

Abstract

Apoptosis is a form of programmed cell death and critical for embryonic development and tissue homeostasis. Dysregulation of apoptosis is associated with various diseases such as oncogenesis and autoimmune disorders; therefore, understanding the mechanism of apoptosis is critical and may promote development of new therapeutics for treatment of diseases. One of the common methods to identify apoptotic cells is to stain them with live/dead, fluorescent apoptotic markers and measure the fluorescence intensity using flow cytometry. Despite wide usage, this method requires cell staining with fluorescent dyes and antibodies which may disturb the functional status of cells and even prevent users from certain downstream applications. Knowing this caveat, we designed a machine-learning algorithm based on label-free images from imaging flow cytometry to identify apoptotic cells. Using a cellular apoptotic model, we captured label-free images in a heterogeneous sample using high-throughput imaging flow cytometry. A novel deep learning algorithm was applied to differentiate the functional status of cells including live, dead and apoptotic cells. Our deep learning algorithm achieved an average F1-score of >90% in comparison to the ground truth labeling. In unsupervised clustering, live, apoptotic and dead cell populations were clearly distinguishable from each other using deep learning-derived imaging features. The label-free identification of live, apoptotic and dead cells by deep learning and imaging flow cytometry provided an accurate, objective and efficient way to define cell functional status. It also raised the possibility of using deep learning methods to differentiate and even isolate cells based on their unique morphology in a label-free manner.

Methods

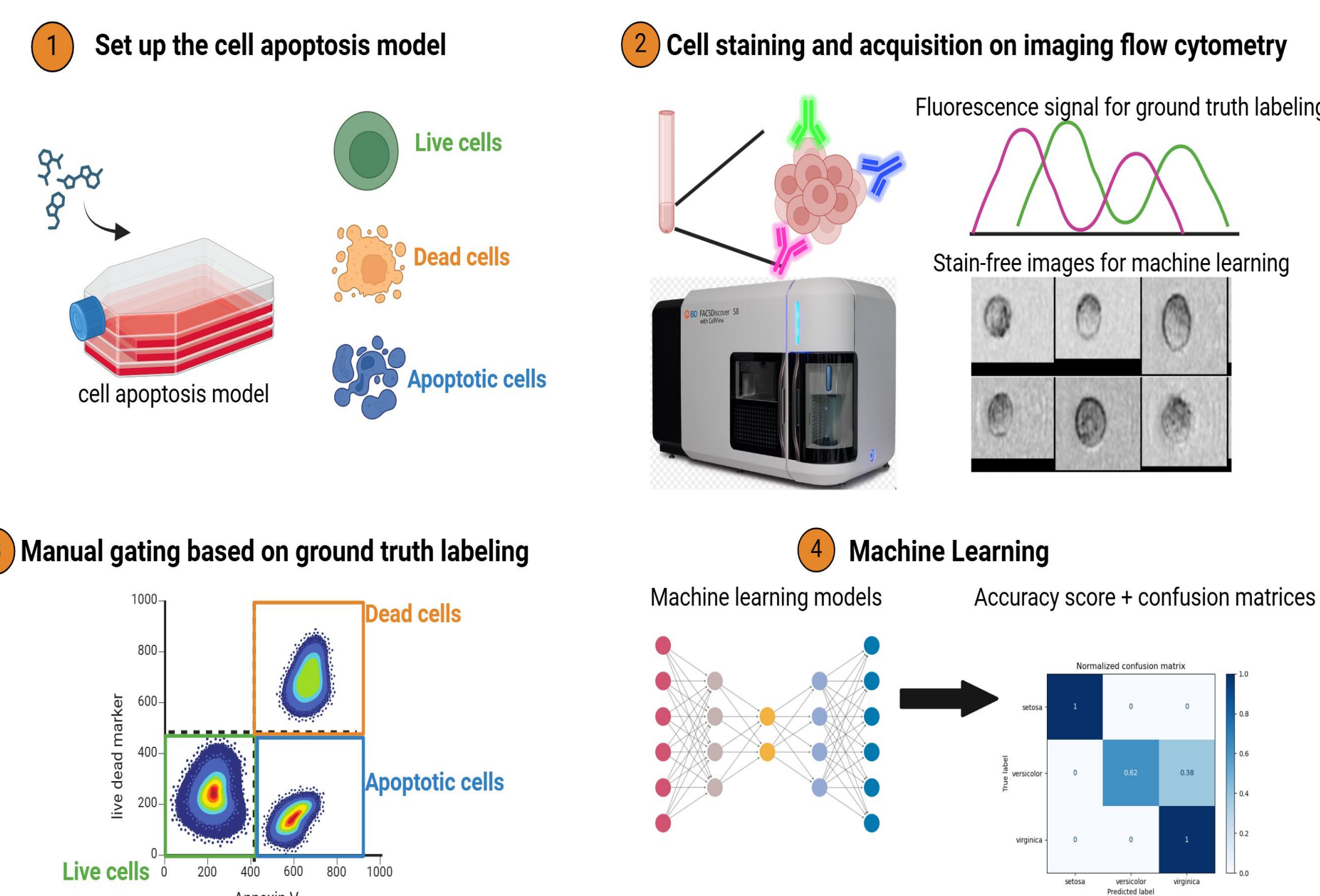


Figure 1. Workflow of label-free cell classification using the deep learning algorithm. (Step 1) Cell apoptosis was induced upon drug treatment. (Step 2) Cell samples containing a heterogeneous mixture of live, dead and apoptotic cells were harvested and stained with the viability and apoptotic marker. Samples were run using the BD FACSDiscover™ S8 Cell Sorter to record both fluorescence signatures and images of all singlets. (Step 3) The fluorescence signal was used to generate the ground truth labeling. (Step 4) Image and fluorescence features were extracted for machine learning training using a deep learning algorithm. During the process, cell classification based on the deep learning algorithm was evaluated against the cell identification classified by the ground truth labeling. Accuracy score and confusion matrices were reported.

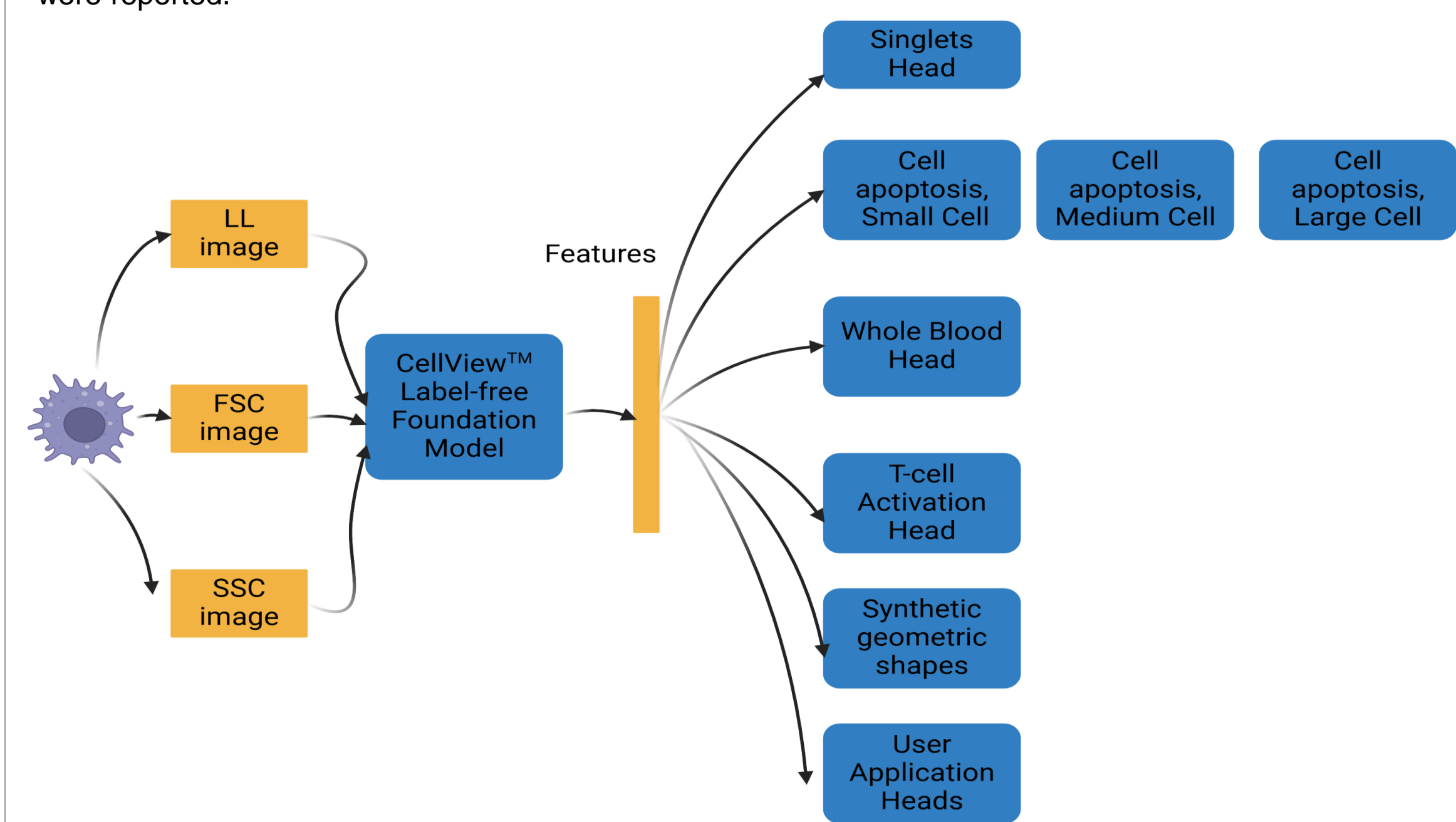
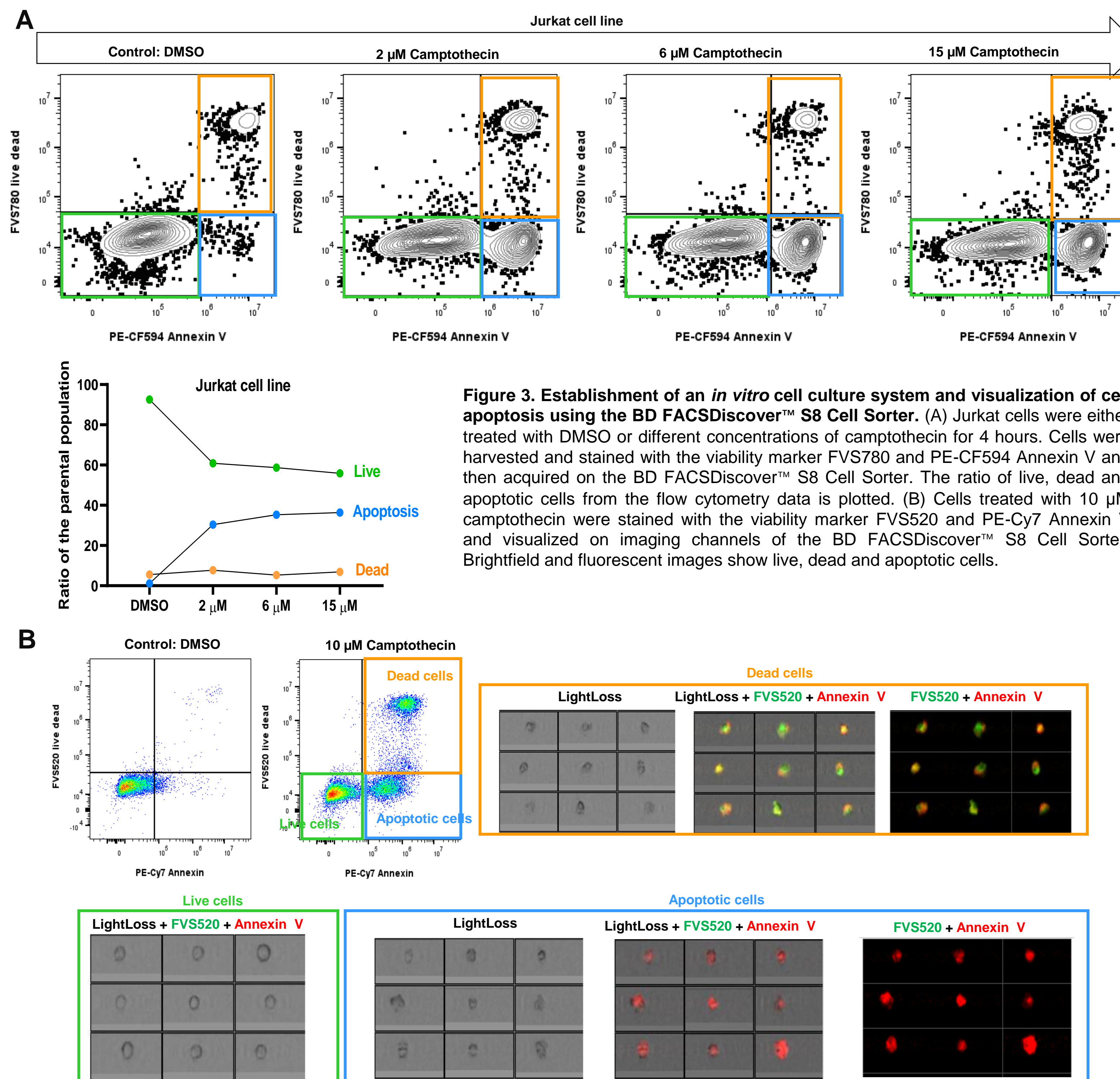
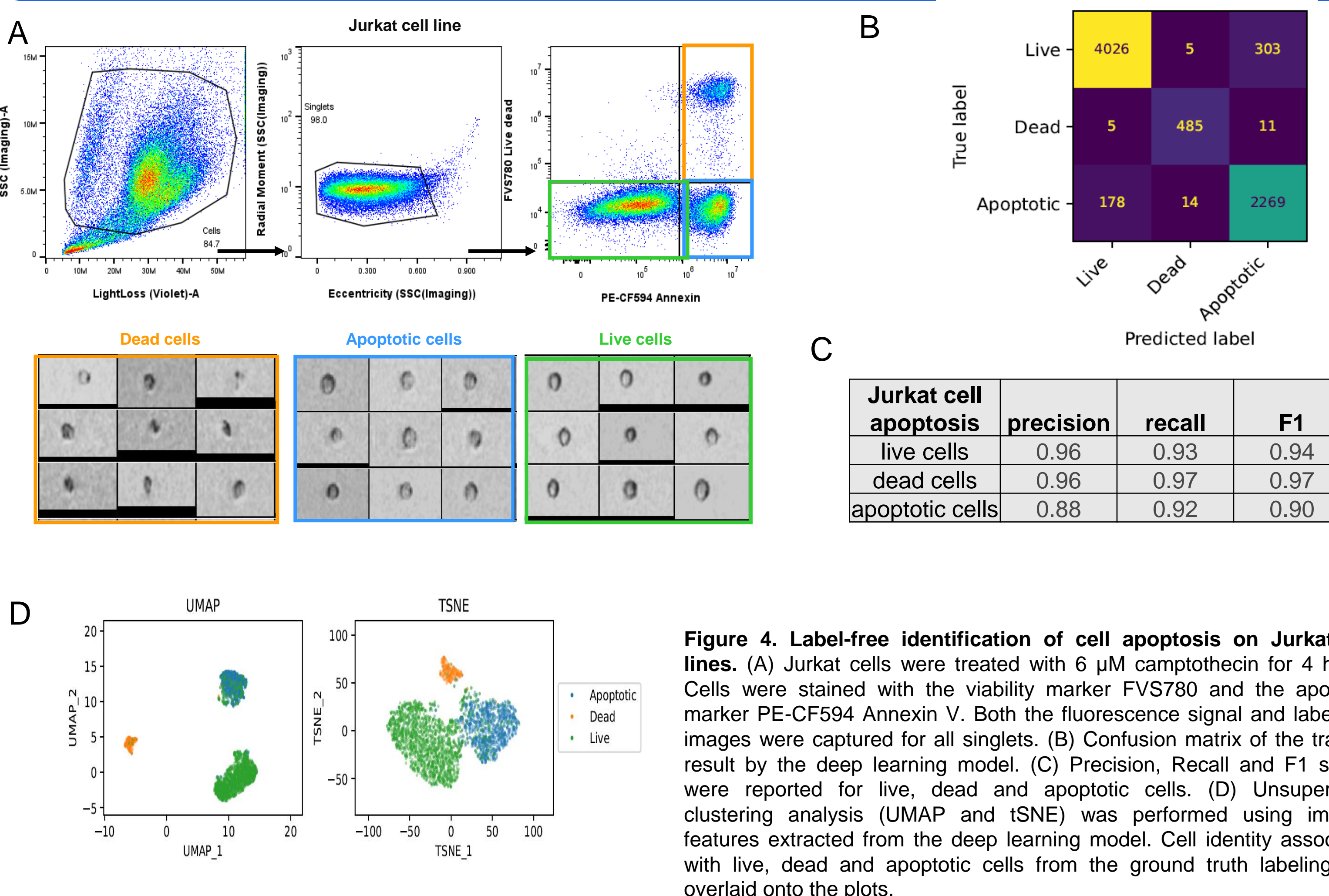


Figure 2. Development of the CellView™ Foundation Model to classify live, dead and apoptotic cells. The BD FACSDiscover™ S8 Cell Sorter produces a BD CellView™ waveform data file (CVW), containing waveforms recorded for each event in each imaging channel. These waveforms encode the spatial information of the images, which can be processed to obtain the image stack. The stack includes three scatter images: Axial Light Loss (ALL), Forward Scatter (FSC) and Side Scatter (SSC). The model consists of a feature extraction network and multiple classification heads. The feature extraction network, based on a modified version of ResNet34, processes the information from the image stack. Different biological datasets were incorporated to train the Foundation Model including the cell apoptosis datasets on both Jurkat cells (medium cell size) and PBMCs (small cell size).

Establishment of a cell apoptosis model



Label-free identification of Jurkat cell apoptosis using deep learning



Label-free identification of cell apoptosis of PBMCs using deep learning

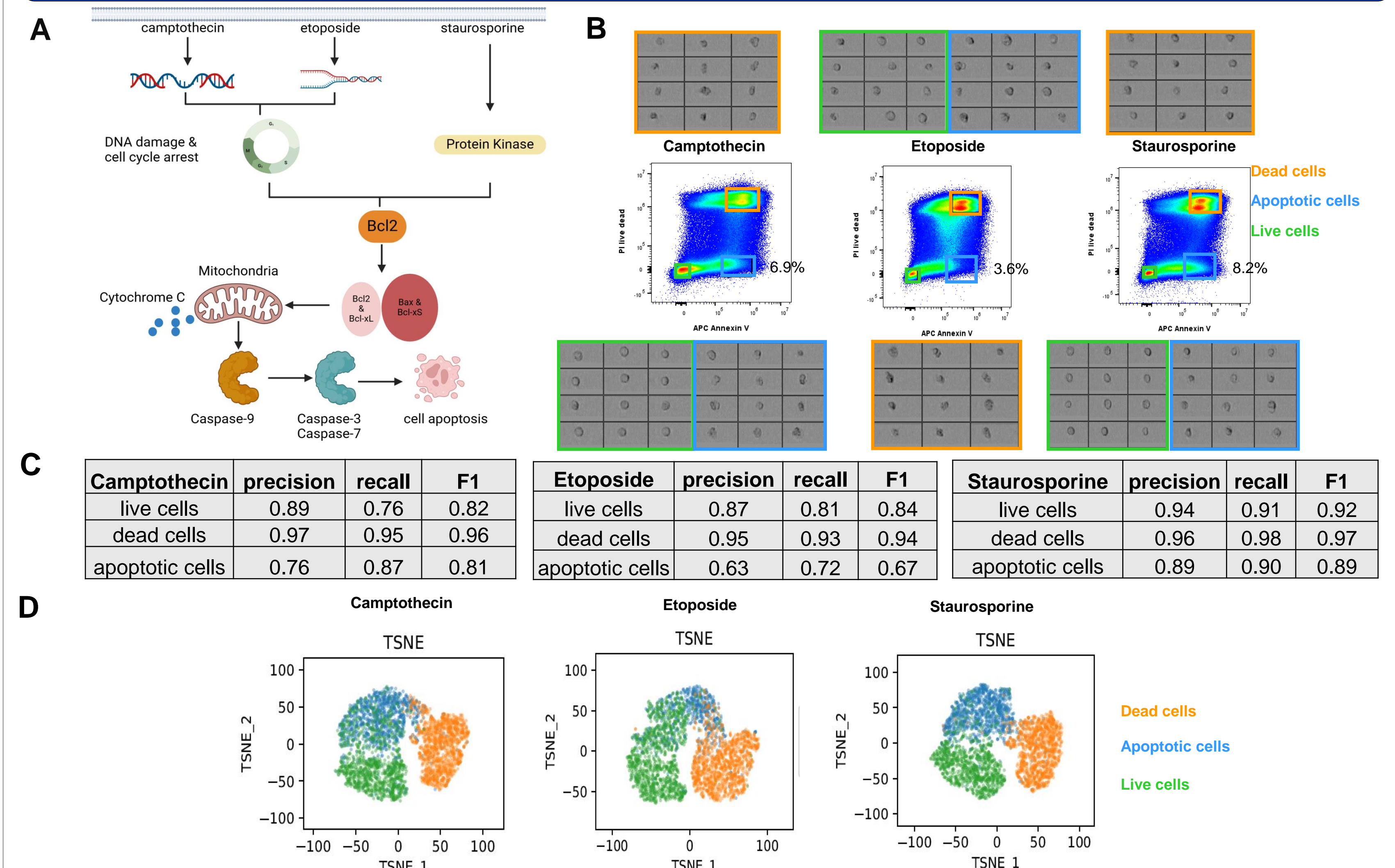
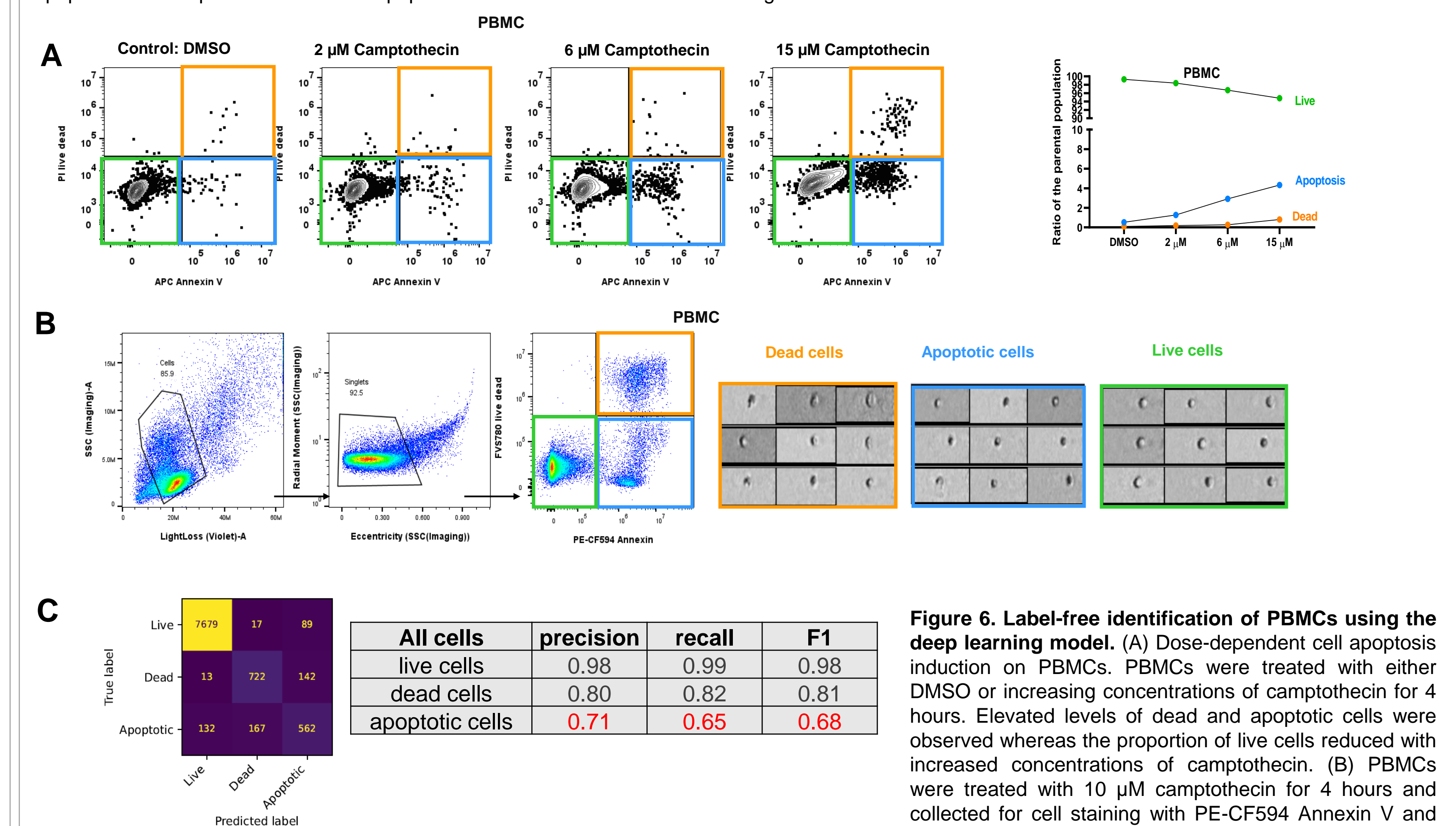


Figure 5. Label-free identification of Jurkat cell apoptosis induced by different drugs. (A) Mechanism of cell apoptosis induction by different drugs. Camptothecin and etoposide promote cell cycle arrest and DNA damage by inhibiting topoisomerases. On the other hand, staurosporine induces DNA damage by blocking protein kinases. In response to DNA damage, the Bcl-2 family proteins modulate the function of mitochondria. Cytochrome C escapes from the damaged mitochondria and activates the protein complex apoptosome, which cleaves pro-caspase-9, triggers the cascade of caspases and eventually induces cell apoptosis. (B) Jurkat cells were treated with 10 μM camptothecin, 10 μM etoposide or 1 μM staurosporine overnight. Cells were stained with PI and Annexin V APC. Images and the fluorescent signal of all single cells were recorded using the BD FACSDiscover™ S8 Cell Sorter. (C) Precision, Recall and F1 scores are shown for the three apoptosis datasets using different drugs. (D) tSNE plots of unsupervised clustering on live, dead and apoptotic cells. Separation of the three populations was observed for all three drugs.



Conclusions

- A CellView™ Foundation Model was developed to train label-free images from FACSDiscover™ S8 Cell Sorter to identify different cell subsets during programmed cell death.
- The deep learning pipeline we developed can successfully differentiate live, apoptotic and dead cells using Jurkat cell lines.
- It is challenging to identify apoptotic PBMCs using the deep learning tool.

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