Machine Learning Framework for Dynamic NIR Fluorescence Based Classification of Congenic Mapping in Rats

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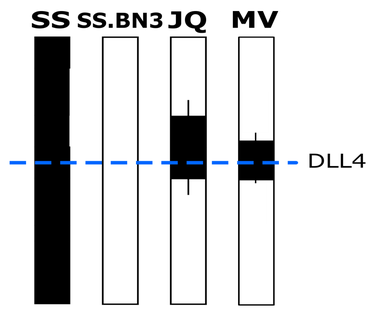
Introduction

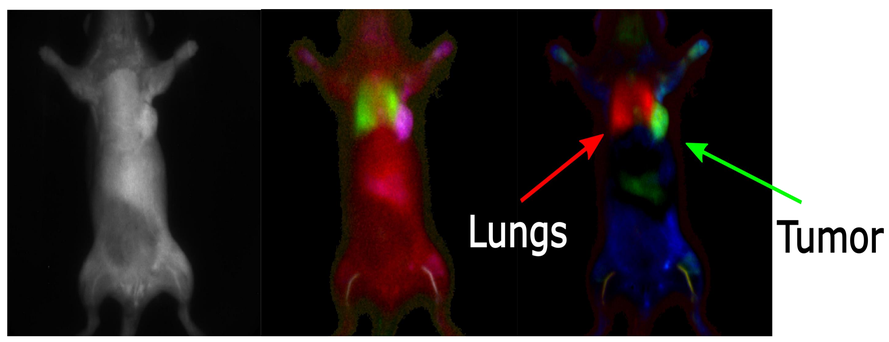
- While there is a large emphasis in current cancer research to understand tumor-cell specific mutations, much less emphasis is placed on understanding germline or host-specific genetic variants which can affect tumor growth and response to therapy.
- Dynamic near-infrared fluorescence (DyNF) imaging provides the ability to measure and characterize the vascular supply in the tumor microenvironment (TME) [1], and herein we report a framework for using DyNF for identifying endogenous genetic modifiers.
- In order to discover and identify subtle phenotypical differences in the TME, we propose and demonstrate the use of machine learning classifiers on Near Infrared (NIR) time series images for the purpose of identifying genotypical differences governing vascular perfusion and permeability.

Methods

- We previously reported vascular function differences in identical tumor xenografts implanted in consomic rats based on immune compromised (IL2Rγ mutant) parental salt sensitive rat strain (SS) with chromosomal substitution from the tumor resistant Brown Norway strain [2].
- To further pinpoint the genes on 3rd chromosome responsible for tumor promoting and therapy resistance driving behavior, congenic mapping was performed to generate rat strains which differ only by cross-overs on the third chromosome (Fig 1B).
- Each of the strains, SS.BN3, JQ, and MV, had MDA-MB-231 cells orthotopically implanted which resulted in differential tumor growth and metastasis.
- After allowing the tumors to grow to a similar size (~10 days), the DyNF imaging of the rats was performed using a deep-cooled intensified charge-coupled device (PiMAX Princeton Instruments) at 50 ms temporal resolution for 6 minutes following a 1 mL bolus injection of 400 µM indocyanine green delivered via a tail vein catheter (λex/λem=785nm/830nm).
- Rat organs, identified using principal component analysis [3], were hand segmented (Fig 1A).
- For each tumor mask, pixels were averaged, and a single time series was recorded (Fig 1C), along with the variance for each time point, resulting in 3,000 features for each tumor.
- These features were then used to train a Gaussian Support Vector Machine using 21 JQ and 17 SS.BN3 rats.

Congenic Rat Model

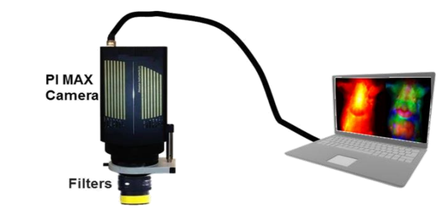


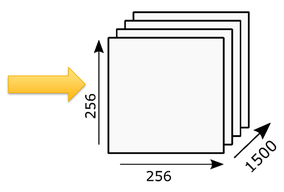


Rat NIR Imaging

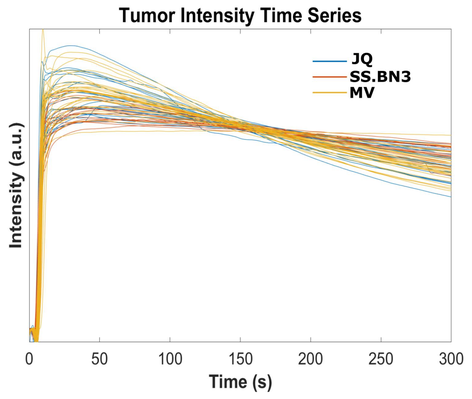
Principal Component Analysis

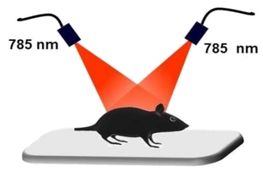
Segment Tumor and Average Pixels





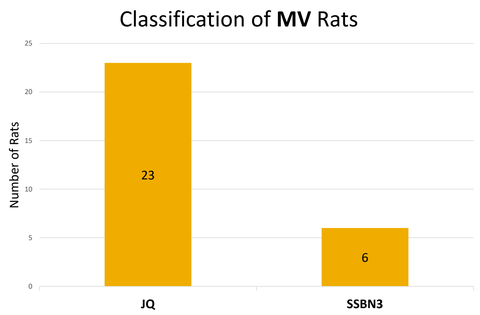
Time series images



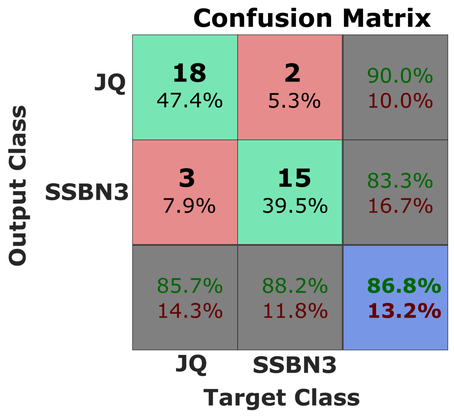


Schematic of Imaging Setup

Train Classifier on 2 of 3 Classes



Classify Untrained (**MV**) Class



Quick Points

- The goal of the project was to develop a robust and objective method of quantifying a phenotype of interest (vascular perfusion)
- The above method can be generalized to multiple classes
- This framework allows for a more refined approach to identifying endogenous genetic modifiers

References

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3. Hillman, E.M. and A. Moore, *All-optical anatomical co-registration for molecular imaging of small animals using dynamic contrast.* Nat Photonics, 2007. 1(9): p. 526-530.
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Results

- The classifier achieved 84% accuracy using 4-fold cross validation.
- The classifier was then presented with 29 NIR time series from MV rats, 23/29 of which it correctly classified as belonging to JQ group.
- Further, this result points to genes underlying the differential vascular function in congenic strains as MV and JQ strains both possess DLL4 containing chromosome 3 region from tumor promoting parental SS strain.
- DLL4 is a notch pathway gene known to be associated with vascular morphology in tumors and has higher expression levels in SS [4].
- The accuracy of the classifier is promising because from visual inspection, it is difficult to identify the rat strain from the time series alone due to the large overlap.
- Even when looking for natural clusters of the data through PCA or t-Stochastic Neighbor Embedding, there doesn't appear to be a strong natural clustering, thus showing the need for advanced classifiers to be used.

Future Focus

- Our result shows that for tumors which all originate from identical tumor lines, differences in the TME as imaged through DyNF, can be combined with machine learning to efficiently classify phenotypical differences resulting from host specific genetics.
- Our plan is to increase the number of classes the classifier is trained on, and to train the classifiers on different organs as well.
- The techniques can be extended to Dynamic Contrast Enhanced MRI as well, which will enable cross-sectional assessment of vascular function.

Funding Resources

Michael J. Flister (NIH R01 CA193343, MCW Cancer Center, and Center for Imaging Research, Radiology
Amit Joshi (Alliance for healthy Wisconsin, Rock River Cancer Research Foundation and MCW Research Affairs Committee Pilot Grant), Wisconsin Breast Cancer Showhouse Award.

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