Integrated Morphology-Transcriptomics Analysis Reveals Functional Cell States in Macrophage Polarization and Tumor-Induced Reprogramming

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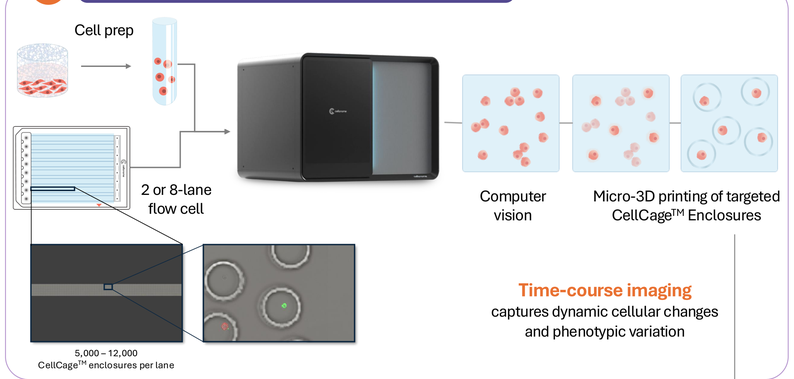
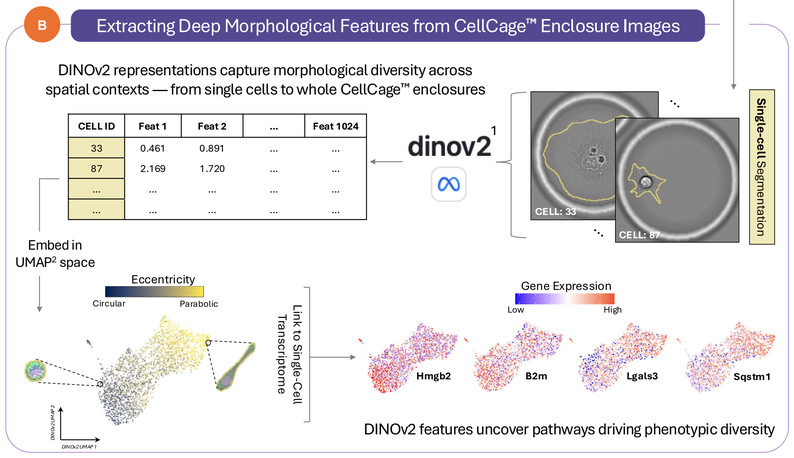
INTRODUCTION Macrophages are highly plastic immune cells whose diverse functional roles in tissue homeostasis, inflammation, and tumor immune evasion are driven by context-dependent cell states. Single-cell transcriptomics has revealed remarkable diversity within macrophage populations, yet it cannot capture morphological features such as cell shape, spreading, pseudopodia, and texture, which relate to functional processes such as phagocytosis, migration, and polarization. Conventional approaches acquire imaging and sequencing data on separate instruments from separate cells, making it impossible to directly link a cell's morphological phenotype to its underlying gene expression program. Cellanome's CellCage™ enclosure technology overcomes this limitation by enabling simultaneous capture of high-resolution images and matched single-cell transcriptomes from the same cells. Combined with DINOv2, a self-supervised vision transformer that extracts rich morphological embeddings from cell images, this platform allows us to systematically relate morphological variation to transcriptomic states at single-cell resolution. Here, we apply this integrated approach to dissect macrophage polarization and tumor-induced reprogramming, revealing functional cell states and morphology–transcriptome relationships that are invisible to either modality alone. This approach provides new insight into the complexity of macrophage phenotypes and how they are shaped by microenvironmental cues, with implications for understanding tumor immune evasion.

Cellanome R3200: A Micro-3D Printing Platform That Unites Morphology and Transcriptomics

A On-Instrument CellCage™ Enclosure Formation



B Extracting Deep Morphological Features from CellCage™ Enclosure Images



C DINOv2 Captures Biologically Relevant Morphology

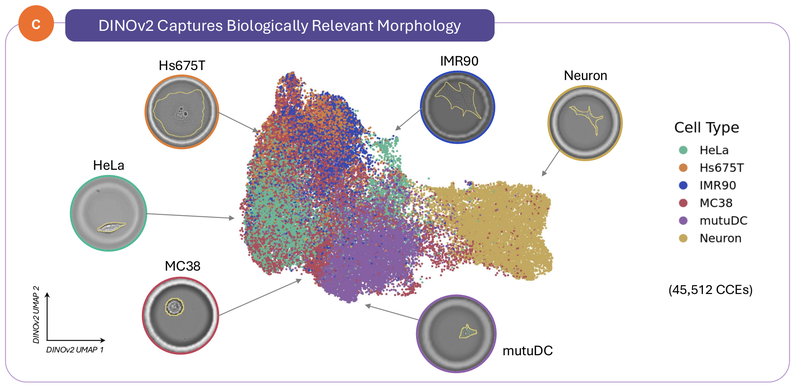
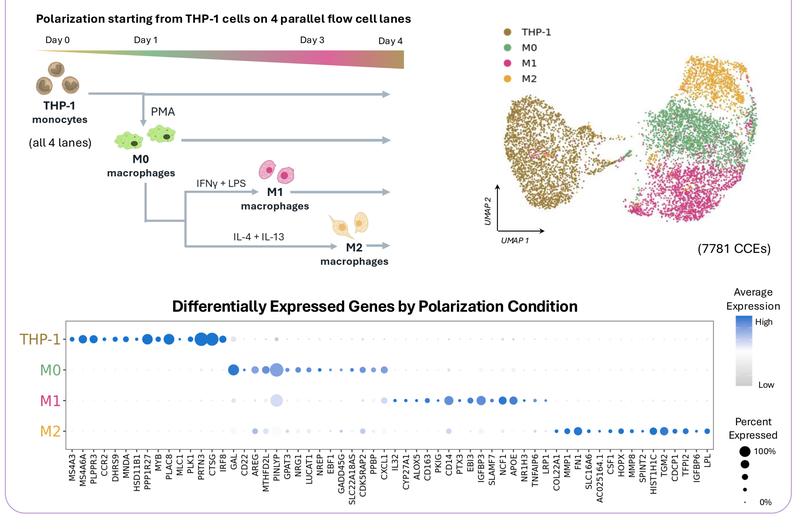


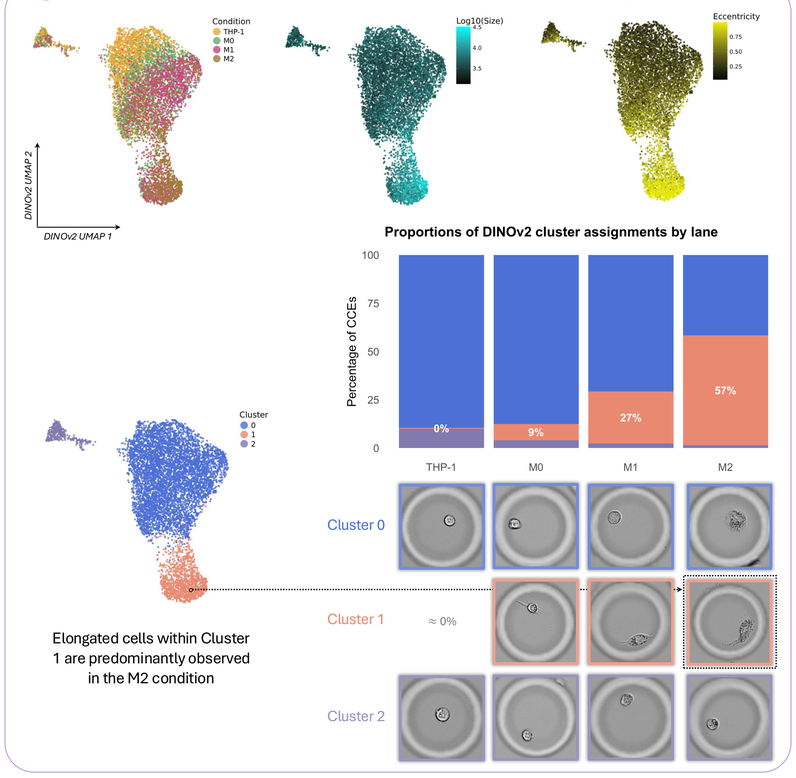
Fig. 1. Integrated experimental and computational workflow linking morphology and transcriptomics. (A) Schematic of Cellanome's CellCage™ enclosure (CCE) technology, which enables multi-modal phenotypic and functional profiling of the same single cells. Tens of thousands of suspended cells are mixed with a hydrogel precursor and loaded into a multi-lane flow cell. Individual cells are detected, and CellCage™ enclosures are automatically generated around them via light-guided polymerization. The CCEs are permeable to small molecules and antibodies, allowing long-term culture and time-course imaging of the same cells. Following imaging-based assays, cells are lysed *in situ*, and mRNA is captured for cDNA synthesis, library preparation, and sequencing. **(B)** DINOv2-based computational workflow for extracting 1024-dimensional morphological embeddings from CCE images. These features capture phenotypic variation across scales — from single cells to entire CCEs — and can be integrated with single-cell transcriptomic data to reveal morphology–gene expression relationships. **(C)** DINOv2 features from six cell lines clustered by cell identity, confirming capture of meaningful morphological differences. Embedding similarity predicted the generalization performance of a Mask R-CNN (Region-based Convolutional Neural Network) segmentation model.

Macrophage Polarization Spans a Transcriptomic and Morphological Continuum

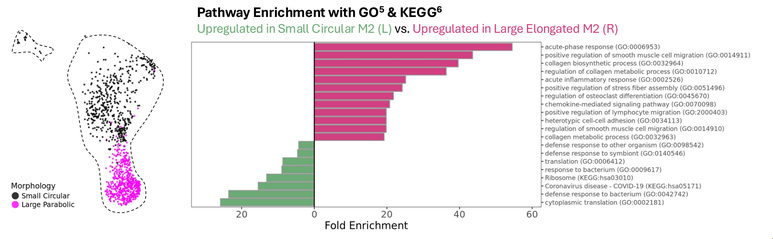
A On-Flow Cell Polarization Leads to Transcriptional Shifts



B Polarization Induces Morphological Heterogeneity Across States



C Morphologically Separated Subsets in M2 have Distinct Transcriptional Signatures



D Morphology Reveals Heterogeneity in Addition to Transcriptomic Pseudotime

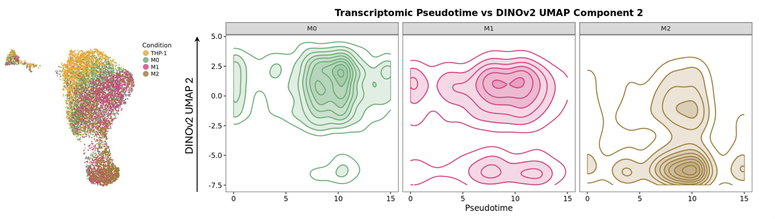
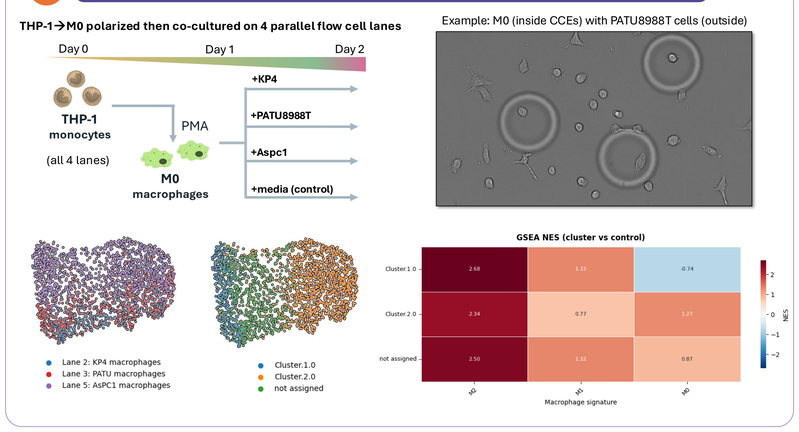


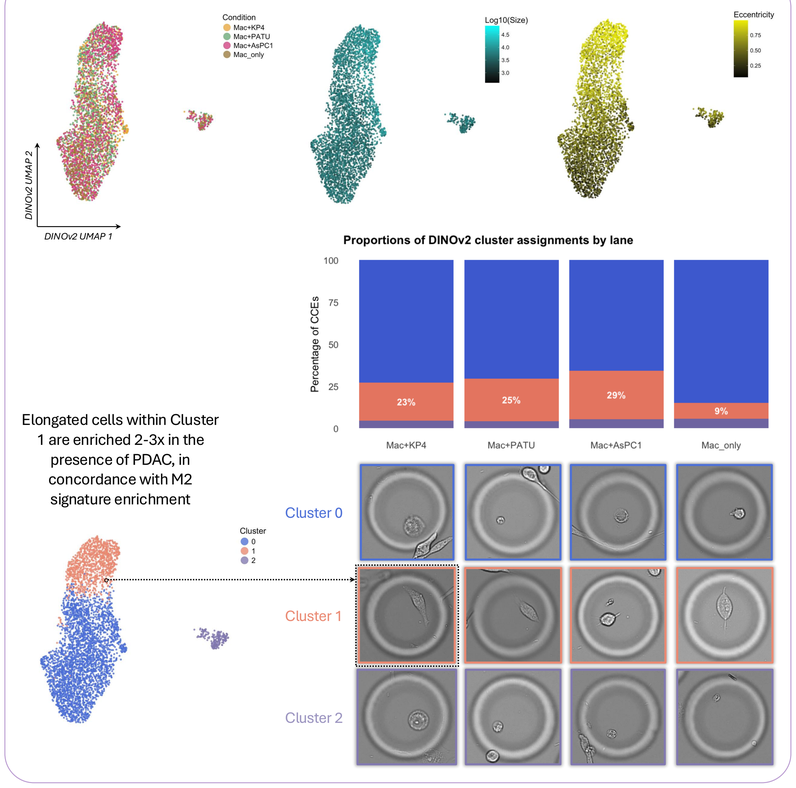
Fig. 2. Morphology adds orthogonal insight to transcriptomic profiling of macrophages. (A) On-instrument polarization of THP-1 monocyte-like cells leads to distinct transcriptional states. **(B)** DINOv2 detected multiple phenotypes that correlated with polarization states[4,5], revealing diverse cellular responses to the same treatment. The UMAP representation of DINOv2 features captured a gradient in size and shape. **(C)** Within the M2 condition, elongated cells had a distinct transcriptional signature from circular cells, enriched for migration, adhesion, and inflammation-related pathways. These morphological fingerprints highlight functional heterogeneity in macrophage responses to polarization. **(D)** DINOv2 tracked morphological shifts with polarization that complement transcriptomic pseudotime[7], further differentiating macrophage subtypes and within-sample variation.

Pancreatic Tumor Cells Differentially Reprogram Macrophages in Co-Culture

A Investigating Pancreatic Tumor Interactions with M0 Macrophages



B Macrophages Exhibit Diverse Morphological Phenotypes with Co-culture



C Tumor-induced Macrophage States Enriched in Elongated Cell Fraction

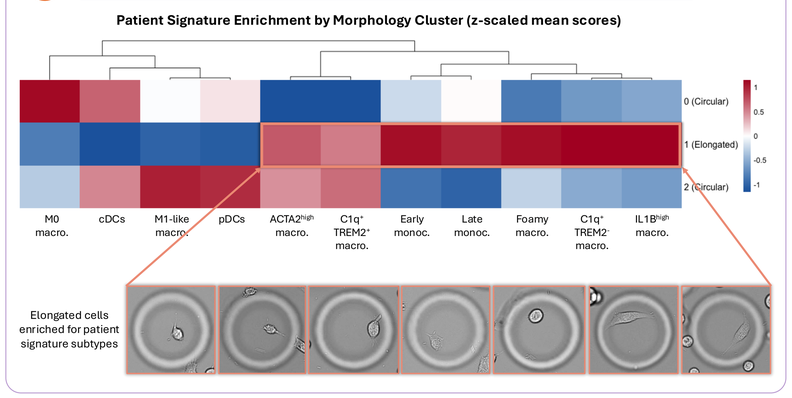


Fig. 3. Macrophage-tumor interactions reveal links between gene and protein expression and morphology. (A) THP-1 derived M0 macrophages were co-cultured on a flow cell with 3 pancreatic ductal adenocarcinoma (PDAC) cell lines: KP4, PATU8988T, Aspc1, or media-only control for 24 hours. Co-cultured macrophages were found to be enriched for M2-like protein-level signatures using VIPER[8]. **(B)** Macrophages in all 3 co-culture conditions were also enriched for elongated morphology (23-29%) compared to control (9%), showing that macrophage morphology is also influenced by tumor cell interactions. **(C)** Elongated macrophages were enriched for specific myeloid cell subtypes from patient-derived signatures (foamy, C1q^{+} TREM2^{-}, and IL1B^{high}), revealing granular phenotypes in these complex populations. Here, morphology and transcriptomics provided complementary views on macrophage plasticity in the context of pancreatic tumors.

[1]**DINOv2**: Oquab *et al.* (2023)
[2]**UMAP**: Uniform Manifold Approximation & Projection; McInnes, Healy, Melville (2018)
[3]**Macrophage morphology**: Selig *et al.* (2024)
[4]**Macrophage morphology**: McWhorter *et al.* (2013)
[5]**Gene Ontology:** Ashburner *et al.* (2000); The Gene Ontology Consortium (2025)
[6]**Kyoto Encyclopedia of Genes and Genomes:** Kanehisa *et al.* (2000, 2019, 2025)
[7]**Monocle**: Cao *et al.* (2019)
[8]**VIPER:** Alvarez *et al.* (2016)

QUESTIONS?