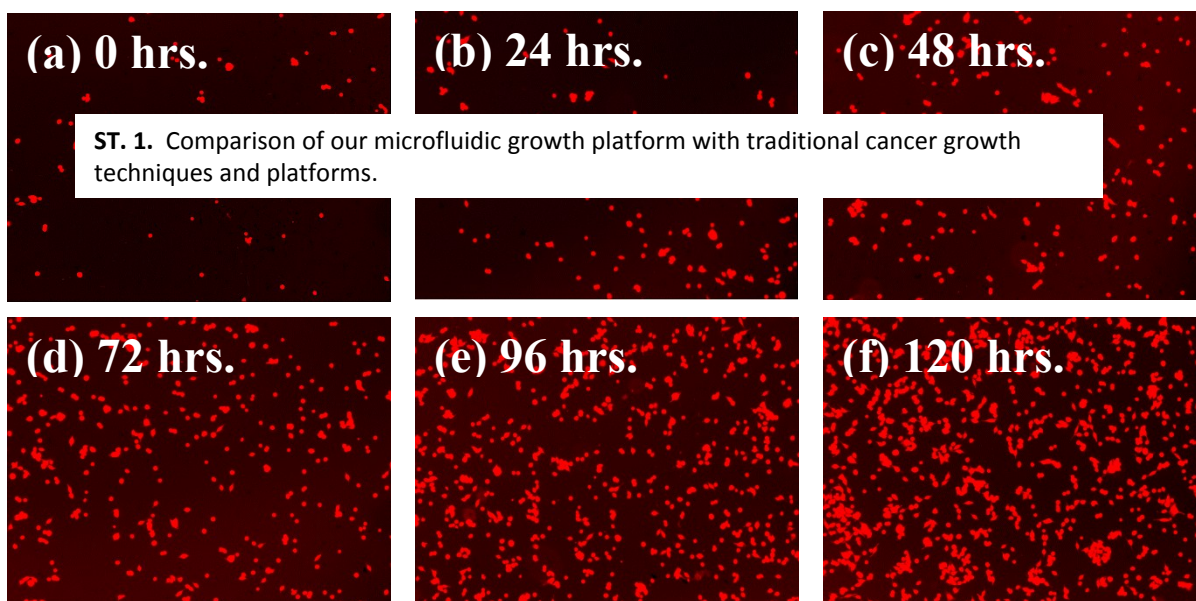


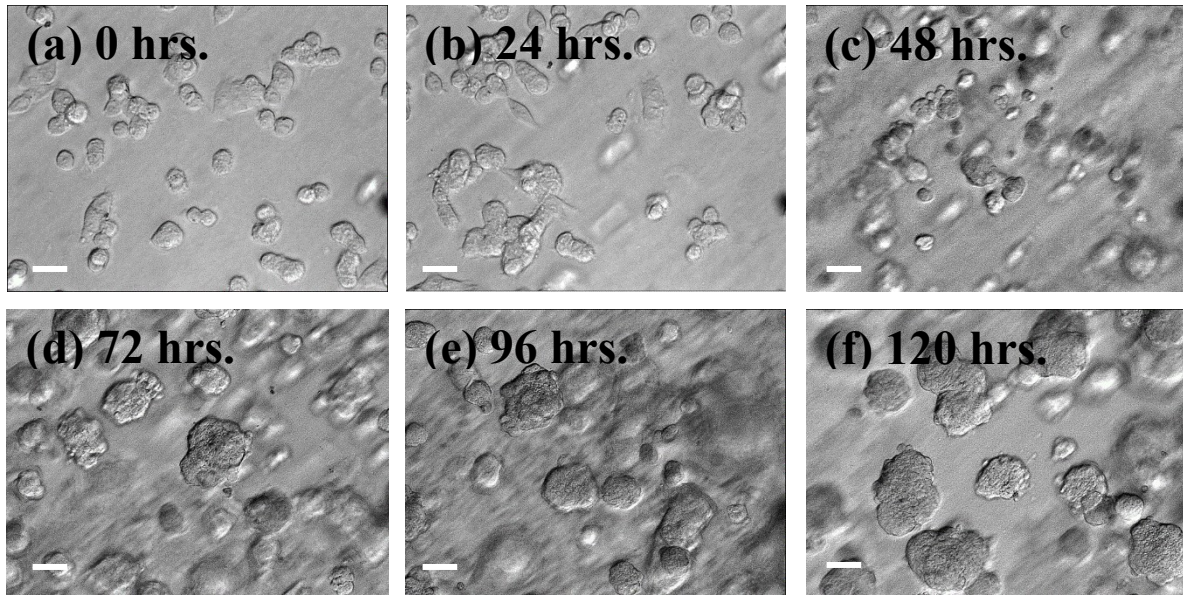
Electronic Supplementary Information

	Imaging Integration	Throughput	Media Circulation	Control over Drug Testing	Cost	Initial Number of Cells Required	Culturing Time Required
Hanging-Drop	Hard	Low	No	None	Low	Low	Long
Animal Models	Very Hard	Low	Yes	Low	High	High	Long
NASA Bioreactor	Hard	High	Yes	Low	High	High	Long
Our Bilayer Microfluidic	Easy	High	Yes	High	Low	Low	Short

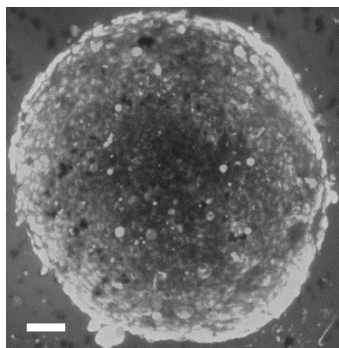
Advantages and improvements over existing methods:



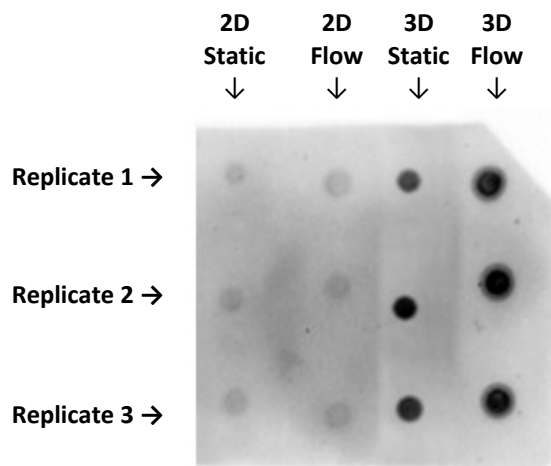
SF. 1. Representative fluorescent images of Leukemia cell static growth suspended in Matrigel stained with CellTrackerRed™. **a**, 0 hrs. **b**, 24 hrs. **c**, 48 hrs. **d**, 72 hrs. **e**, 96 hrs. **f**, 120 hrs. All white scale bars are 100µm in length.



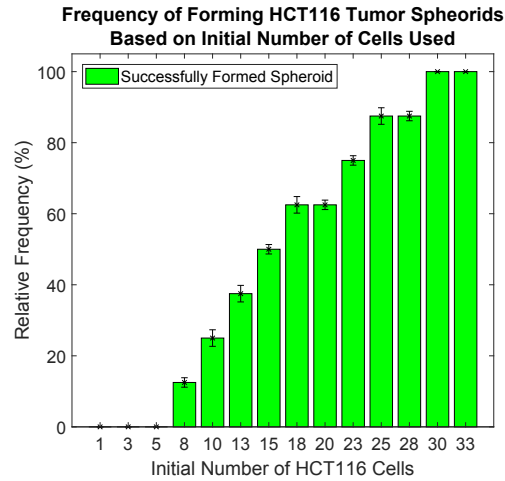
SF. 2. Representative brightfield images of HCT116 cell static growth suspended in Matrigel. **a**, 0 hrs. **b**, 24 hrs. **c**, 48 hrs. **d**, 72 hrs. **e**, 96 hrs. **f**, 120 hrs. All white scale bars are 100 μ m in length.



SF. 3. HCT116 tumor spheroid grown in microfluidic device displaying high degree of sphericity. White scale bar is 60 μ m in length.



SF. 4. Dot plot testing results for E-Cadherin screening. Similar levels of E-Cadherin expression are observed for static and flow conditions for both two-dimensional and three dimensional cancer cell growth.



SF. 5. Relative frequency of HCT116 tumor spheroid formation based on the initial number of cancer cells introduced into the microfluidic device (n=24). No spheroid formation is observed between 1 and 5 cells. The smaller number of cells required to form a spheroid is 8 cells with roughly a 12.5% likelihood of success. Consistent (100%) spheroid formation occurs when using 30 cells or higher.