Membrane-localized Keratin-14 promotes invasion

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Overview

- Objective
- Methods
- Results
- Discussion
- Future Directions

Quantifying the Invasive Potential of Tumors

- Why would we need to make a system that quantifies the invasive potential of tumors?
  - Quantitative phenotypes are more amenable to systemic analysis
  - They can be used in population genetics methods, where genetic variations and invasive potential of tumors can be studied by directly perturbing candidate genes
  - Such studies can lead to the discovery of new drugs for cancer therapy which can ultimately have a positive impact on the poor prognosis seen in patients with metastatic carcinoma

Phenotype quantification allows us to pull out the molecular correlates

- Invasivenes quantification allows us to calculate the genetic contribution of individual genes
  - Quantitive characterization allows us to detect even minor contributors
  - Quantification of phenotype allows us to calculate the interaction between different genes
  - The fine-grainness of the quantification allows us to perform genetic correlation at the genome scale

Why we use Keratin 14 as a proof of expression?

- Keratin 14 is a know biomolecular marker for metastasis
- [Shamir .. Bader Ewald 2014] challenge the epithelial-to-mesenchymal transition requirement for metastasis, and instead, provide evidence in support of a model based on Twist1 induced dissemination of cytokeratin positive epithelial cells
- [Cheung .. Bader Ewald 2016] demonstrated that tumor cells only can invade and metastasize in clusters
- in the same paper, Cheung et al. suggested Keratin 14-associated pathways are key regulators of metastasis

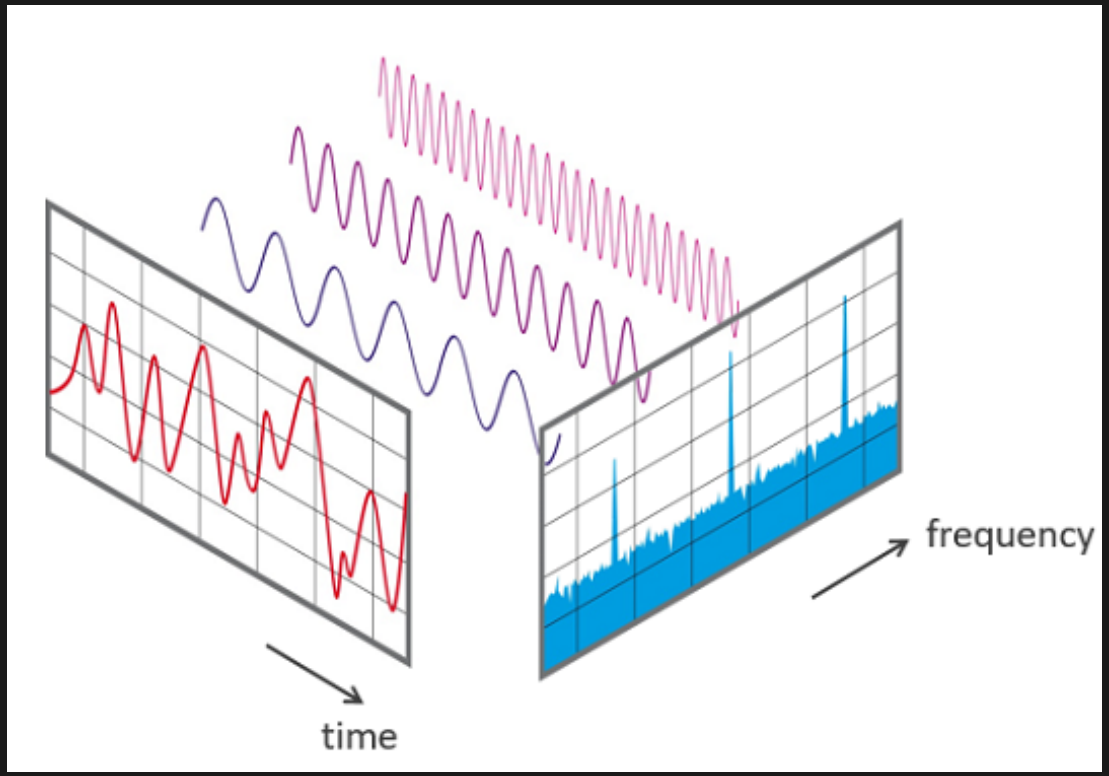
Project Objective

- We will test whether K14 is correlated with invasiveness:
  1. Total K14
  2. Peripheral K14
  3. Central K14

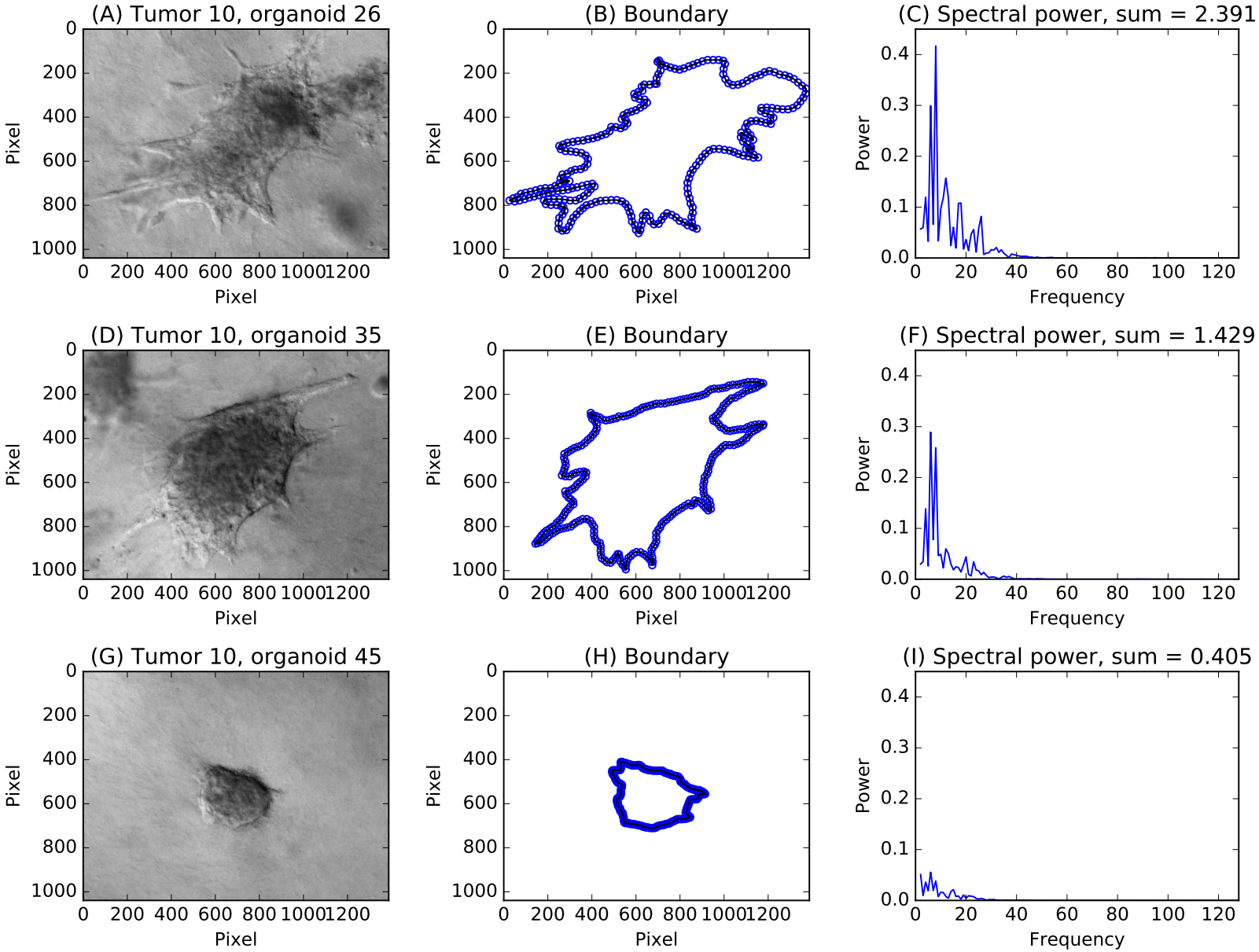
Our Data

- MMTV-PyMT transgenic mice were be used to produce a supply of organoids
- Tumors were removed from 4 mice and a total of 90 - 150 small and large organoids were generated per tumor ($\approx$ 300 organoids in total per tumor)
- The organoids were imaged using differential interference contrast (DIC) microscopy
- The images were manually traced using IMAGEJ to define organoid boundaries
- corresponding K14 images are available

Fourier Transform reminder



Example


(A) Tumor 10, organoid 26
(B) Boundary
(C) Spectral power, sum = 2.391
(D) Tumor 10, organoid 35
(E) Boundary
(F) Spectral power, sum = 1.429
(G) Tumor 10, organoid 45
(H) Boundary
(I) Spectral power, sum = 0.405
Pixel
Power
Frequency

Analysis

- Features that we considered to compare against our model include the following:
  - Fractional area of the organoid
  - K14 Mean
  - K14 Sum of pixels intensities in the periphery
  - K14 Sum of pixels intensities in the center
  - K14 total pixel intensity sum

An Efficient Way to Get Peripheral Pixels

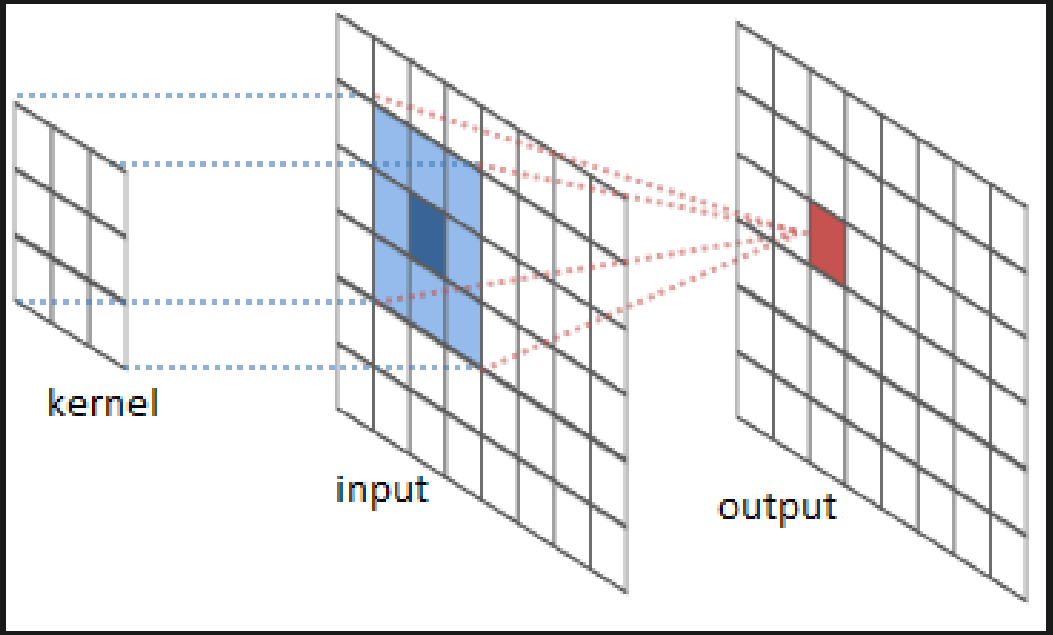
1. Generate a mask from the boundary
2. make a disk to be used as a kernel with which to convolve the mask

Example

A disk with a radius of 3 is:

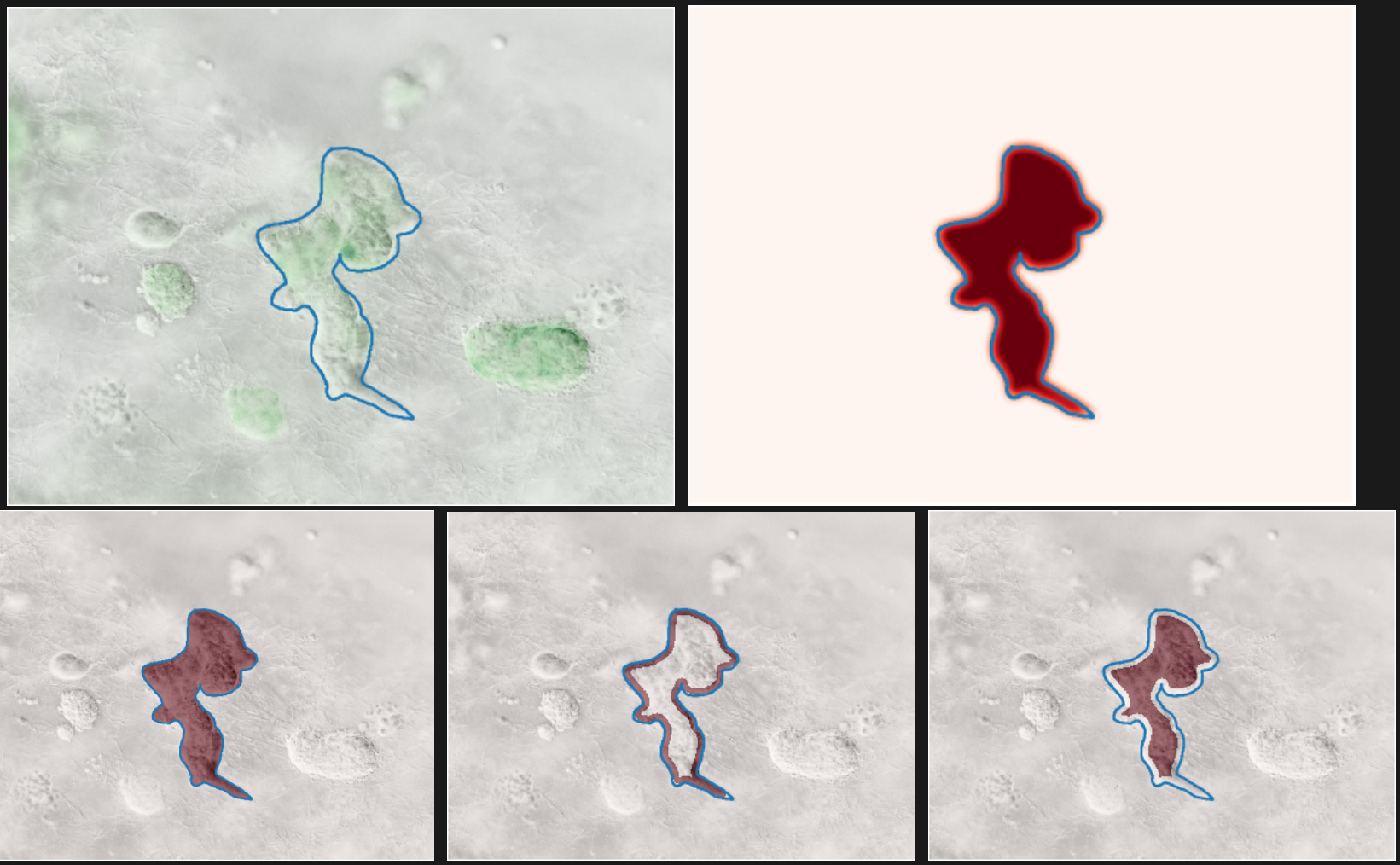
$$k = \begin{bmatrix} 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix}$$

Convolution



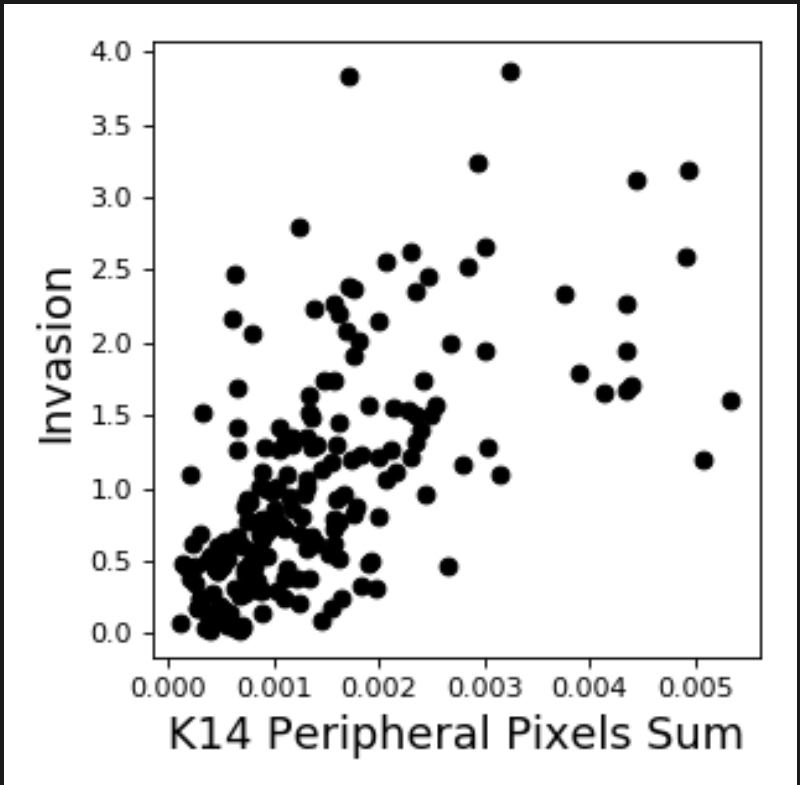
$$(f * g)[i, j] = \sum_{k=minW_{kernel}}^{maxW_{kernel}} \sum_{l=minH_{kernel}}^{maxH_{kernel}} f[i-k, j-l]g[k, l]$$

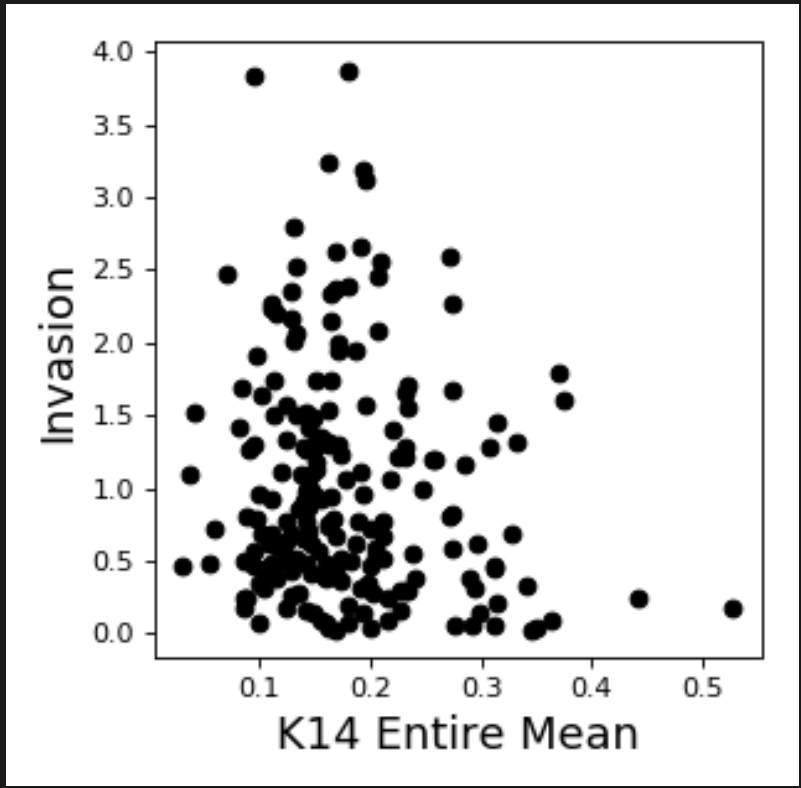
Convolution for efficient organoid segmentation



radius, $r = 20$ pixel size $\sim 0.5\mu$

Peripheral K14 expression correlates with invasive potential





Peripheral K14 explains correlates with invasive potential the best

	r^2 by mouse		
Organoids (PyMMT mice)	1	2	3
Peripheral sum K14	**0.39**	**0.43**	**0.29**
Centeral sum K14	0.21	0.19	0.11
Entire sum K14	0.22	0.22	0.12
Size	0.27	0.33	0.27

Peripheral K14 explains correlates with invasive potential the best

	r^2 by mouse			
Organoids (C1tag mice)	1	2	3	4
Peripheral sum K14	**0.11**	**0.25**	**0.06**	**0.03**
Centeral sum K14	0.01	0.03	0.06	0.00
Entire sum K14	0.01	0.00	0.01	0.00
Size	0.07	0.01	0.07	0.06

Discussion

- Looking at K14 expression in the periphery of the organoids is promising
- K14 expression in the center of the organoids correlates less with spectral score than peripheral K14 expression
- K14 Mean doesn't seem to contribute much
- Using peripheral K14 expression seems to have more significance for large organoids
- Fractional area and peripheral K14 expression give comparable r^2 values

Future Directions

- Apply our model on human organoids generated from breast tumors

Thank you!

- Joel Bader
- Joel Bader lab members

- Andrew Ewald
- Veena Padamanaban (data)
- Andrew Ewald members

- Cancer Target Discovery and Development