

## Introduction

Glioblastoma Multiforme (GBM) is a highly aggressive form of brain tumor with variable local invasion of the brain's extracellular matrix (ECM)<sup>1</sup>.

The cellular protrusions that "find" a path through the ECM and subsequently attach and degrade it are called invadopodia<sup>2</sup>.

Visualization of invadopodia can be achieved by allowing GBM cells to invade a fluorescently labeled gelatin coating. The degradation creates a dark spot in the gelatin matrix. The presence of invadopodia are confirmed by colocalization of matrix degradation and actin puncta<sup>2</sup>.

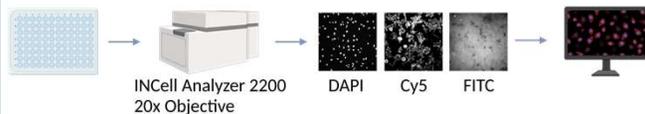
The most common image analysis is a threshold-based method performed in the NIH ImageJ program. This method sets low intensity thresholds to capture dark spots of degradation.<sup>3</sup>

Although useful, ImageJ is user intensive and limited to analyzing a single image at a time. It may also overestimate degradation in unevenly illuminated microscope images.

## Project Goal

This project establishes a method for microscopy image analysis using the CellProfiler software to objectively measure invasion behaviors of glioblastoma cancer cells.

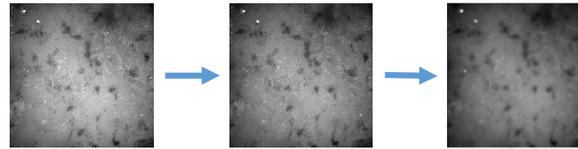
## Imaging Methods



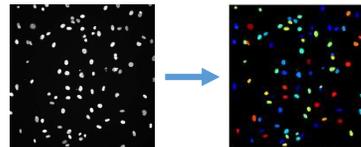
Created with BioRender.com

## The Pipeline

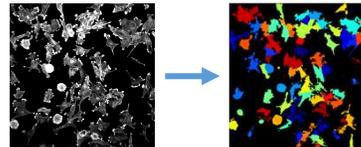
### 1. Correct Illumination and Smooth Gelatin (FITC)



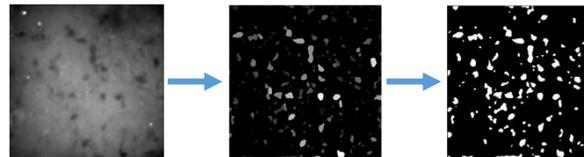
### 2. Identify Nuclei (DAPI)



### 3. Identify Cell Bodies (Cy5)



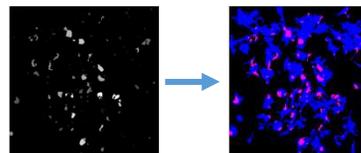
### 4. Enhance and Threshold Dark Holes in Gel



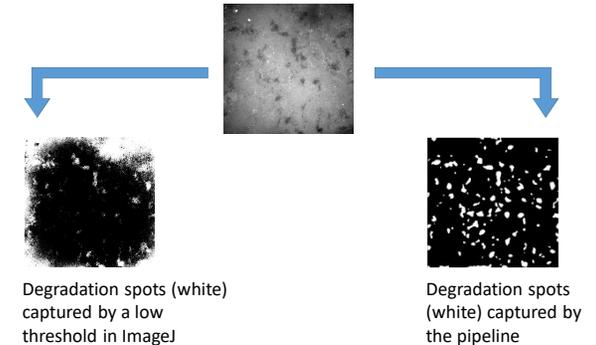
### 5. Mask Invasion with Cell Area



### 6. Measure Colocalization of Cell Area and Invasion



## The Pipeline



## Conclusions

This pipeline has the capacity to provide count measurements of nuclei/cell bodies and area measurements of cells and degradation.

Future methods of imaging may be specified to visualize individual invadopodia per cell<sup>4</sup>. Identifying invadopodia with this pipeline would follow the same procedure already used to identify nuclei and cell bodies.

With this high throughput pipeline, high content screening can be used to save time and remove user bias from the imaging process.

## References and Funding

### References

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- Murphy, D. A.; Courtneidge, S. A. The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. *Nat Rev Mol Cell Biol.* 2011, 12, 413-426. DOI: 10.1038/nrm3141
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- Chen YC, Baik M, Byers JT, Chen KT, French SW, Diaz B. TK55-positive invadopodia-like structures in human tumor surgical specimens. *Exp Mol Pathol.* 2019; 106, 17-26. DOI: 10.1016/j.yexmp.2018.11.005.

This work was supported by NIH award R01- NS103486 (Lawrence)