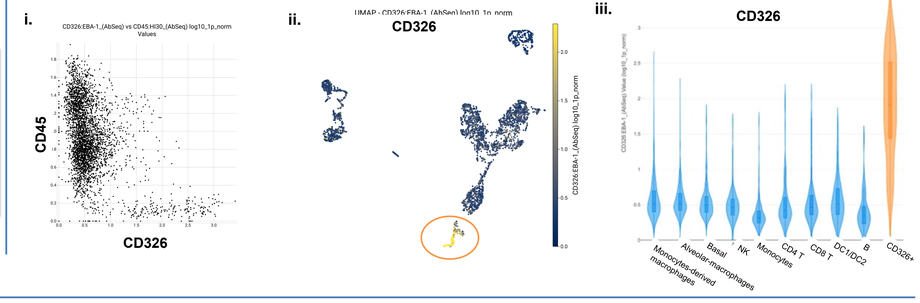

**Figure 3**. Cells dissociated from human lung cancer tissue were cryopreserved in BD® OMICS-Guard™ CRYO Preservation Buffer and analyzed by whole-transcriptome and cell surface protein profiling (CITE-seq). (i) Sequencing read quality and mapping efficiency for mRNA and protein was high. (ii) Alignment metrics were as expected and similar to what is typical of a fresh sample. (iii) WTA assay was sensitive with robust molecule recovery and gene detection. (iv) Reads assigned to putative cells indicates intact and well-preserved cell inputs.

## Cell clustering and annotations show successful preservation of TILs and tumor cells

### Figure 4A. UMAP and Cell Annotations




### Figure 4B. Identification of tumor cells




### Figure 4C. Protein profiling of CD326+ cells

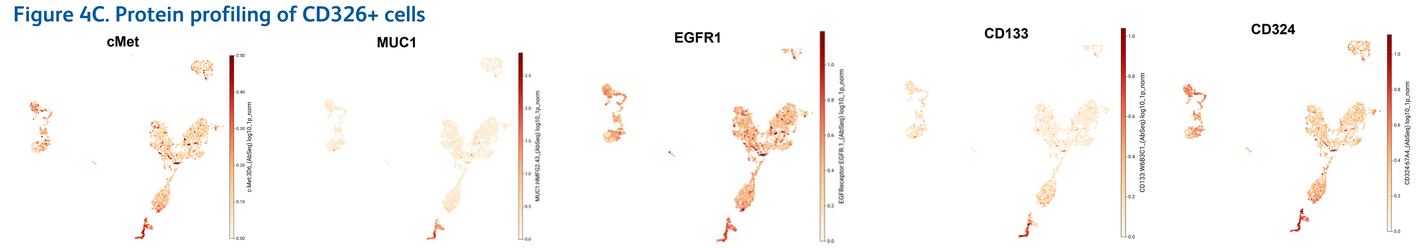



**Figure 4.** (A) Joint mRNA and protein-driven UMAP revealed well-defined cell clusters, including tumor-infiltrating lymphocytes and CD326+ cells. (B) Protein profiling showed increased CD326 expression in non-immune cell populations. (C) Expression of key protein markers within the CD326+ population enabled classification of the lung tumor as non–small cell lung cancer.

## Cite-seq revealed sub-populations of macrophages

### Figure 5. Identification of macrophage cell subtypes

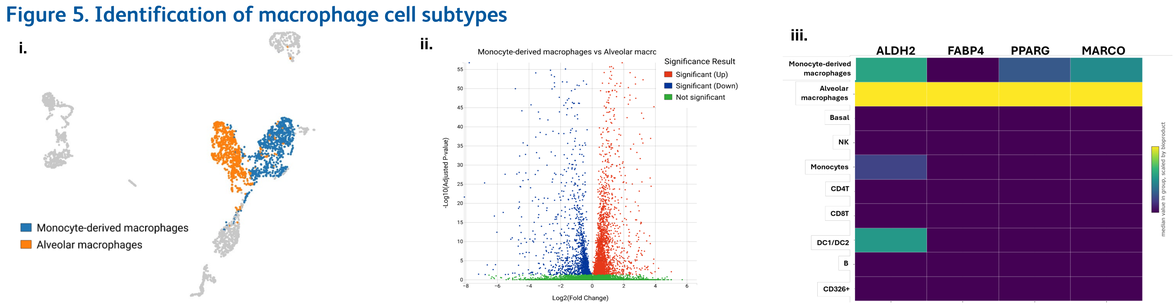



**Figure 5.** Figure 5. (i) Joint mRNA and protein profiling identified two distinct macrophage subpopulations. (ii) Volcano plots illustrate differentially expressed genes between the two macrophage populations using the BD Cellismo™ Data Visualization tool. (iii) Expression of key mRNA and protein markers was enriched in the alveolar macrophage population, supporting population-specific characterization.

## Conclusions

- Cryopreserved lung tumor–derived cells generated sequencing and protein profiling metrics that demonstrated preservation of RNA quality, protein epitopes, and cell-type composition.
- Joint mRNA and protein profiling supported robust cell clustering and cell annotation, including identification of tumor-infiltrating lymphocytes, epithelial tumor cells, and macrophage subpopulations.
- Multiomic analysis enabled discrimination of immune and non-immune cells, with protein marker expression supporting tumor classification.
- These results demonstrate that cryopreservation using BD® OMICS-Guard™ CRYO Preservation Buffer can preserve key biological features to support high-quality single-cell multiomic analysis of complex clinical samples.