FlowCam as a Platform for Assessing Heterogeneity in Low-Passage Tumoroid Models

A Promising New Method for 3D Cell Culture Analysis

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1. Overview

Many 3D tissue culture protocols use scaffold-based systems to provide the structural support cells need to achieve physiologically relevant spatial organization and contact-based biochemical signaling.

Removing tumoroids from their supportive 3D microenvironment for image analysis introduces several risks that can affect the structural integrity, viability, representativeness, and reproducibility of the sample.

In this study, we demonstrate the value of FlowCam® for analyzing low-passage canine tumoroids, cultured in Matrigel®, by enabling rapid, objective measurement of quantity, size, morphology, and structural complexity across hundreds of individual 3D cell structures in a single run.

2. Introduction

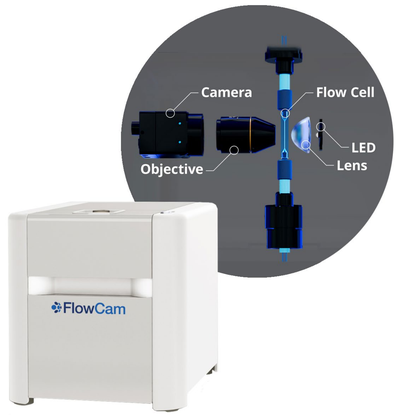
Patient-derived tumoroids represent a powerful translational model for comparative oncology. In vitro cultivation of tumoroid subtypes in the presence of extracellular matrix components creates scalable, patient-specific 3D models that vastly increase the material available for scientific study. Monitoring growth is important for understanding the biological variability and relevance of the developing model, however, visualization can be difficult in the presence of scaffolding matrices.

Flow imaging microscopy (FIM) using Yokogawa FlowCam® technology provides a high-throughput solution for quantitative characterization of precious and complex 3D structures previously grown in scaffolding matrices.

FlowCam image-based analysis visualizes how tumoroid subtypes respond to their in vitro environment and supports refinement of culture and treatment conditions to better preserve clinically-relevant phenotypes.

3. Methods

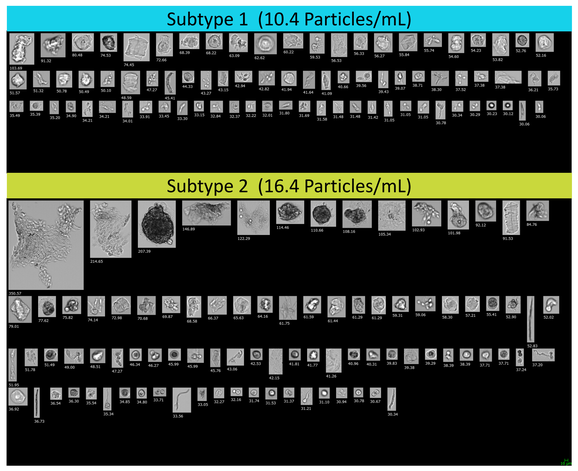
- Low-passage canine tumoroids, cultured in Matrigel® were collected and dissociated from the scaffold matrix for real-time image and data analysis.
- FIM of ~10 mL sample volumes was performed with FlowCam 8000 configured with a 4X objective, FOV 300 flow cell, a sample flow rate of 2mL/min, and ~5 min run time.
- Images of 3D structures were also obtained with an inverted microscope using a 4X objective in brightfield mode.

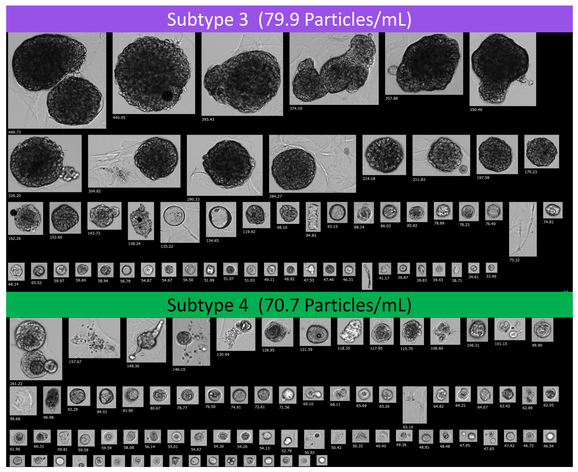


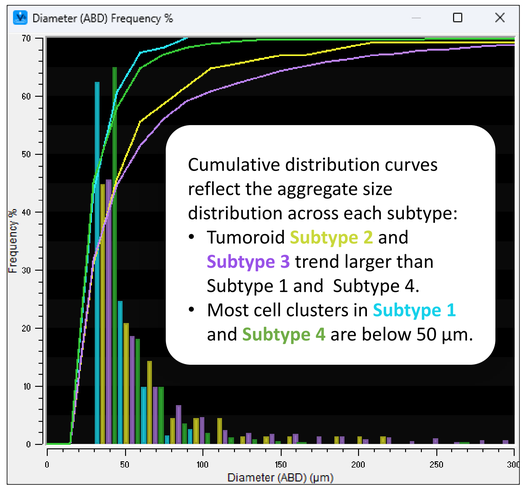
4. Results

Four patient-derived tumoroid subtypes grown under the same culture and treatment conditions showed distinct differences in count and size distributions. Heterogeneous cell cluster and sheet-like structures were semi-automatically classified using morphological particle properties.

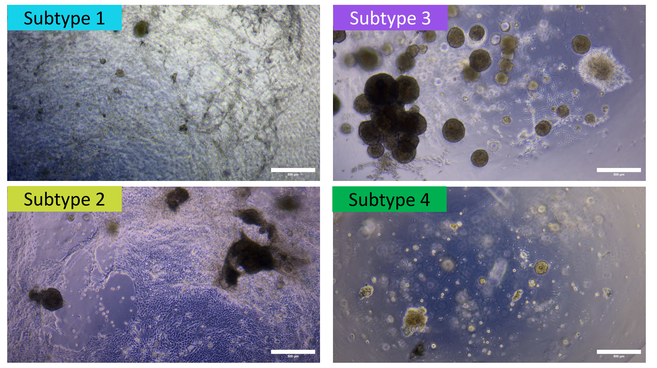
Rapid, high-resolution FlowCam images of cell clusters from four patient-derived tumoroid subtypes show morphological heterogeneity and real-time size distributions.





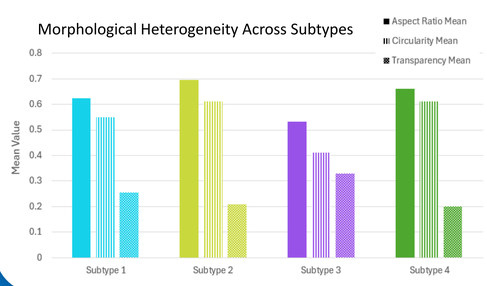


FlowCam quantifies observations made by qualitative manual microscopy.

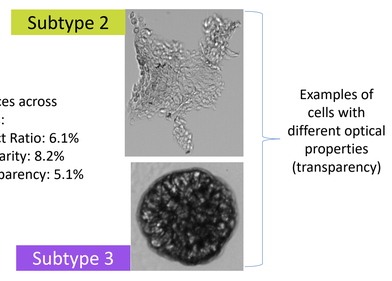


- FlowCam images show morphological features consistent with microscope images.
- Manual brightfield microscopy provides image data only.

Particle property measurements made directly from FlowCam images enable separation of tumoroid cell structures by morphology and optical properties.



Differences across subtypes:
- Aspect Ratio: 6.1%
- Circularity: 8.2%
- Transparency: 5.1%



Examples of cells with different optical properties (transparency)

5. Conclusions

FIM with FlowCam 8000 adds significant value to tumoroid workflows by maximizing data output from limited samples, improving biological insight towards tumor heterogeneity, and delivering scalable, standardized analysis for translational and commercial applications.

6. Get in Touch

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Questions about flow imaging microscopy?

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