

## Abstract

Image-based cell phenotyping has emerged as a powerful approach for extracting morphological insights from cells. This study introduces a deep learning framework that leverages multi-channel label-free images generated by BD CellView™ Image Technology for cell phenotyping. By integrating brightfield, side scatter, and forward scatter images, the framework captures detailed morphological features. Demonstrated applications include singlet discrimination, cell viability assessment, whole blood cell classification, and activated T cell determination, offering a versatile solution for cellular analysis. The model's performance is evaluated using standard classification metrics such as precision, recall, and F1-score, showcasing its robustness and accuracy across diverse applications. Supported by BD FACSDiscover™ instruments, this framework offers three key advantages: (1) high-throughput capability for rapid data generation; (2) spectral functionality for simultaneous ground-truth label generation within a single experiment; and (3) a multi-channel label-free imaging setup that enhances morphological detail, providing comprehensive insights into cellular characteristics. This study highlights the potential of integrating deep learning with advanced multi-channel imaging modalities of BD CellView™ to streamline and enhance phenotypic analysis

## Methods

### 1. Viability Assay:

- Reagents:** Staurosporine, etoposide, and camptothecin were used to induce apoptosis. Ground truth was measured with Annexin V (APC Annexin V and PE-Cy7 Annexin V)
- Cell Lines:** HT29, HeLa, Jurkat, OVCAR3, RPMI-8226, Raji, U226, HCT116, HEL, and A549 were used to train classification heads for various cell sizes. PBMCs were isolated from fresh healthy donor whole blood using SepMate-50 and Ficoll density gradient medium.
- Analysis:** Multiparameter flow cytometry data with images were collected on BD FACSDiscover™ S8 and A8 instruments, and analyzed in FlowJo™ Software.

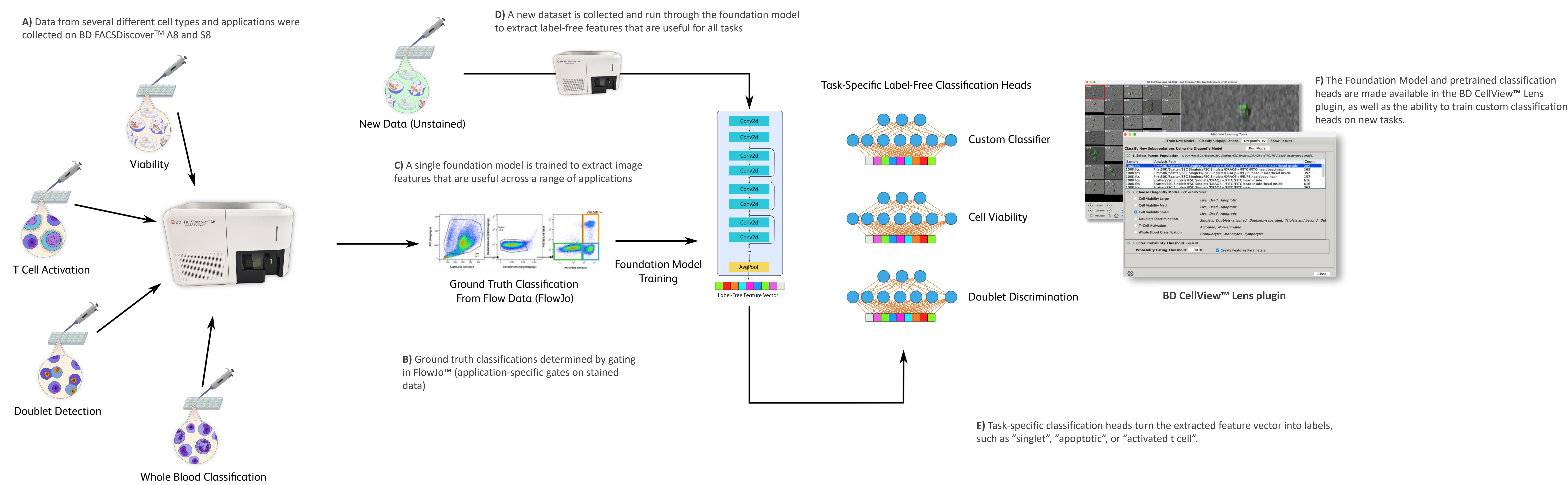
### 2. Doublet Discrimination:

- Reagents:** Propidium Iodide was used to stain DNA for ground truth cell counts.
- Cells:** PBMCs isolated from healthy donors as above.
- Analysis:** PI staining used to separate 2N singlets from 4N, 6N doublets and multiplets. Attached doublets were manually identified by inspection of the images.

### 3. Model Training:

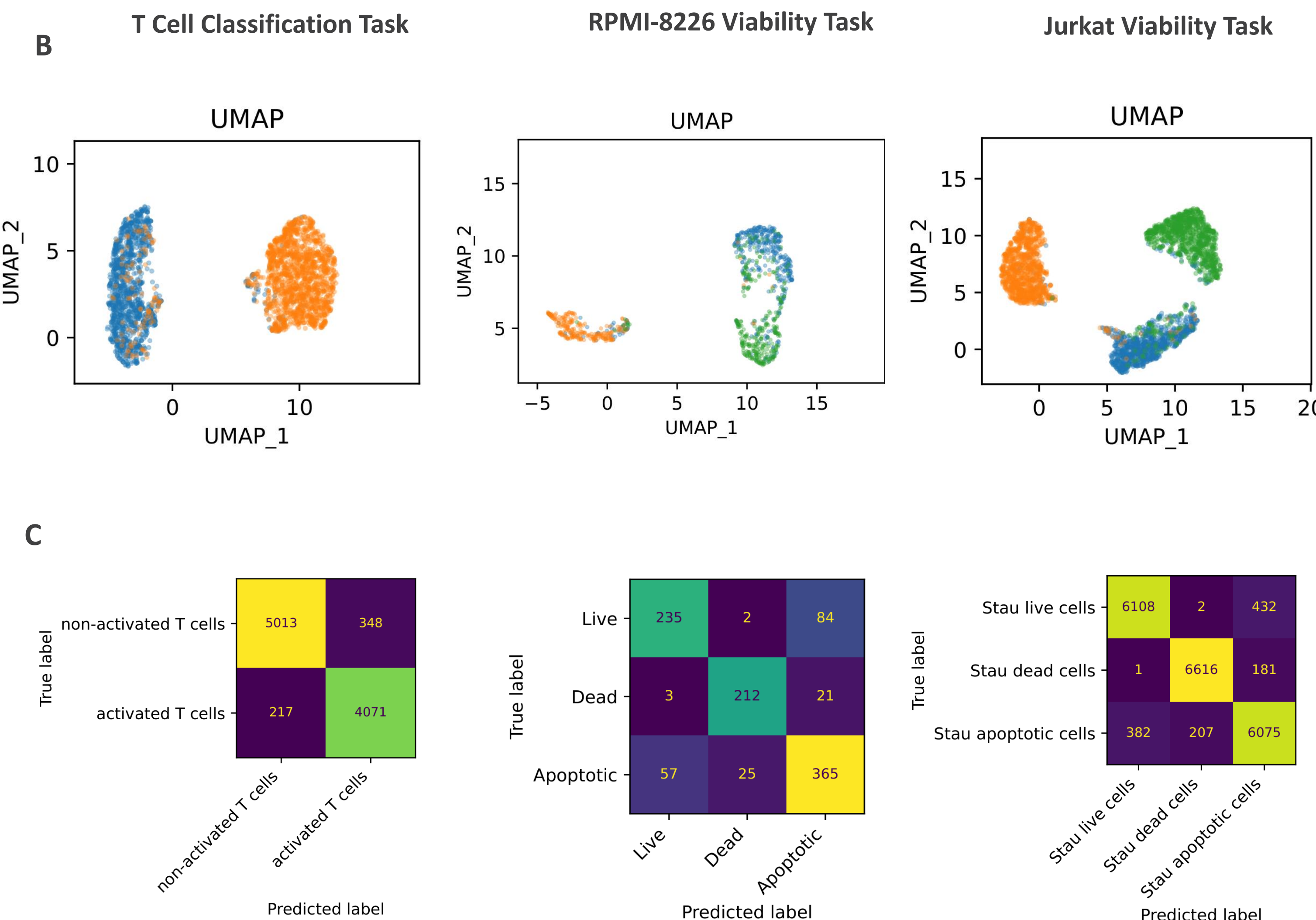
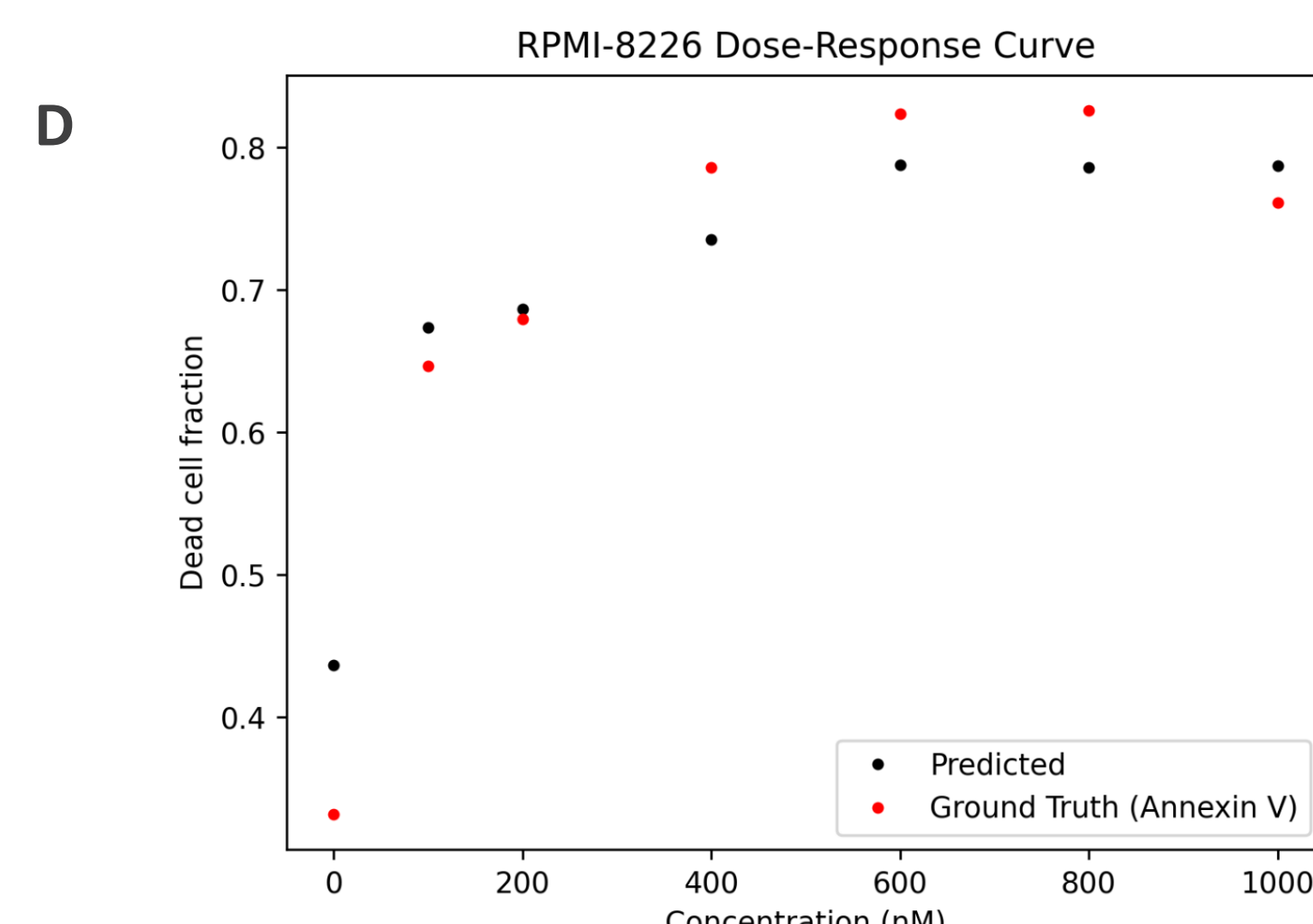
- Foundation model trained based on pretrained ResNet34 ImageNet1K\_V1 weights.
- Classification heads are simple single-layer perceptrons.
- Models were trained with cross-entropy loss on all tasks simultaneously

## Workflow



## Results

APPLICATION	CLASS	PRECISION	SENSITIVITY	F1-SCORE
SINGLET DISCRIMINATION (PBMC)	Singlets	1.00	1.00	1.00
	Doublets Attached	0.95	0.93	0.94
	Doublets Separated	0.98	0.97	0.97
	Triples and Beyond	0.85	0.89	0.87
VIABILITY (JURKAT CELLS)	Live	0.96	0.93	0.94
	Dead	0.96	0.97	0.97
	Apoptotic	0.88	0.92	0.90
WHOLE BLOOD CELL CLASSIFICATION	Granulocyte	1.00	1.00	1.00
	Monocyte	0.95	0.96	0.96
	Lymphocyte	0.99	0.99	0.99
T-CELL ACTIVATION	Non-activated T-cell	0.93	0.94	0.94
	Activated T-cell	0.93	0.91	0.92



- Summary of performance of the foundation model across a variety of datasets and application types. Performance varies between tasks but is acceptable for most classes.
- Dimensionality reduction on the feature extracted by the foundation model shows that interesting classes separate well in the extracted feature space, regardless of cell type or class of interest. Columns show activated T cell classification, RPMI-8226 cell viability, and Jurkat viability, respectively.
- Shows confusion matrices for each task.
- The model was used to calculate dead cell fraction as a function of Camptothecin concentration in a killing assay, showing agreement between predicted dead cell fraction and ground truth Annexin V staining.

## Conclusions

- We trained a generalist cell foundation model capable of performing multiple cell classification tasks simultaneously.
- We show that the viability classifier can classify cells of different cell types, collected on different instruments, with different treatment conditions.
- Using dimensionality reduction, we see that features extracted by the foundation model naturally separate classes of interest, enabling a simple linear classifier to perform the classification.
- The workflow, including the foundation model and pre-trained classification heads will soon be available on the FlowJo™ Plugin Exchange portal, as part of an update to BD CellView™ Lens.