Oncology · **Application Note**

Deepcell

Label-free multi-dimensional morphology profiling *identifies and enriches* tumor cells in heterogeneous populations

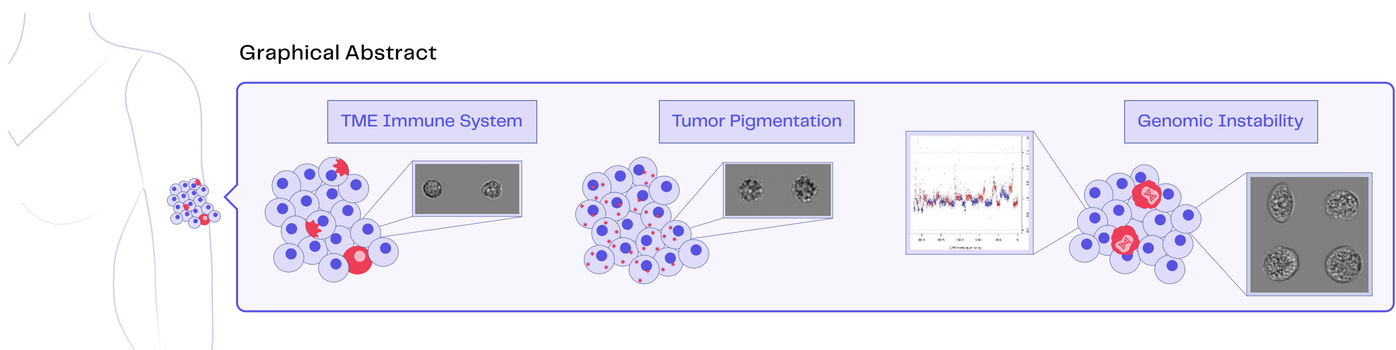
Highlights

- Tumor cell detection often relies on biomarker labels, limiting analysis to biomarker-specified populations.
- The REM-I platform characterizes and sorts cells without labels based on multi-dimensional morphometric and deep learning derived features of morphology.
- Quantitative multi-dimensional morphology information at the single cell level provides an additional analyte to resolve cancer heterogeneity.

Introduction

Tumors are composed of heterogeneous assortments of cells with distinct genetic and phenotypic characteristics that may drive therapeutic resistance, immune evasion, and disease progression[1, 2]. The advent of single cell technologies has enabled deep profiling of individual cells within a tumor microenvironment, leading to a better understanding of tumor biology and subsequently more effective cancer treatment strategies[3, 4]. While profiling technologies such as flow cytometry and single cell sequencing yields insight on tumor composition, cells are sometimes no longer amenable to additional downstream studies after being subjected to antibody staining or destructive analytical processes such as cell lysis[5]. Traditional sorting methods such as fluorescence-activated cell sorting (FACS) rely on a limited set of biomarkers, which cannot cover the full extent or be readily available for all distinct cell

Graphical Abstract



properties. Additionally, dependence on antibodies, dyes/stains, and biomarkers to denote cell identity may inadvertently create sampling bias by depleting biomarker-negative but potentially biologically interesting cell populations.

Hidden in plain sight is cell morphology information, which has historically been both the gold standard for cell and disease characterization, as well as hard to objectively and reproducibly quantify. Traditionally, cell morphology is studied qualitatively through microscopes, a process that's inherently slow, difficult to scale, and relies on human interpretation. Multi-dimensional morphology analysis enabled by AI and computer vision morphometrics provides higher resolution and biological insight while reducing labor-intensive cell processing manipulations. The REM-I platform from Deepcell performs multi-dimensional morphology analysis and sorting of unlabeled single cells using AI, advanced imaging, and microfluidics to assess population heterogeneity beyond biomarkers.

Methods

The REM-I platform consists of a benchtop instrument for sample imaging and sorting, a self-supervised deep learning foundation model (Human Foundation Model; HFM), and Axon, the Deepcell data suite for cloud-enabled data storage and analysis. To begin an experimental run, samples from established human cell lines or dissociated tissue biopsies in single cell suspension are loaded onto a microfluidic chip. Images of single cells are captured and analyzed in real-time by a combined deep learning and morpho-metric (computer vision) foundation model to generate multi-dimensional quantitative morphological profiles. User-defined cell clusters can then be sorted for downstream functional or molecular analysis. In addition, the collected morphology data (referred to as embeddings) can be further analyzed as a unique modality, and users can continuously train customized models for specific applications.

Results

Characterization of tumor cell population composition by morphology

Melanoma lesions are generally composed of primary tumor cells as well as a diverse set of immune cells in various activation states. To simulate these tumors and assess our ability to differentiate cells based on morphology alone, we created an in-silico mixture of cell types typically represented in solid tumors, including: human melanoma cell lines (SK-MEL-1, SK-MEL-3, MNT-1), in vitro activated T cells from PBMC, plasma cells from purified bone marrow, stromal cells from patient lymph nodes, and CD45+ immune cells (activated T, T, B, and NK cells) and macrophages isolated from metastatic melanoma biopsies. (Fig. 1). A total of 2.12 x 10^6 cell images were captured, then combined deep learning and morphometric features

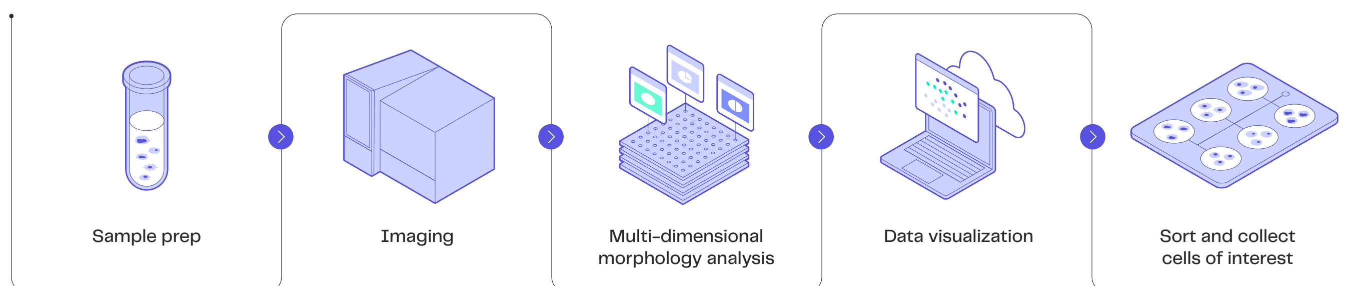


Figure 1. *REM-I platform workflow and AI image analysis. Single cell suspensions are loaded onto a microfluidic chip for high-speed, high-resolution imaging. Images are analyzed in real-time by self-supervised deep learning to extract morphological features and inform sorting decisions based on predicted cell class. Data is stored and analyzed on Axon, the Deepcell data suite, while sorted cells of interest can be assayed further with molecular or functional studies.*

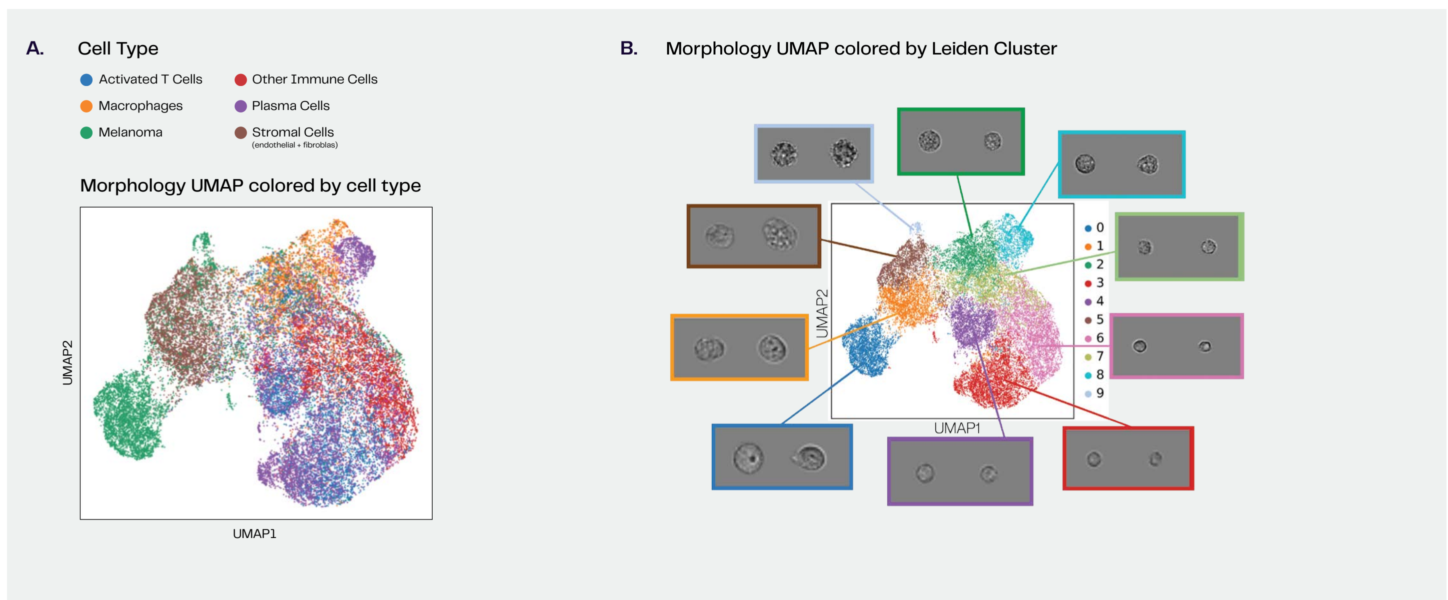


Figure 2. Morphologically distinct cells cluster separately. A. Morphology UMAP from cell image feature embeddings colored by cell type, which is a heterogeneous collection of melanoma, immune, and stromal cells derived from cell lines and patient biopsies. B. Morphology UMAP colored by cluster imputed using the Leiden algorithm, with randomly chosen representative images from each cluster shown.

were extracted and combined for embeddings analysis based on the Deepcell HFM v1. Features were standardized, then data were projected into a lower dimensional PCA basis. Nearest neighbors were computed in the PCA space, then these neighbors were used to identify clusters (Leiden algorithm) and compute 2D Uniform Manifold Approximation and Projections (UMAP, Fig. 2A). Notably, melanoma cells are morphologically distinct and cluster separately from non-tumor cells. Tumor cell lines SK-MEL-1 and SK-MEL-3 (cluster 10) are distinct from MNT-1 (cluster 3) (Fig. 2B), as seen in images showing cluster 3 cells with higher granularity and cluster 10 cells exhibiting a smoother appearance. Furthermore, distinct immune cell types are variably located in the morphology UMAP, indicating cells have subtle but separable morphologies. Larger immune cells with more granular features (macrophages) cluster toward the top of the morphology UMAP, while smaller cells with no granularity (activated T cells, plasma cells) are located in the lower clusters of the UMAP. Together, the data suggest the REM-I platform can differentiate cell types represented in melanoma tumors based solely on multi-dimensional morphological profiles. These include tumor versus non-tumor cells, activated versus quiescent lymphocytes, and different immune cells (plasma cells, macrophages, and lymphocytes) with varying granularity features.

Computer vision morphometrics reveals differential cell features in melanoma cell lines

Skin and hair pigmentation is the result of melanosome melanin biosynthesis in epidermal melanocytes[6]. The multi-step melanin biosynthesis process is highly regulated, and has recently been implicated in melanoma progression[7-11]. Despite the implications of melanin biosynthesis with melanoma and melanoma pathogenesis, existing technologies rely on pathway biomarkers to measure pigmentation levels rather than a direct quantification of melanin granules in melanocytes[12]. Currently, it is difficult to non-destructively sort cells with pigmentation. One option is to utilize intracellular biomarkers followed by FACS, but this method is not common and renders cells incompatible for additional functional studies. Using Axon, the Deepcell data suite, we isolated the three established melanoma cell lines with varying degrees of pigmentation[13] from the larger dataset shown in Fig. 2, and used computer vision combined with deep learning to extract pigmentation features (Fig. 3). Pigmented melanoma cells (MNT-1) were

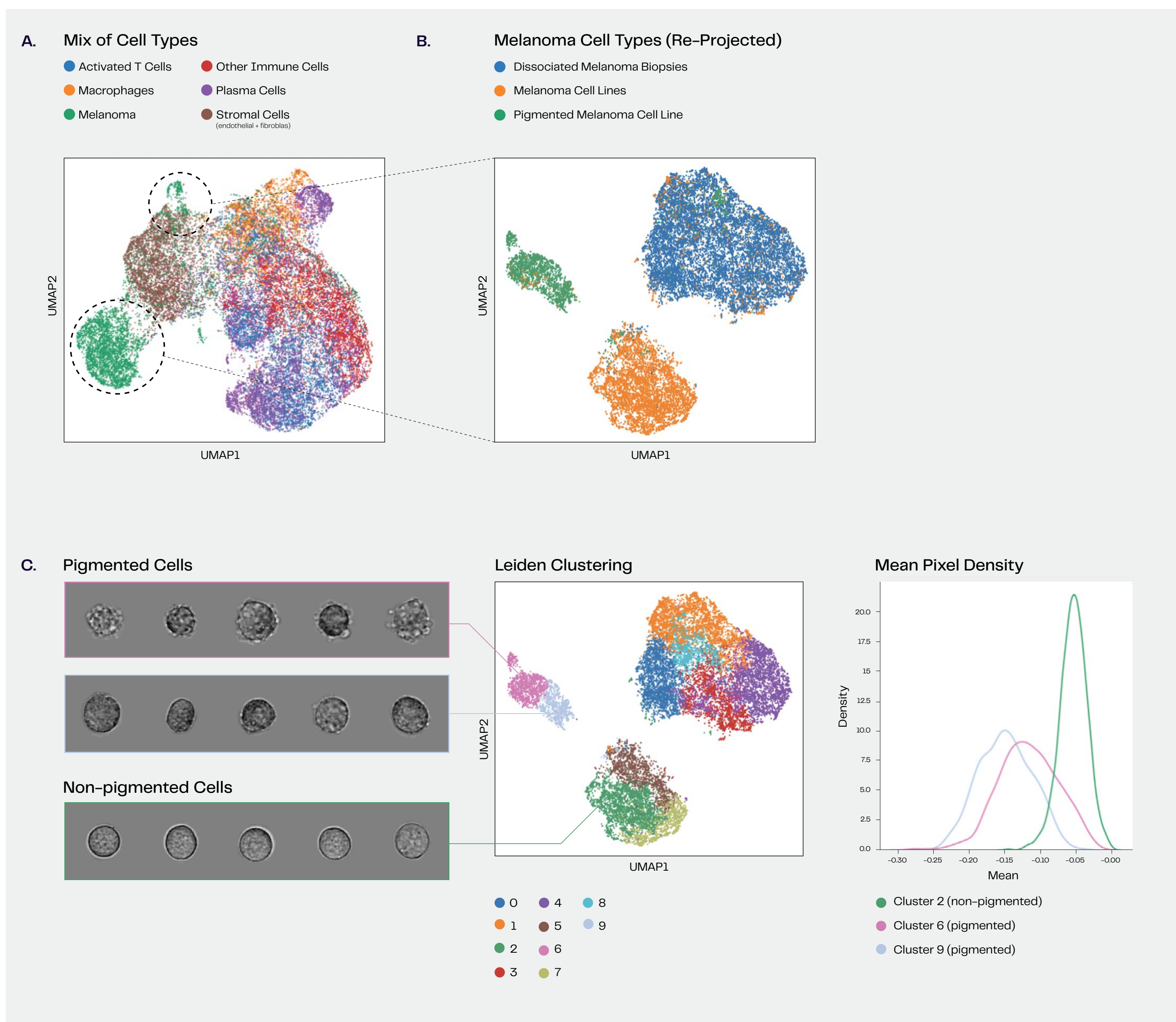


Figure 3. Pigmentation is a separable morphological feature distinguishing melanoma cells. A. Morphology UMAP of a heterogeneous collection of melanoma cell lines and immune/stromal cells derived from patient biopsies (as seen in Fig. 2A). B. Re-projected morphology UMAP using filtered data from Fig. 3A showing only melanoma cells colored by cell line. C. (Left) Morphology UMAP colored by cluster imputed using the Leiden algorithm, with randomly chosen representative images from each cluster shown. (Right) Density plots showing mean pixel intensity, with lower mean value (i.e., darker pixels) linked to more pigmentation.

morphologically distinct and clustered separately from other melanoma cells (SK-MEL-1 and SK-MEL-3) with low levels of melanin (Fig. 3A–B). Morphometric analysis further showed the population of highly pigmented cells exhibit heterogeneity with differing levels of pigmentation (Fig. 3C), as shown by quantification of this morphological feature. These results indicate the REM-I platform can clearly detect pigmented cells and different levels of pigmentation, and allows for subsequent cell sorting based on user interest for downstream studies (e.g., cell culture, drug studies, and treatment response).

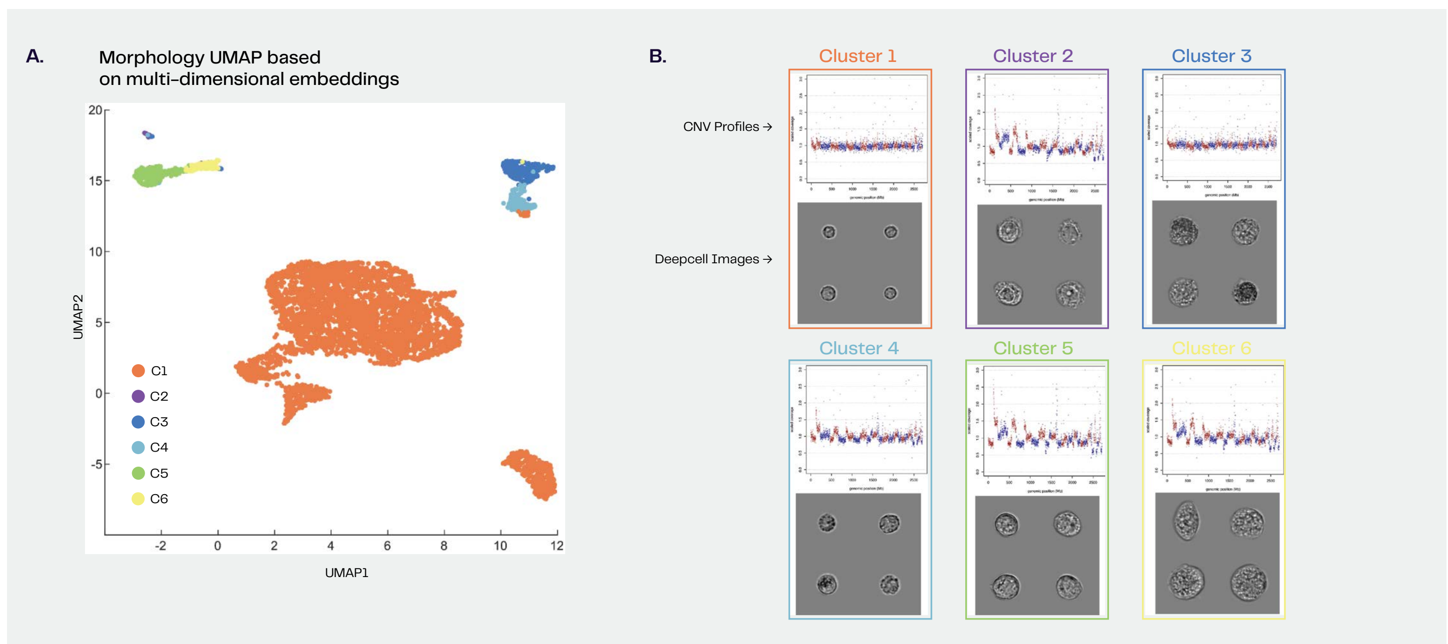


Figure 4. *Morphologically distinct cell clusters from human NSCLC tissue.* ***A.*** *Morphology UMAP of cells residing in a NSCLC DTC biopsy sample. Six morphologically distinct clusters (1, 2, 3, 4, 5, 6) were identified and colored by the assigned cluster number.* ***B.*** *Clusters were isolated by the Deepcell platform via user-defined sorting and processed for CNV profiling. Results showed sorted morphology clusters exhibited distinct CNV patterns consistent with cancer cell profiles in clusters 2, 5, and 6.*

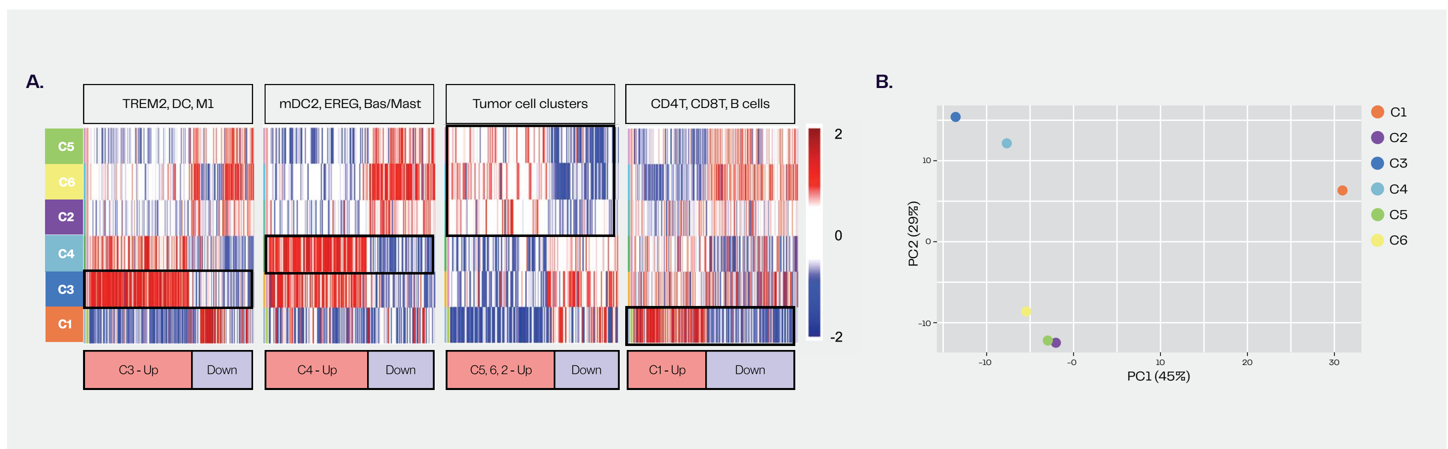


Figure 5. *Transcriptomic characterization of morphology clusters.* ***A.*** *Bulk RNA-seq analysis followed by* ***B.*** *principal component analysis (PCA) was performed on each morphology cluster.*

User-defined multi-dimensional morphology clusters have distinct molecular profiles

Lung cancer is highly heterogeneous, and generally divided into two groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC makes up approximately 15% of lung cancer, while NSCLC comprises 85%[14–15]. To perform multi-dimensional morphology analysis in NSCLC, we developed an AI model using pure cell populations derived from dissociated tumor cells (DTC) biopsies. User-defined clusters were sorted and subjected to copy number variation (CNV) analysis and bulk RNA-Sequencing (RNA-Seq). Isolated morphology clusters were viable and showed high concordance with CNV and bulk RNA-Seq expression profiles (Fig. 4-5). Notably, sorted morphology clusters were composed of increased

populations of respective cell classes. For instance, clusters 2, 5, and 6 showed enrichment of tumor cells while clusters 1, 3, and 4 showed enrichment of immune cells (Fig. 5A-B), indicating morphology may be reflective of cell identity and function. Notably, detection of multiple cancer cell clusters based on multi-dimensional morphology may be reflective of the heterogeneous nature of the tumor sample, which may not have been revealed by a constrained panel of biomarkers. Together, this data suggest cell morphology can be leveraged to characterize and isolate cells of interest based on morphology and enable integrative multi-omics analysis workflows.

Conclusion

- The REM-I platform combines label-free imaging, deep learning, computer vision morphometrics, and gentle cell sorting to harness multi-dimensional single cell morphology as a quantitative biological readout.
- Pigmentation, among other morphological features extracted by combined AI and morphometrics, can be used in the assessment of melanoma cells using the REM-I platform.
- Cell populations characterized with specific morphological profiles have distinct molecular profiles.
- Morphologically distinct cells (normal vs. tumor) are distinguishable in AI and morphometric embedding space, suggesting morphology may be used to profile cell type and function.

Resources

Analyze high-dimensional morphology enabled by self-supervised deep learning by exploring the following datasets at https://exploredata.deepcell.com/.

→ Melanoma cell identification

→ Heterogeneity in melanoma

→ Lung cancer tumor microenvironment

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