High dimensional morphology analysis reveals new insights in melanoma cell heterogeneity and enables label-free phenotyping

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KEY POINTS

- The Deepcell platform characterizes & sorts cells based on multi-dimensional morphology analysis without labels, eliminating the need for specific biomarkers.
- We demonstrate that multi-dimensional morphology can distinguish mesenchymal vs melanocytic cells in both cell lines and dissociated tumor biopsies
- An AI-based random forest classifier can predict cell phenotype based on morphology alone, verified by single-cell transcriptional information

INTRODUCTION

- Melanomas are the deadliest skin cancers, in part due to cellular plasticity and heterogeneity within the tumors.
- The Deepcell platform enables multi-dimensional morphology analysis and enrichment of unlabeled single cells using artificial intelligence (AI), advanced imaging, and microfluidics, enabling high resolution profiling of population heterogeneity.
- We imaged and analyzed 18 patient-derived melanoma cell lines representing both mesenchymal and melanocytic phenotypic states.
- High-dimensional morphological analysis showed distinct clusters for each phenotype, indicating distinct morphotype for each phenotype.
- We developed a random forest classifier to identify the top differential morphological features between the different cell lines, thereby providing a label-free means of phenotyping melanoma samples.
- The morphology analysis of the cell lines uncovered significant variability in pigmentation; a random forest classifier distinguished pigmented vs non-pigmented cells with >90% accuracy.
- Application of the classifier to images of dissociated tumor biopsies identified their phenotype, which was verified by scRNASeq, demonstrating the application of a label-free phenotyping using morphology alone in clinical samples.

METHODS

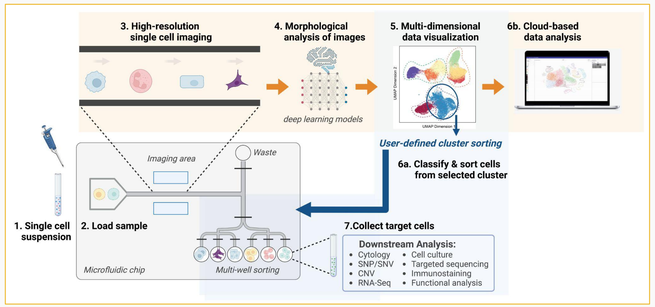

Figure 1. The Deepcell workflow. We analyzed each of the sample types using the Deepcell Workflow. **1)** Single cell suspension is **(2)** loaded onto a microfluidic chip. **(3)** Images of single cells are captured and analyzed in real-time by **(4)** deep learning and morphometric (computer vision) models to generate **(5)** multi-dimensional quantitative morphological profiles. User-defined cell clusters can be **(6a)** sorted for **(7)** downstream functional or molecular analysis. **(6b)** Morphology descriptions (embeddings) are extracted for data analysis/exploration and custom model training. The embeddings were used to profile the morphological heterogeneity of the cells, identify which morphology features differentiate each condition, and train a random forest classifier to predict each class of cells.

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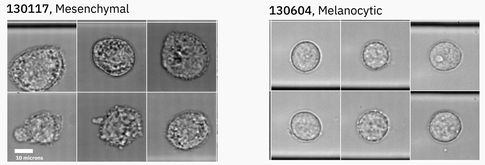
RESULTS

Multi-dimensional morphology distinguishes melanocytic vs mesenchymal cell lines

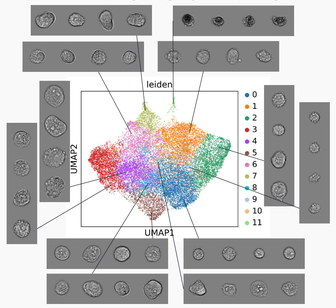
A Patient-derived melanoma cell lines included in study

Phenotype	Total cell lines	Cell lines
Mesenchymal	9	120815, 121008, **130117**, 130107, 150922, 170314, 150507, 150672, 181004
Melanocytic	9	**130604**, 160411, 160526, 161201, 170123_2, 991104, 140307, 990922, WM983B

B Morphological difference in phenotype is perceivable by eye



C Representative images highlight morphological diversity in cell lines



D Density plots show unique morphotypes for each phenotype

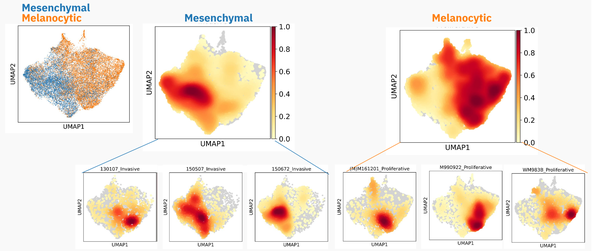
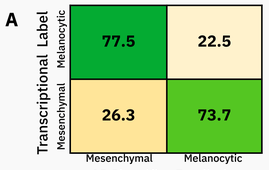


Figure 2. Multi-dimensional morphology distinguishes mesenchymal vs melanocytic cell lines. (A) 18 patient-derived cell lines from metastatic melanomas representing mesenchymal and melanocytic phenotypes were imaged and analyzed on the Deepcell platform. The phenotype of each cell line was based on mesenchymal or melanocytic gene expression signatures from bulk RNA-Seq data. **(B)** Images of cells indicate qualitative morphology differences detectable by eye, with melanocytic cells appearing smoother and mesenchymal cells are more granular. **(C)** Leiden clustering highlights the morphological diversity across the patient derived cell lines. **(D)** Multi-dimensional morphology UMAP showing distinct clusters for mesenchymal (orange) and melanocytic (green) cells, further illustrated by density plots. Inspection of the individual cell lines shows multiple morphotypes within each phenotype.

A random forest classifier can predict phenotype based on morphology alone

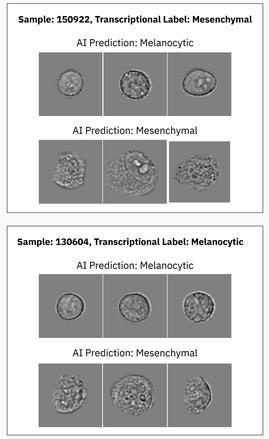
A



B

Sample ID	Transcriptional Label	Classifier Prediction	
		Mesenchymal (% of cells)	Melanocytic (% of cells)
M130604	Melanocytic	16.33	83.67
M170123-2	Melanocytic	15.85	84.15
M991104	Melanocytic	20.03	79.97
M160526	Melanocytic	20.85	79.15
M161201	Melanocytic	25.61	74.39
M160411	Melanocytic	27.03	72.97
M990922	Melanocytic	34.18	65.82
M140307	Melanocytic	41.01	58.99
WM983B	Melanocytic	35.37	64.63
M120815	Mesenchymal	44.40	55.60
M170314	Mesenchymal	65.92	34.08
M130107	Mesenchymal	77.51	22.49
M130117	Mesenchymal	84.08	15.92
M150922	Mesenchymal	75.02	24.98
M150507	Mesenchymal	82.62	17.38
M181004	Mesenchymal	88.41	11.59
M170617	Mesenchymal	88.74	11.26
M150672	Mesenchymal	90.63	9.37

C



D Mesenchymal vs Melanocytic

Feature	Importance
LBP_CENTER_09	3.04
DL FEATURE 11	2.73
DL FEATURE 49	2.6
LBP_CENTER_08	2.45
LBP_CENTER_04	2.42
PERCENTILE_75	2.18

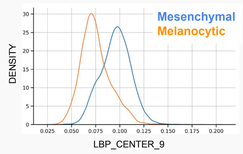
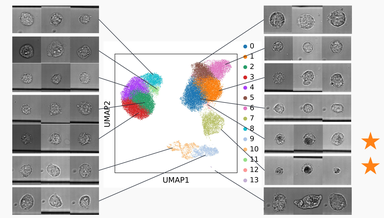


Figure 3. A random forest classifier can predict phenotype of melanoma cells with up to 77% accuracy. (A) We developed a random forest classifier to predict the phenotype of melanoma cells based on the images taken on the Deepcell platform. 8 cell lines with the most clear transcriptional score were used to train the classifier, which was then tested on an additional 12 cell lines. The classifier can predict the phenotype with up to 77% accuracy. **(B)** The classifier is concordant with transcriptional phenotype for 17/18 cell lines. The classifier provides single-cell phenotype information, noted in the percent of cells classified as each phenotype for each cell line. **(C)** Inspection of the images verified the predicted heterogeneity. **(D)** Local binary pattern (LBP) features, a metric of texture and granularity, are the top differential morphometric features distinguishing mesenchymal and melanocytic phenotypes. The distribution of the top feature is shown, suggesting mesenchymal cells (blue) have higher measures of granularity compared to melanocytic cells (orange). DL; deep learning.

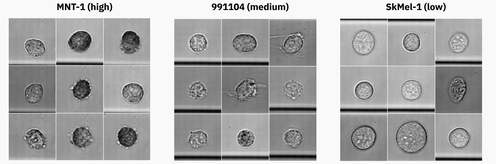
RESULTS cont.

Morphometric analysis of morphology elucidates differences in pigmentation in phenotypic states

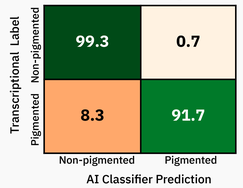
A Morphometrics features UMAP colored by Leiden clustering identifies a unique pigmented population. Representative images from each cluster are shown



B Representative images of cell lines exhibiting high, medium, and low pigment



C AI Classifier Predicts Pigmentation



D Top differential morphometric features

Non-pigmented vs pigmented	
Feature	**Importance**
PERCENTILE_25	11.66
NEGATIVE FRACTION	10.65
MEAN	9.07
PERCENTILE_75	7.90
MIN_FERET	6.23
PERIMETER	5.91

UMAPs colored by feature

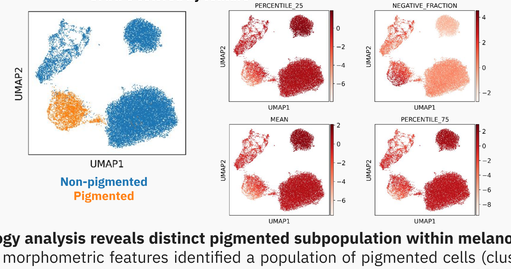


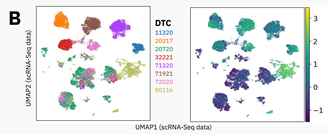
Figure 4. High dimensional morphology analysis reveals distinct pigmented subpopulation within melanoma cell lines. (A) Leiden clustering using morphometric features identified a population of pigmented cells (cluster 7). **(B)** Visual inspection and morphometric quantification of individual melanoma cell lines (MNT-1, 991104, SkMel-1) showed high, medium, and low pigmentation. **(C)** A random forest classifier using the morphometric features predict pigmented populations with up to 99% accuracy. **(D)** Top differential features distinguishing pigmented versus non-pigmented cells include pixel contrast and intensity values, as expressed by 25th/75th percentile of pixel grayscale values (Percentile_25/75), negative fraction, and mean pixel intensity.

scRNA-Seq verifies AI predictions of phenotype on melanoma DTCs

A

DTC Sample ID	Tumor Location	Melanocytic Transcriptional Score	Mesenchymal Transcriptional Score
11320	Lymph nodes	0.79	-0.30
20317	Metastatic tissue to lymph nodes	0.62	-0.39
20720	Lymph nodes	0.80	-0.43
32221	Lymph nodes	0.74	-0.35
71320	Lymph nodes	0.71	-0.37
71921	Lymph nodes	0.78	-0.51
72020	Lymph nodes	0.80	-0.31
80116	Axillary lymph node metastatic from melanoma	0.79	-0.40

B



	DTC 20317	DTC 72020
Composition (scRNA-Seq)	95% Tumor 5% Immune	60% Tumor 40% Immune
Transcriptional Score ("Bulk" Analysis)	0.62 Melano, -0.39 Mesen, Phenotype: Melanocytic	0.8 Melano, -0.31 Mesen, Phenotype: Melanocytic
Classifier Prediction	63% Melanocytic 37% Mesenchymal	65% Melanocytic 35% Mesenchymal

Figure 5. The phenotype classifier can predict the morphology of dissociated tumor biopsies. (A) The Melanoma Phenotype Classifier was run on dissociated tumor cell (DTC) samples, and AI predictions identified all DTCs as melanocytic. **(B)** We performed scRNA-Seq on the DTCs and the resulting UMAPs are shown. We analyzed the composition of the DTCs with sufficient malignant cell abundance for statistical analysis (labeled in blue). Results verified the AI predictions that the melanocytic phenotype was more prevalent in these samples.

CONCLUSIONS

- High dimensional morphology analysis shows multiple morphotypes for melanocytic and mesenchymal cells
- These morphological differences were used to develop a Melanoma Phenotype Classifier that can predict phenotype with up to 77% accuracy
- The morphometric features also identified a unique pigmented population with up to 99% accuracy
- The Melanoma Phenotype Classifier can be used to predict the phenotype of dissociated melanoma biopsies.
- These results suggest that high dimensional morphology can be used to characterize phenotype in a label-free manner, and provide new insights into tumor biology.