A novel platform using deep learning models to perform multi-dimensional morphology analysis for biological discoveries

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KEY POINTS

- The Deepcell platform performs realtime deep learning interpretations of cell morphology.
- Multi-dimensional morphology profiling shows high concordance with cell images, gene expression profiles, flow cytometry, and genomic sequencing.
- Multi-dimensional morphology information represents a novel modality to describe cell morphology and complex cell biology processes.

INTRODUCTION

- Morphology is a fundamental cell property associated with identity, state, & function, but there is a need for multi-dimensional, unbiased, & quantitative assessment.
- Single cell multi-dimensional morphology analysis on the Deepcell platform provides a deeper assessment of heterogeneity by augmenting classical single cell multi-omics data (mRNA, genome, chromatin accessibility, protein).

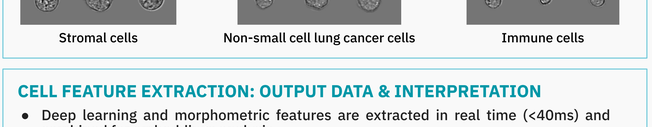


Figure 1. "-omics" modalities to characterize cells. Integration of the "morpholome" with other multi-dimensional evaluations can provide more comprehensive understanding of cell biology.

METHODS

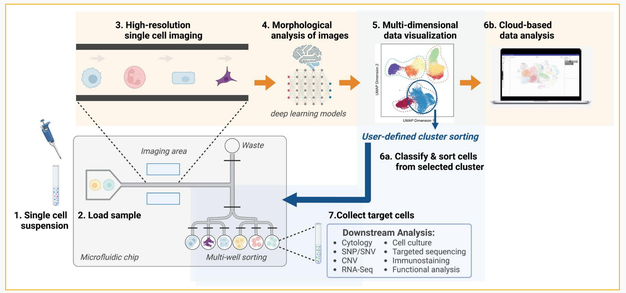


Figure 2. Deepcell Workflow. (1) Single cell suspension is (2) loaded onto a microfluidic chip. (3) Images of cells are captured and analyzed in realtime by (4) deep learning and morphometric models to generate (5) multi-dimensional morphological profiles. User-defined cell clusters are (6a) sorted for (7) downstream analysis. (6b) Morphology descriptions (embeddings) are extracted for data analysis and custom model training.

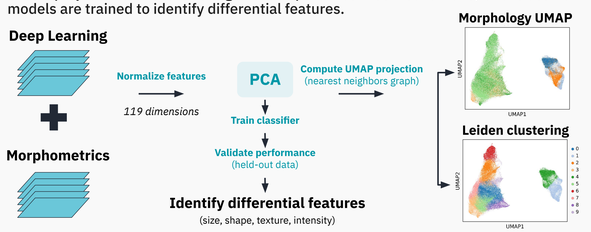
CELL FEATURE EXTRACTION: INPUT DATA

- Unlabeled brightfield images of single cells

Stromal cells | Non-small cell lung cancer cells | Immune cells

CELL FEATURE EXTRACTION: OUTPUT DATA & INTERPRETATION

- Deep learning and morphometric features are extracted in real time (<40ms) and combined for embeddings analysis.
- UMAP projections and clustering are compared between conditions, and classifier models are trained to identify differential features.



RESULTS

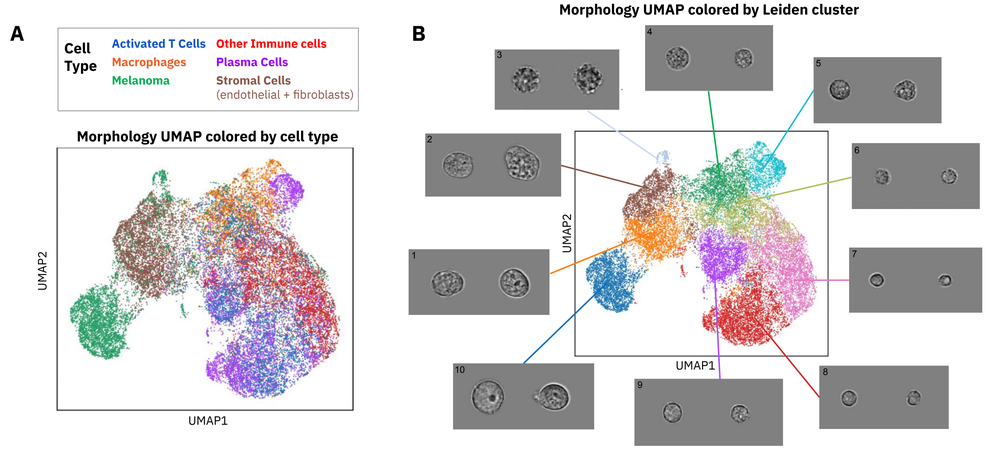


Figure 3. Morphology UMAP based on multi-dimensional embeddings. (A) Human melanoma, immune, and stromal cell lines and dissociated biopsies were combined to recapitulate heterogeneous tumor samples. Morphology UMAP from cell image feature embeddings colored by cell type. **(B)** Morphology UMAP colored by cluster imputed using the Leiden algorithm, with randomly chosen representative images from each cluster shown. Melanoma cells are morphologically distinct & cluster separately from non-tumor cells. Immune cells have subtle but separable morphologies.

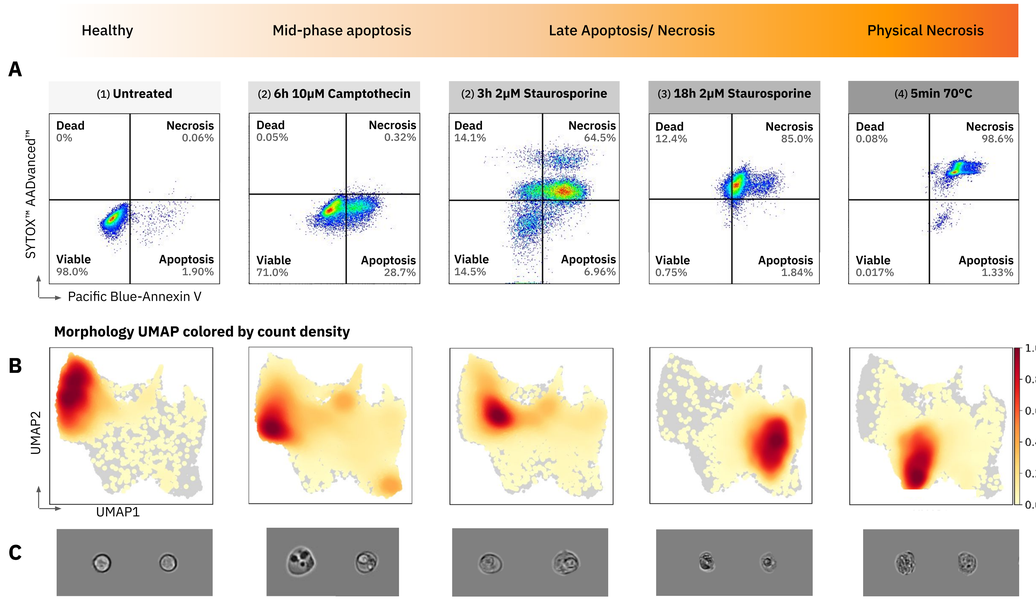


Figure 4. Cell health states correspond to distinct morphological profiles. Cell death was induced in Jurkat T lymphocytes. **(A)** Flow cytometry plots with percentage of healthy and dying populations based on membrane stains (Annexin V) and DNA intercalator (SYTOX™). **(B)** Morphology UMAP visualization of image feature embeddings from Deepcell platform imaged cells, colored by count density. **(C)** Representative images of each condition. Cells in various health and death pathways cluster separately based on morphology. Notably, cells occupying the same **(A)** flow cytometry gate segregate in distinct clusters based on **(B-C)** morphological traits, indicating ability of multi-dimensional morphology to detect and/or monitor subtle changes in sample health status and assess sample quality.

RESULTS cont.

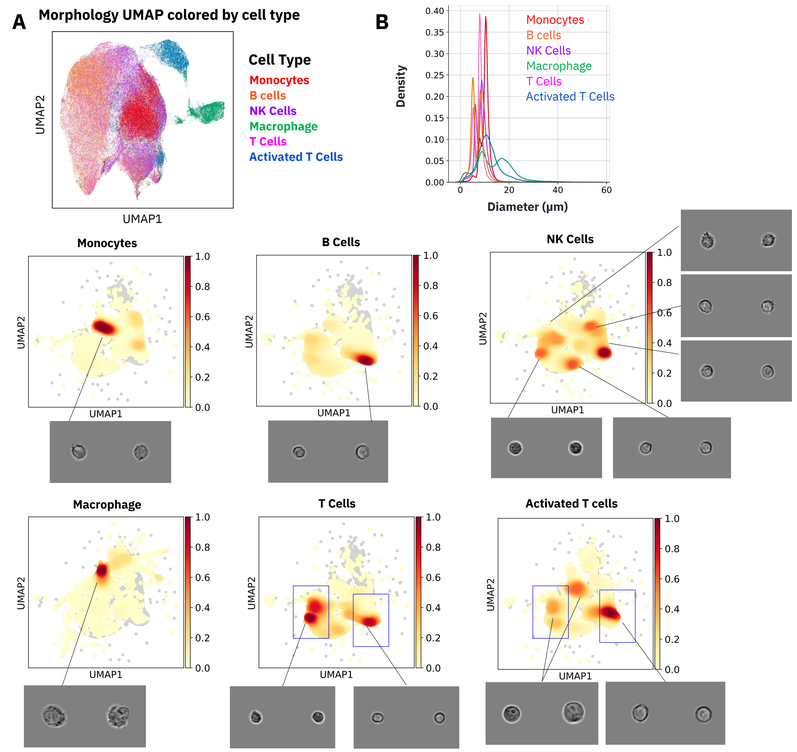


Figure 5. Mixed immune cell populations are morphologically distinct. Donor derived PBMCs and isolated immune cells including monocytes, B cells, NK Cells, macrophages, T cells and *in vitro* activated T cells were imaged on the Deepcell platform. **(A)** Isolated primary immune cell morphology UMAP from cell image feature embeddings, colored by cell type. **(B)** Computer vision morphometrics plot showing diameter of corresponding cell types. **(C)** Morphology UMAP of indicated immune cell type (orange) projected on top of PBMCs from 3 donors (gray). UMAP visualization shows image feature embeddings from imaged cells, colored by count density of indicated cell type. Representative images of high density regions are shown. Non-overlapping high density regions across the immune cell types indicate distinct morphological profiles in immune cells with differing functions/identities. Of note, individual cells have varied morphologies within the same cell class, suggesting heterogeneity in immune cell subclasses. For instance, the "T cell" population may contain some activated T cells, and "activated T cell" population may contain some non-activated T cells, as seen in the bottom right two panels (blue squares). Future work includes downstream functional analyses of these intra-population clusters.

CONCLUSIONS

- The Deepcell platform combines label-free brightfield imaging, deep learning, computer vision morphometrics, and gentle cell sorting to harness multi-dimensional single cell morphology as a biological readout.
- Morphologically distinct cells (normal vs tumor) cluster separately in AI embedding space, suggesting morphology may be used to profile cell type.
- Cells undergoing cell death are morphologically distinct from healthy cells. Multi-dimensional morphology profiles detected by the Deepcell platform provide more information on dying cells to better discern cell health status compared to marker-based flow cytometry gating.
- Quantitative multi-dimensional morphology information at single cell level provides an additional modality to understand heterogeneity.