Engineering High-Fidelity Early Cancer Models: Single-Cell Bioprinting in 2D and 3D to Mimic the Native Tumor Microenvironment

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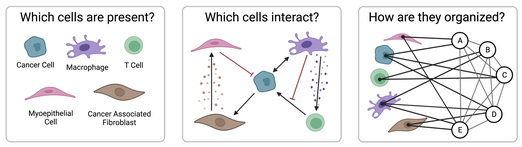
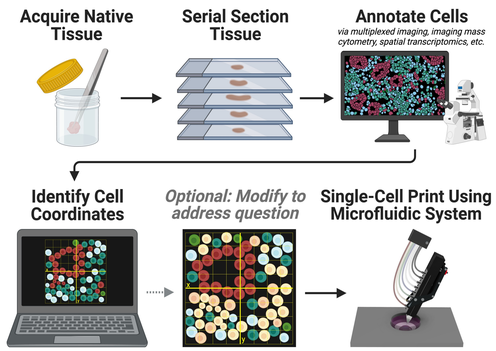
Engineered Tumor Avatars

- Spatial features of the tumor microenvironment, such as cellular organization, have been linked to clinical outcomes. Yet much remains unknown about the specific contribution of various cell types and their signaling to cancer evolution



- Engineered tumor avatars provide opportunities to identify mechanisms of cancer initiation, progression, and treatment resistance
- However, current biofabrication approaches are unable to control the exact location of multiple cell types, thus limit their ability to precisely address spatially driven questions
- **Here we present two high-fidelity engineered cancer models in which we can controllably manipulate the cellular and extracellular environment to systematically identify which conditions promote or inhibit cancer initiation and progression**

Approach to Mimic the Native TME



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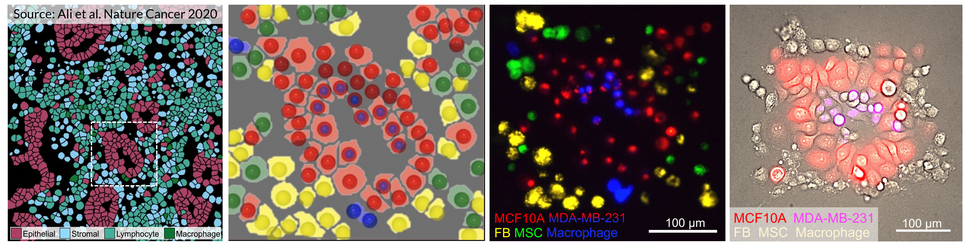
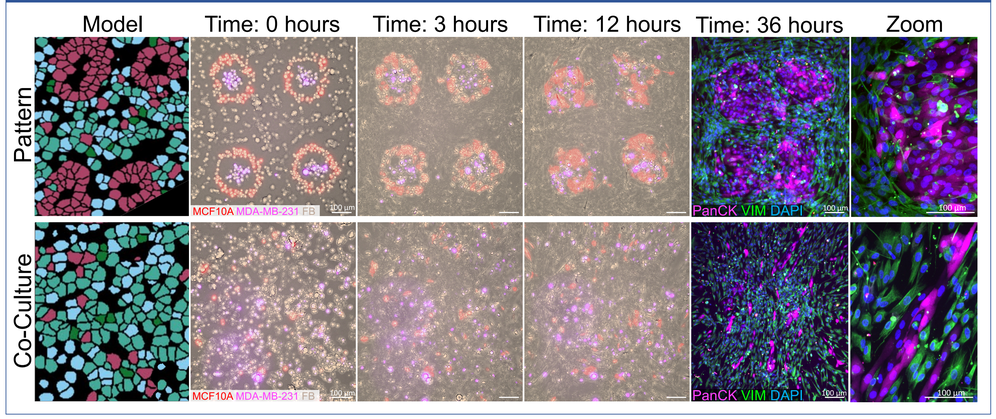
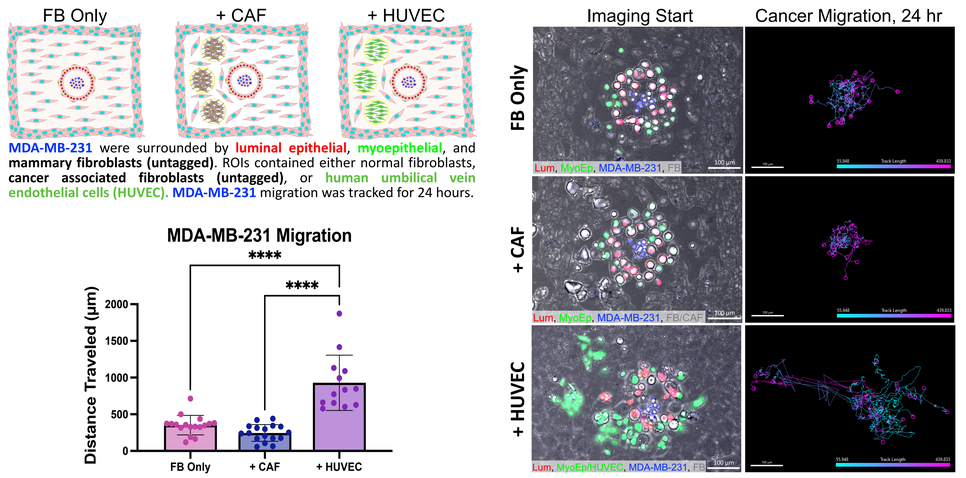
Reconstructing the TME with Single-Cell Resolution in 2D



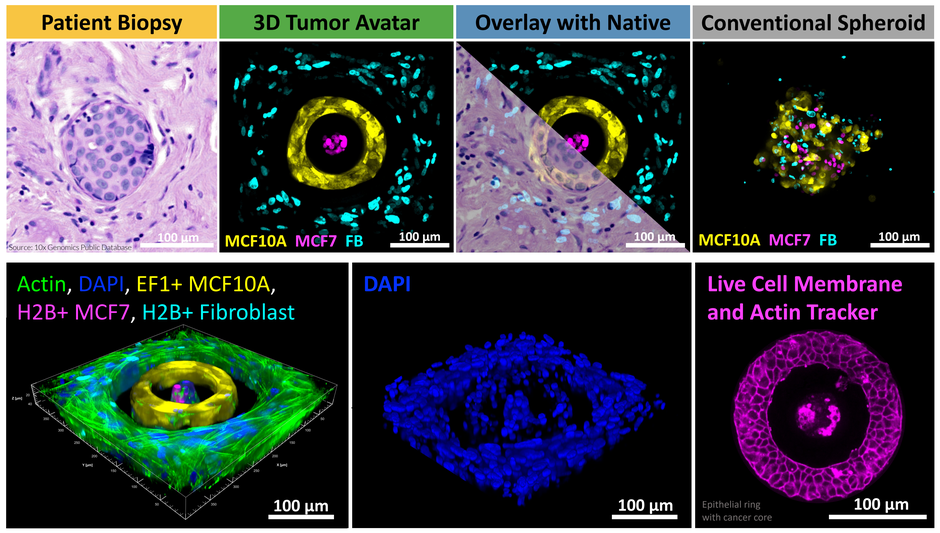
Spatial Arrangement Influences Cell Morphology

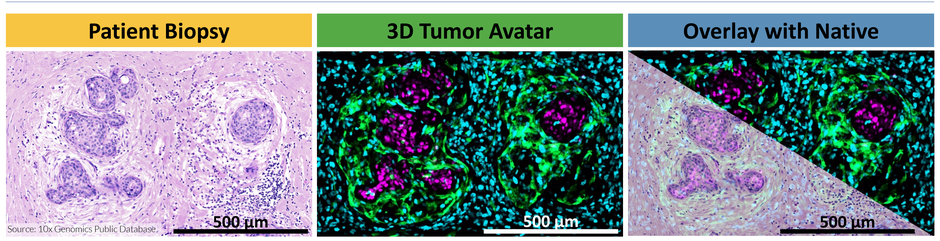


TME Composition Influences Cancer Migration



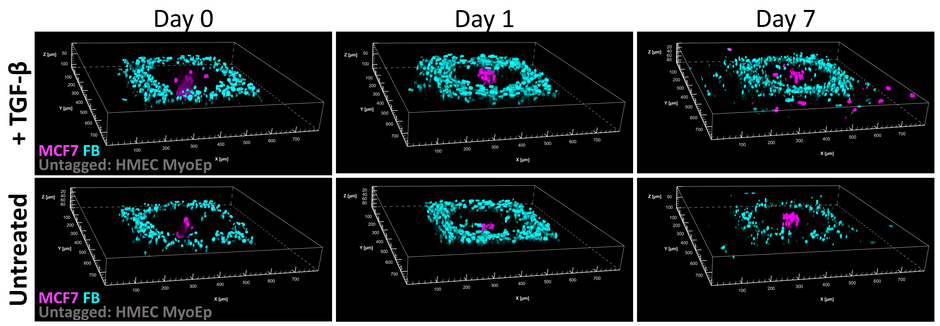
Fabricating 3D, Cell Dense, and Spatially Resolved Tumor Avatars





Avatar Containing 7 Cell Types: **H2B iRFP+ MDA-MB-231**, **H2B mTurquoise+ Mammary Fibroblasts**, **GFP+ Endothelial Cells (HUVECs)**, **Mammary Epithelial Cells** (HMEC, untagged), **Myoepithelial Cells** (untagged), **Mesenchymal Stromal Cells** (untagged), and **Macrophages** (untagged). 24 hours post print.

3D Tumor Avatars Responds to TGF-β Stimulation



Avatars Containing 4 Cell Types: **H2B+ MCF7**, **Mammary Epithelial Cells** (HMEC, untagged), **Myoepithelial Cells** (untagged), **H2B+ Mammary Fibroblasts**. TGF-β treatment (10 ng/mL) for 7 days promotes cancer cell migration and sustained fibroblast population, compared to the untreated control.

Conclusions

We have demonstrated the ability to reproducibly fabricate highly heterogeneous, cell dense, and spatial resolved tumor microenvironments using up to 7 cell types with single-cell resolution in 2D and 60 µm resolution in 3D. Initial studies have confirmed spatial arrangement, TME composition, and external stimuli influence cell behavior. This high-fidelity engineering approach enables the systematic manipulation of the cellular composition and arrangement within the tumor microenvironment to dictate specific cell-cell interactions. Unlocking the relative contributions of each cell in the microenvironment to tumor evolution may hold the answer for many questions in early detection, intervention, and predictive tumor models.

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