Tumor Invasive Scores vs. K14 Expression

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Overview

- Background Information
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- Methods
- Results
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- Future Directions

Background

- Metastasis is one of the main determinants of patient prognosis
- Mortality is often due to systemic spread of tumor and not in situ proliferation

Example

5-year survival rate for invasive breast cancer at diagnosis is:

- 99% for patient with local tumors
- 85% for patients with regional spread
- 26% for those with tumors that have spread to distance organs [Tarver, 2012]

- Currently, the molecular mechanism of metastasis is not well understood
- Neoplastic drug interventions mostly target proliferation, not metastatic transformation

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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

Related Research Work and Current Developments

- A quantitative model to study and characterize metastasis has yet to be developed
- [Shamir et al., 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 et al., 2016] demonstrated that tumor cells can invade and metastasize in clusters challenging the single-cell/single-metastasis model
- They further study how Keratin 14, a known molecular biomarker for metastasis, expressing cells intiate and complete the metastatic process

Project Objective

The first aim of the project will be to test the hypothesis that K14 is directly correlated with invasiveness versus the alternative hypothesis that organoid size drives both K14 expression and invasion

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

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Our Computer Model (Spectral Power)

- the manually traced boundary is defined by a pair of points $\{x_v, y_v\}$ for $p \in [0, P-1]$, where P is the total number of points
- First the overall length L was calculated
- $L = \sum_{v=1}^{V-1} \sqrt{(x_v - x_{v-1})^2 + (y_v - y_{v-1})^2}$
- Next, an equally spaced grid was defined with distance L/M between adjacent points, where $M = 128$
- Then, linear interpolation was used to generate a new boundary with equally spaced points $\{x_j, y_j\}$, where $j \in \{0, 1, ..., M-1\}$

- Then, a fast Fourier transform on the newly generated boundary is performed according to:

$$\hat{x}_k = \sum_{j=0}^{M-1} e^{-2\pi ijk/M} x_j$$

$$\hat{y}_k = \sum_{j=0}^{M-1} e^{-2\pi ijk/M} y_j$$

- where $k \in \{0, 1, ..., M/2 + 1\}$

- The spectral power P_k is given by:
  $P_k = |\hat{x}_k|^2 + |\hat{y}_k|^2$

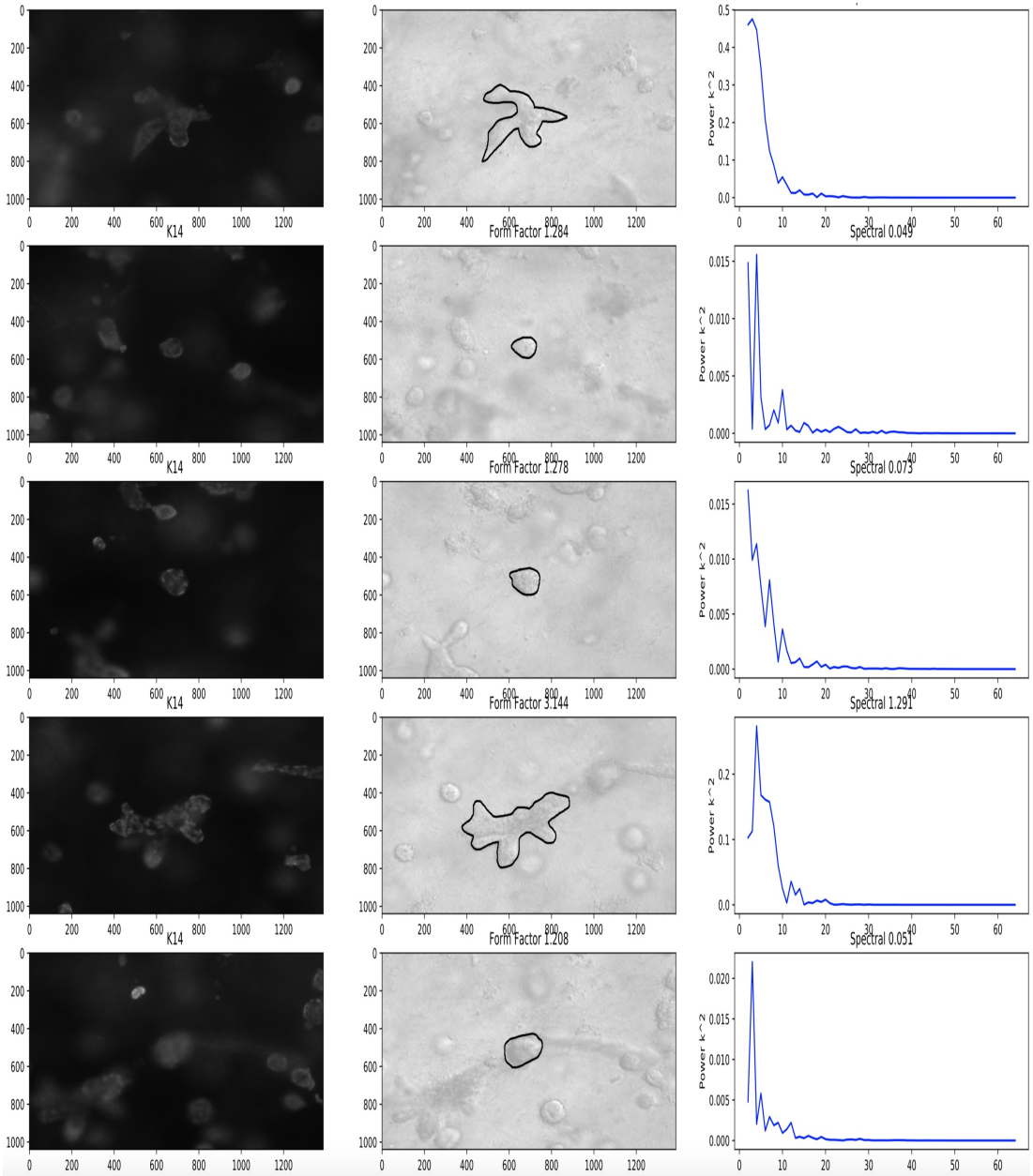
- where $k \in \{0, 1, ..., M/2 + 1\}$
- P_0 is the center and P_1 is used to normalize the total power given by:

$$P_{total} = \sum_{k=2}^{M/2+1} P_k / P_1$$

- this produces a spectral power that is scale invariant in addition to being transilation and rotation invariant.

- Means and parametric derivative transforms are estimated
- This is done to smooth out artifacts in the boundary
- The transform for $\bar{x}_{j\prime} \equiv (x_{j\prime+1/2} + x_{j\prime-1/2})/2$ is:
  $\hat{\bar{x}}_k = \cos(\pi k/M)\hat{x}$

- The transform for $\dot{x}$ is:
  $\hat{\dot{x}} = (iM/\pi)\sin(\pi k/M)\hat{x}$
- Therefore, the final weighted spectral power becomes:
  $$w \equiv \sum_{k=2}^{M/2} (M/\pi)^2 \sin^2(\pi k/M)\cos^2(\pi k/M) P_k/P_1$$



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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:

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

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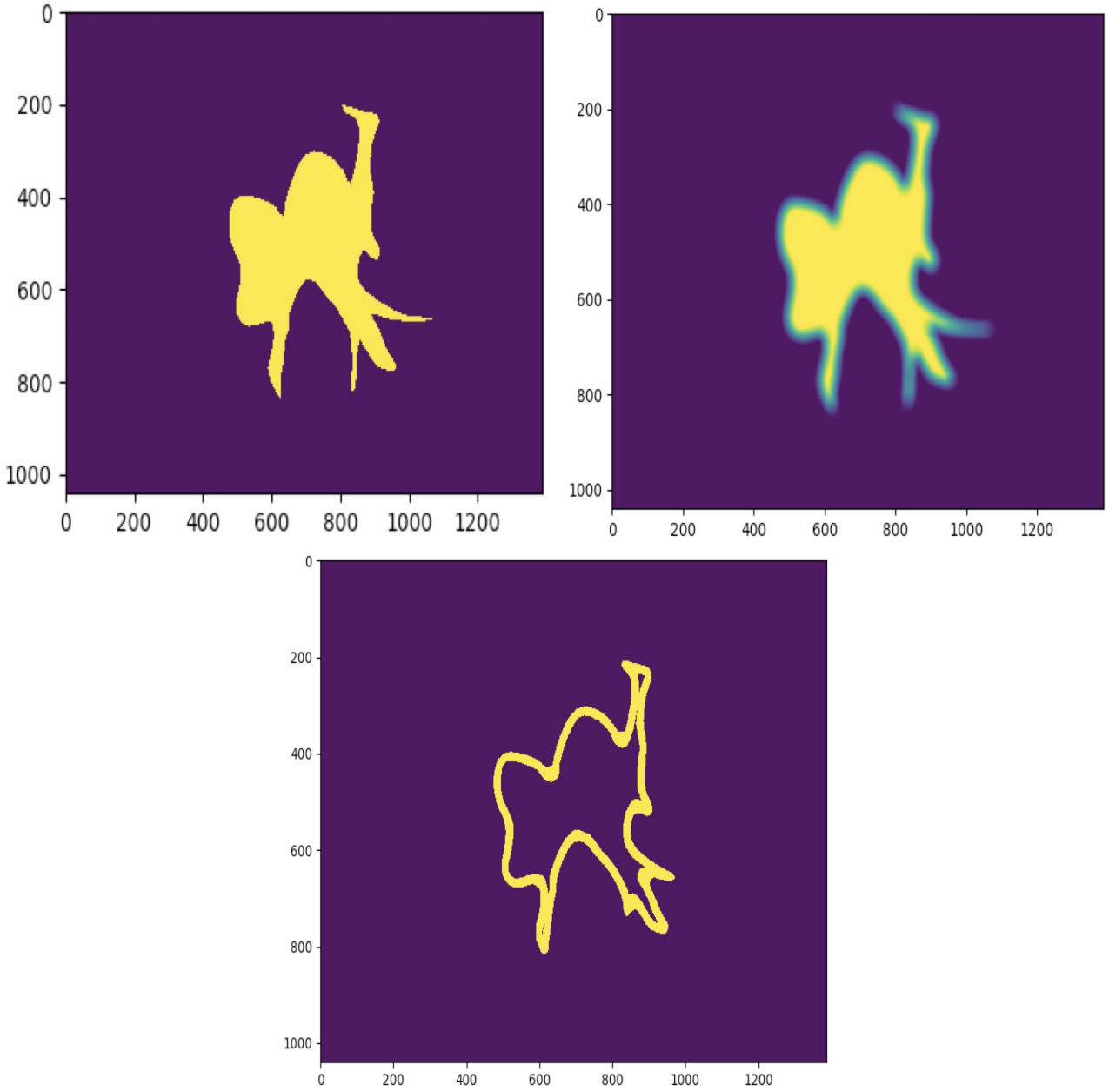

radius, $r = 20$.

$pixelsize \sim 0.5\mu$

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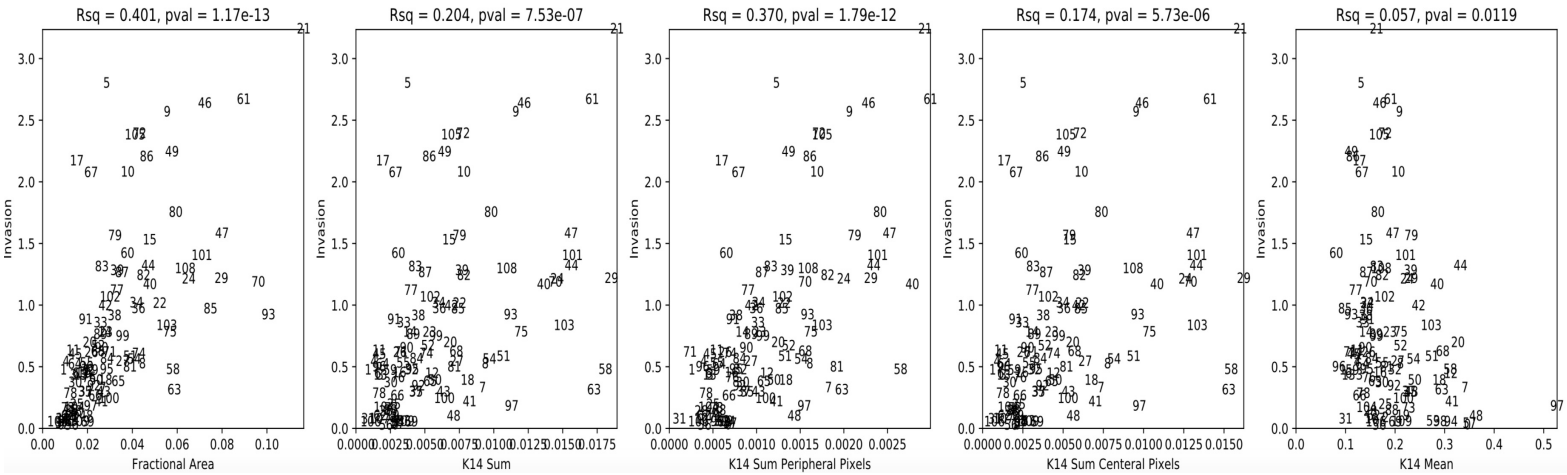
Small Organoids



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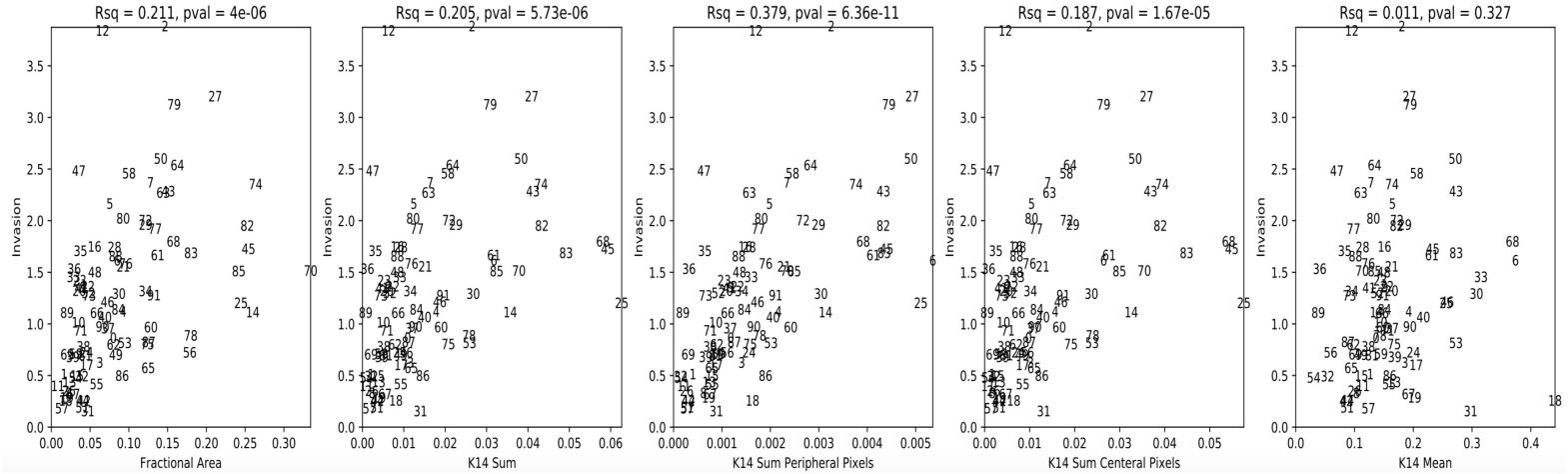
Large Organoids



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Organoids (mouse# Size)	1 Small	1 Large	2 Small	2 Large	3 Small	3 Large
Fractional Areav(r^2)	**0.401**	0.211	**0.518**	0.239	**0.493**	0.110
Peripheral K14(r^2)	0.370	**0.379**	0.464	**0.339**	0.333	**0.179**
Centeral K14(r^2)	0.174	0.187	0.337	0.093	0.251	0.013
Total K14(r^2)	0.204	0.205	0.361	0.110	0.264	0.019
K14 Mean(r^2)	0.057	0.011	0.023	0.239	0.011	0.023

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Discussion

- Looking at K14 expression in the periphery of the organoids holds some promise
- 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

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Future Directions

- Mouse #4 data seems to be slightly off, so fix it
- Establish statistical significance for our hypothesis
- Apply our model on human organoids generated from breast tumors

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Thank You

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