



QUANTITATIVE ANALYSIS OF MOLECULAR ULTRASOUND DATA

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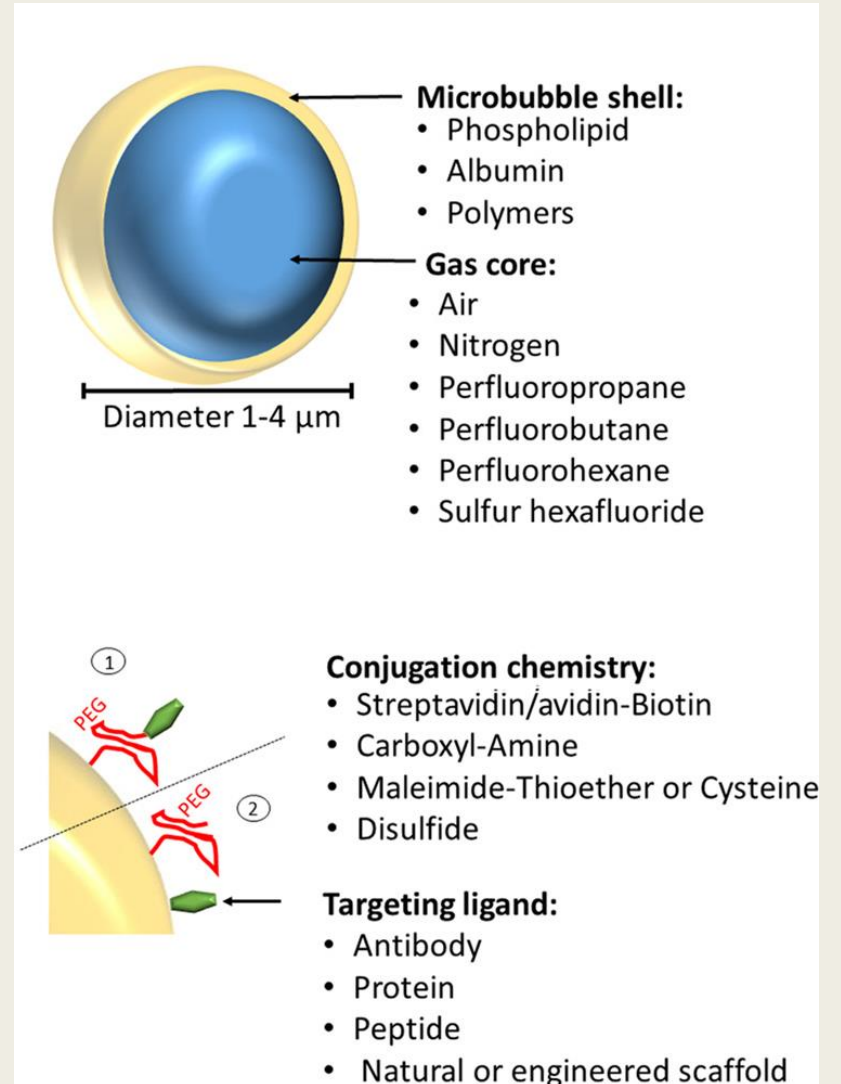
October 5, 2017

Background

- Ovarian cancer is the leading cause of gynecologic-cancer associated mortality; most women have advanced disease (stage III) at diagnosis.
- Over the course of a lifetime, one in eight women develop breast cancer.
- Ultrasound is a relatively inexpensive, non-invasive, and real-time imaging modality.
- Traditional ultrasound is widely used to assess potential ovarian tumors; it is used in conjunction with mammography for breast lesions.
- However, radiologists are often presented with lesions that represent a diagnostic dilemma.

Contrast Enhanced and Molecular Ultrasound

- Dynamic contrast-enhanced ultrasound (CEUS) has recently emerged as a tool to aid in the characterization of lesions.
- Imaging can be performed within minutes of administration of the molecular agent.
- Microbubbles are predominantly cleared by the liver and spleen, rather than the kidneys.
- Molecularly targeted microbubbles involve binding a ligand to the microbubble shell, causing them to accumulate where the ligand naturally binds.



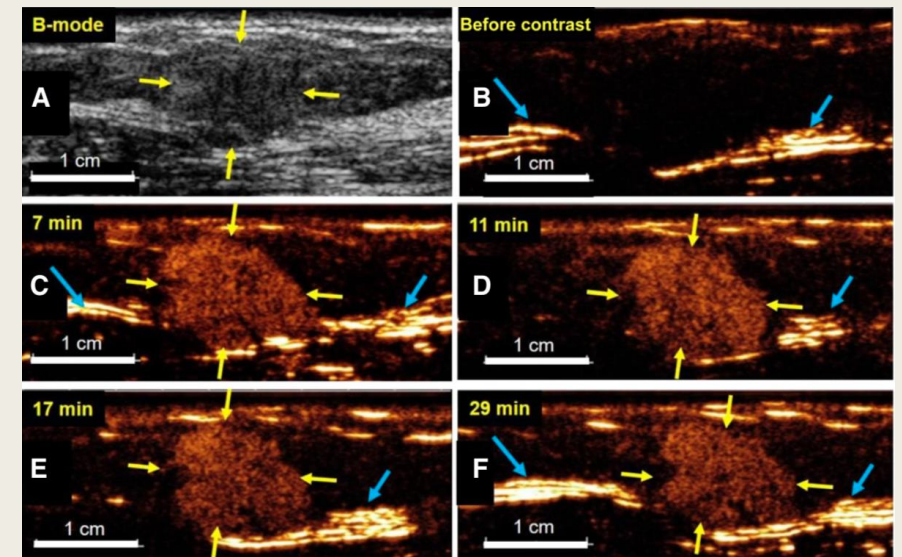
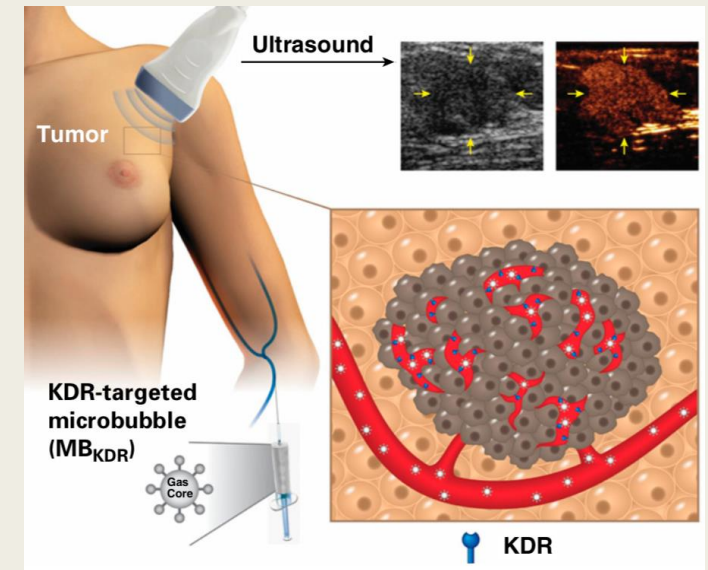
Abou-Elkacem, 2015.

Selected prior experiences with molecular ultrasound

- Ultrasound molecular imaging (USMI) has been used to study inflammatory bowel disease using targeted microbubbles designed to attach to P-selectins and E-selectins, allowing for quantification of inflammation in a porcine model of terminal ileitis (Wang, 2013).
- USMI has been used in a primate model of myocardial ischemia, in which selectin-targeted myocardial contrast echocardiography showed a significant increase in signal within ischemic myocardium (Davidson, 2014).
- The USMI agent BR55 was used to detect submillimeter foci of pancreatic cancer in a transgenic mouse model (Pysz, 2015).
- Another study using USMI agent BR55 analyzed mice with human colon cancer xenografts and showed that molecular imaging signal decreased by 57% after antiangiogenic treatment compared to the control group (Want, 2015).

Molecular Agent BR55

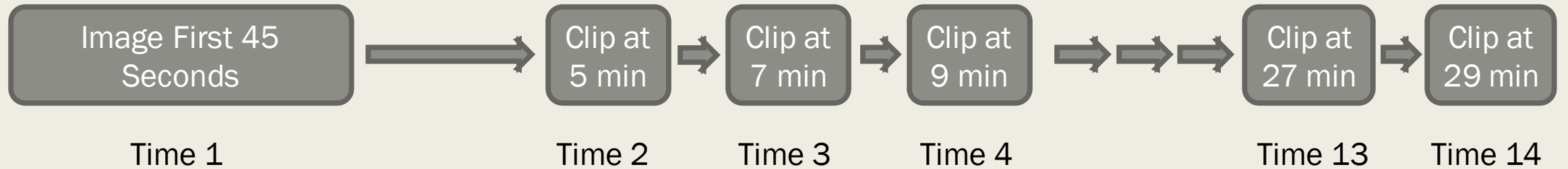
- VEGF is a signaling protein related to angiogenesis; it is highly expressed in the lumen of endothelial cells in multiple cancer types.
- BR55 is a VEGF-targeted molecular contrast agent that specifically targets the kinase domain receptor (KDR).
- Strong molecular ultrasound imaging signal persists for much longer than it does for CEUS, due to the molecular agent continuing to bind to its receptor.



Clinical Trial Data

- The first-in-human clinical trial of ultrasound molecular imaging in breast and ovarian cancer patients was spearheaded by Dr. Willmann and performed using clinical-grade contrast agent BR55.
- 24 women with ovarian lesions and 21 women with breast lesions were enrolled in the trial.
- Pathological outcomes were obtained, including whether the lesion was benign or malignant and KDR expression.
- Experienced radiologists qualitatively assessed the presence of molecular ultrasound imaging signal, defined as focal enhancement visible after freely circulating microbubbles had disappeared.

Imaging Sequence



Initial Arrival of Contrast Agent.

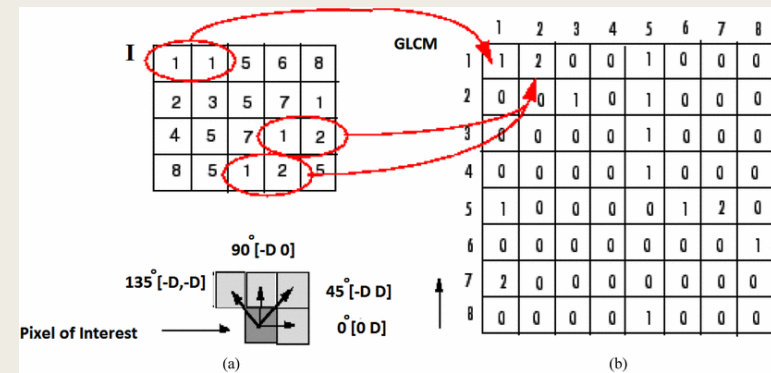
Persistent binding of the molecular agent should represent true molecular imaging signal after the disappearance of freely circulating microbubbles.

Final timepoint.

Data Analysis Methods: Analyzing US Molecular Imaging Signal

- Label the timepoint for each DICOM series for each patient. Import the segmentation of the region of interest (ROI) into Matlab as well as the DICOM data for each timepoint.
- Focus on the texture of the lesion at later timepoints, when all freely circulating microbubbles have cleared. Take a single-frame snapshot of the lesion within the ROI in the middle of this timepoint, as well as the average of a small time of frames.
- Extract texture features (i.e. Haralick texture features). Standardize features and perform principal components analysis. Use support vector machines for classification, and validate using *leave-one-out cross validation* to obtain a score for each patient.
- Use scores to generate receiver operating characteristic (ROC) curves.

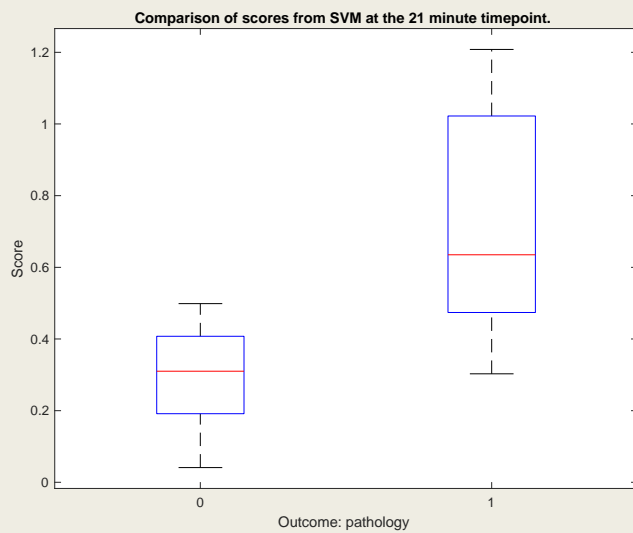
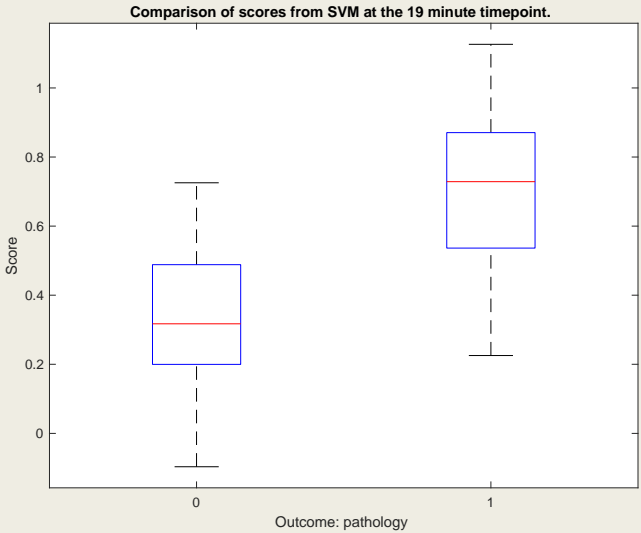
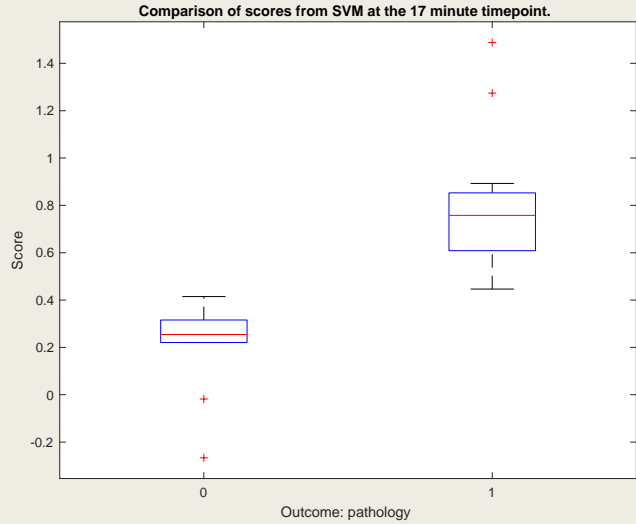
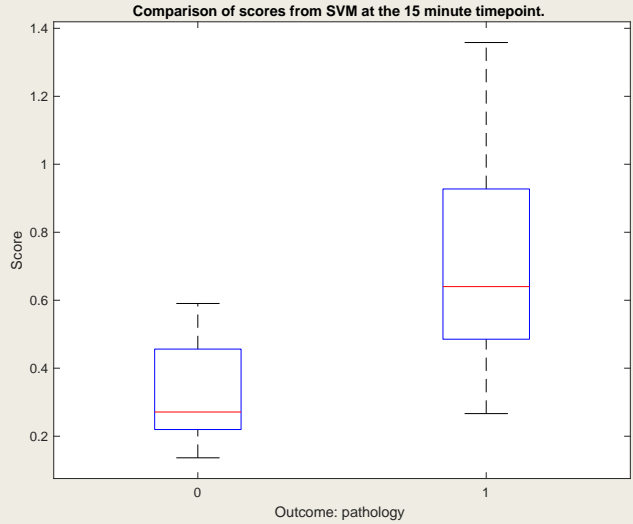
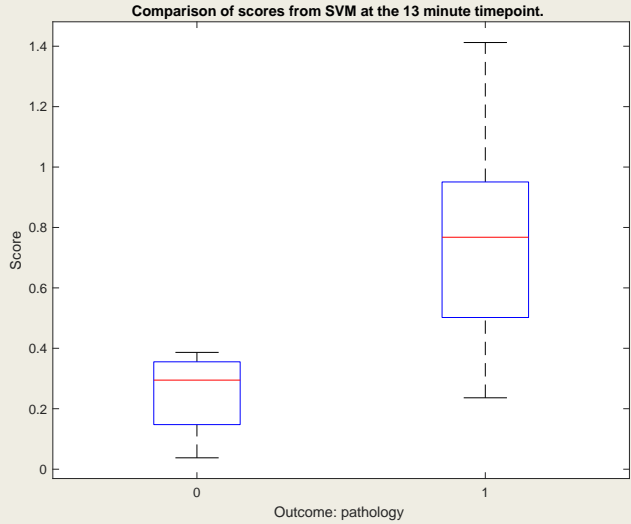
Texture Analysis



Haleem, 2015

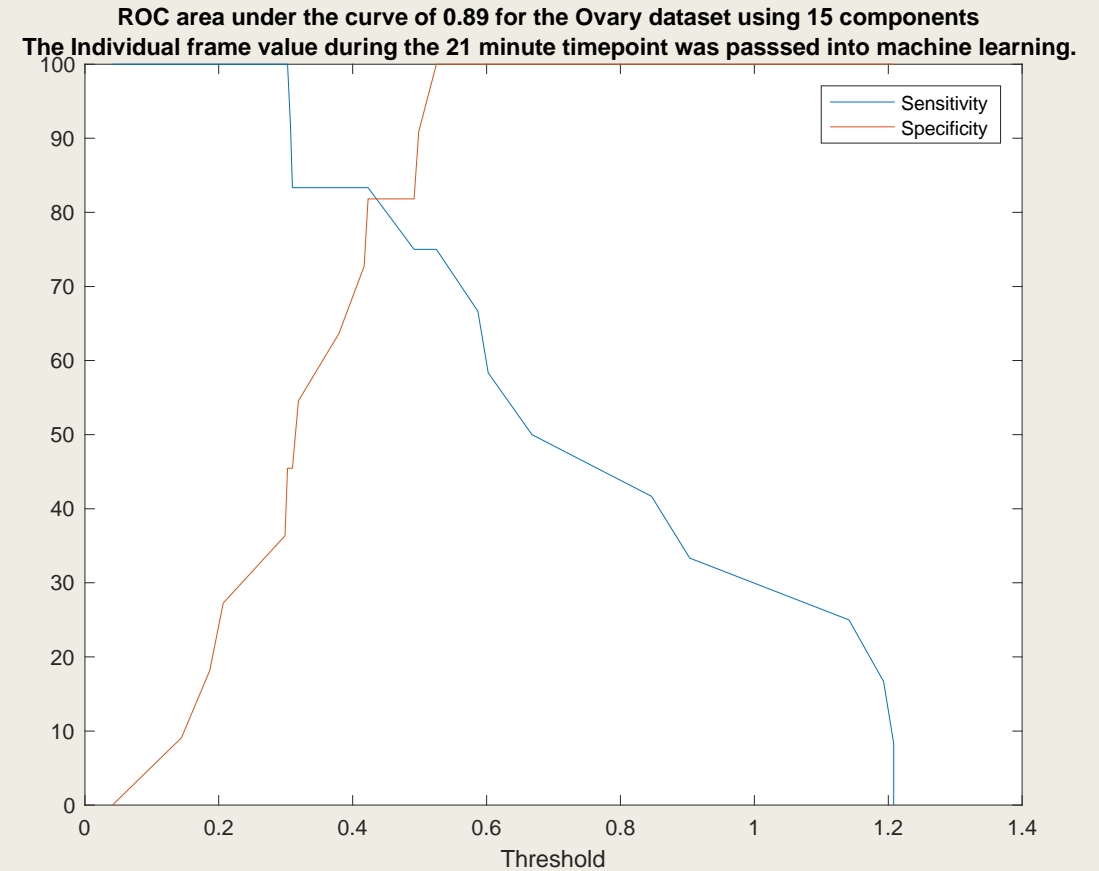
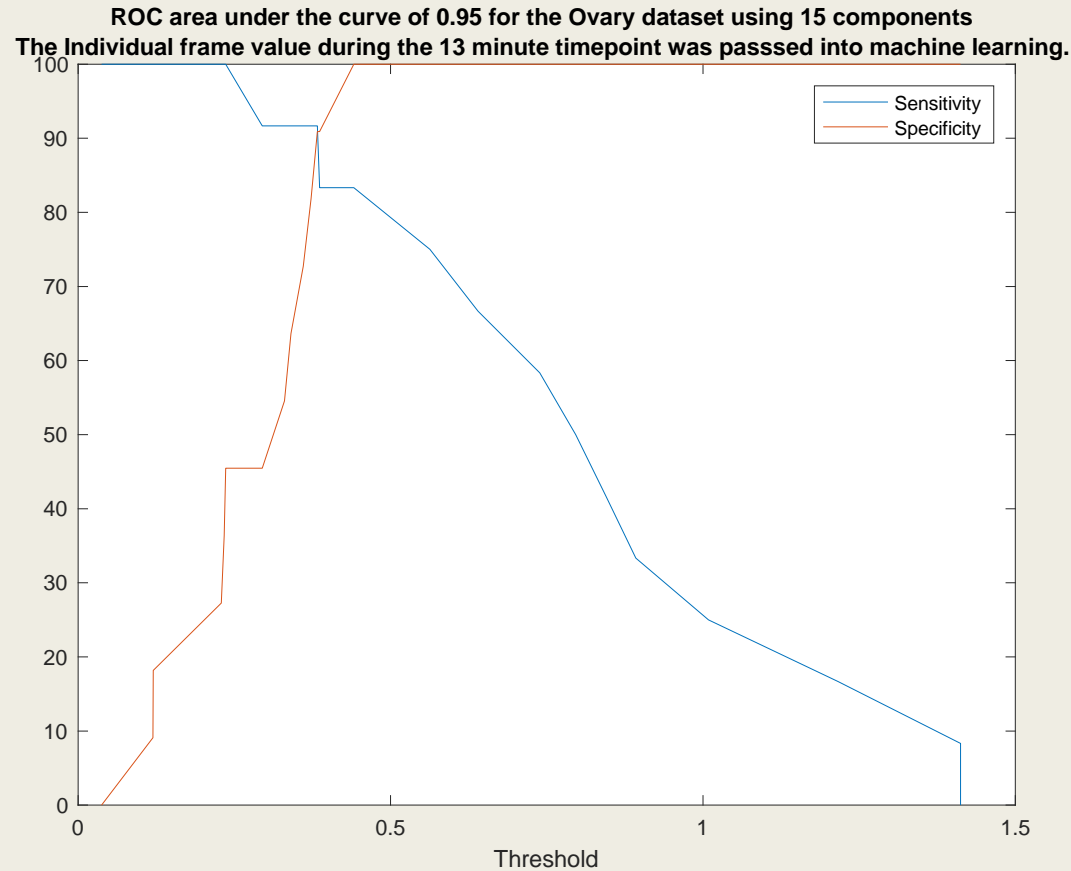
- A gray-level co-occurrence matrix (GLCM) examines the spatial relationships between pixels by calculating how often pairs of pixels with specific gray level values occur at specified spatial relationships (Haralick, 1973); this procedure creates a gray level co-occurrence matrix.
- Haralick then described statistics that could be calculated from the co-occurrence matrix, (contrast, homogeneity, entropy, energy, correlation, variance, etc.)
- We also performed initial analyses using other texture analysis techniques, including gray level histograms, Haar wavelets, and Canny edge detection. While those did not perform as well as the GLCM in our initial tests, we will analyze more techniques in the future (e.g. Reisz wavelets).

Scores from SVM at different times vs. Pathology.



Note: leave-one-out cross validation was used to generate scores.

Example ROC curves at 13 minutes and 21 minutes.



Experiment was repeated averaging 10 frames within each timepoint.

Time:	13 min	15 min	17 min	19 min	21 min	Avg.
Individual Frame	0.95	0.87	1.00	0.85	0.89	0.91
Average 10 frames	0.88	0.67	0.84	0.70	0.89	0.80

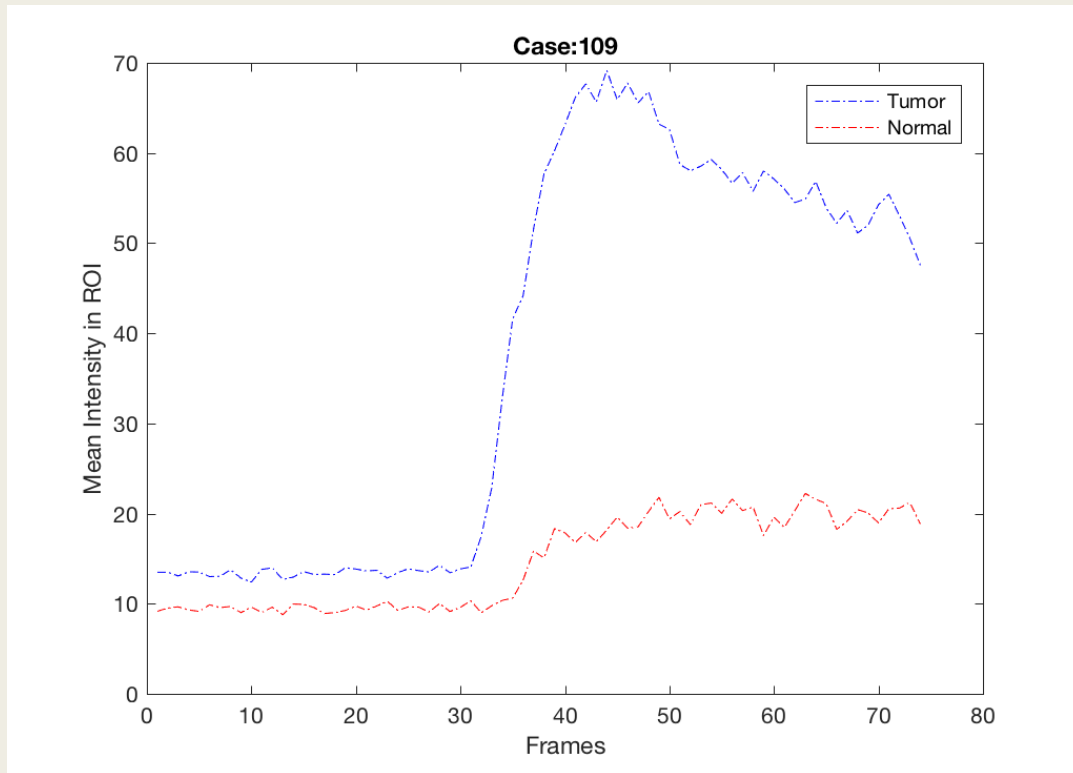
Hypothesis:

Averaging frames should lower performance, as texture will not be as reliably captured due to motion, which will affect the performance of the GLCM, which looks at relationships between gray values.

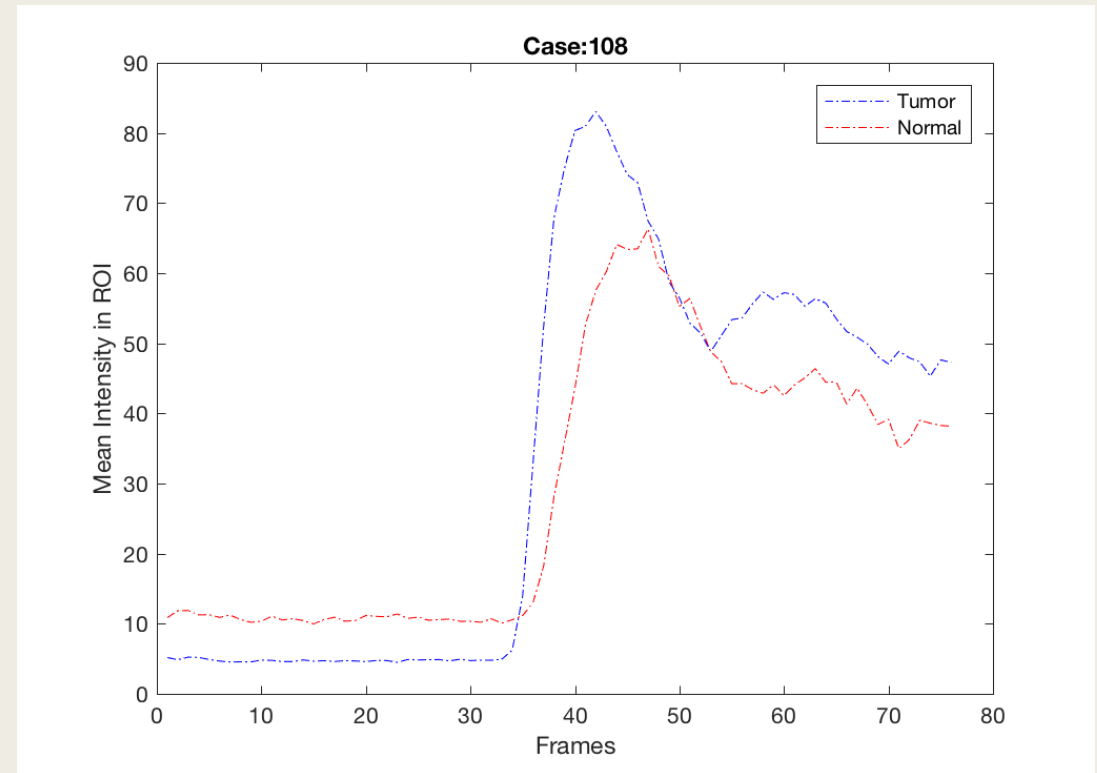
Data Analysis Methods: Perfusion Parameters

- Label data and import into Matlab.
- Generate time-intensity curves and parametric maps.
- For time-intensity curves, analyze the first time-point separately, as well as the entire 30 minute duration. Analyze individual values of PE/AUC/TTP within an ROI as well as their combination. Using logistic regression with the pathologic outcome (benign vs. malignant) as the response variable, obtain scores for each patient.
- For parametric maps, extract texture features (i.e. Haralick texture features). Standardize features and perform principal components analysis. Use support vector machines for classification, and validate using leave-one-out cross validation to obtain a score for each patient.
- Use scores to generate receiver operating characteristic (ROC) curves.

Examples: Comparison of time-intensity curves in the ROI over the first 45 seconds.

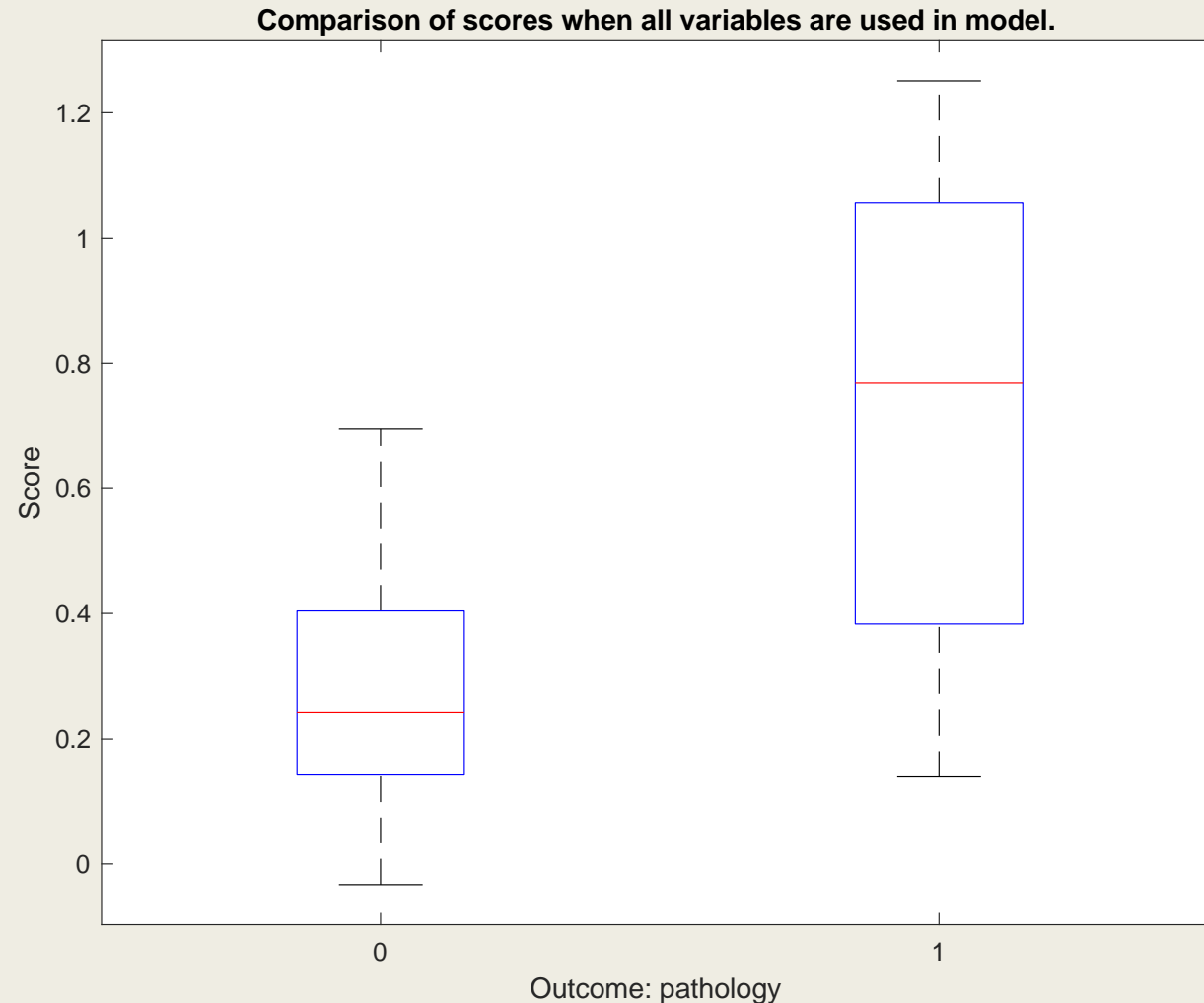


Sometimes curves very clearly separate in the first time point.



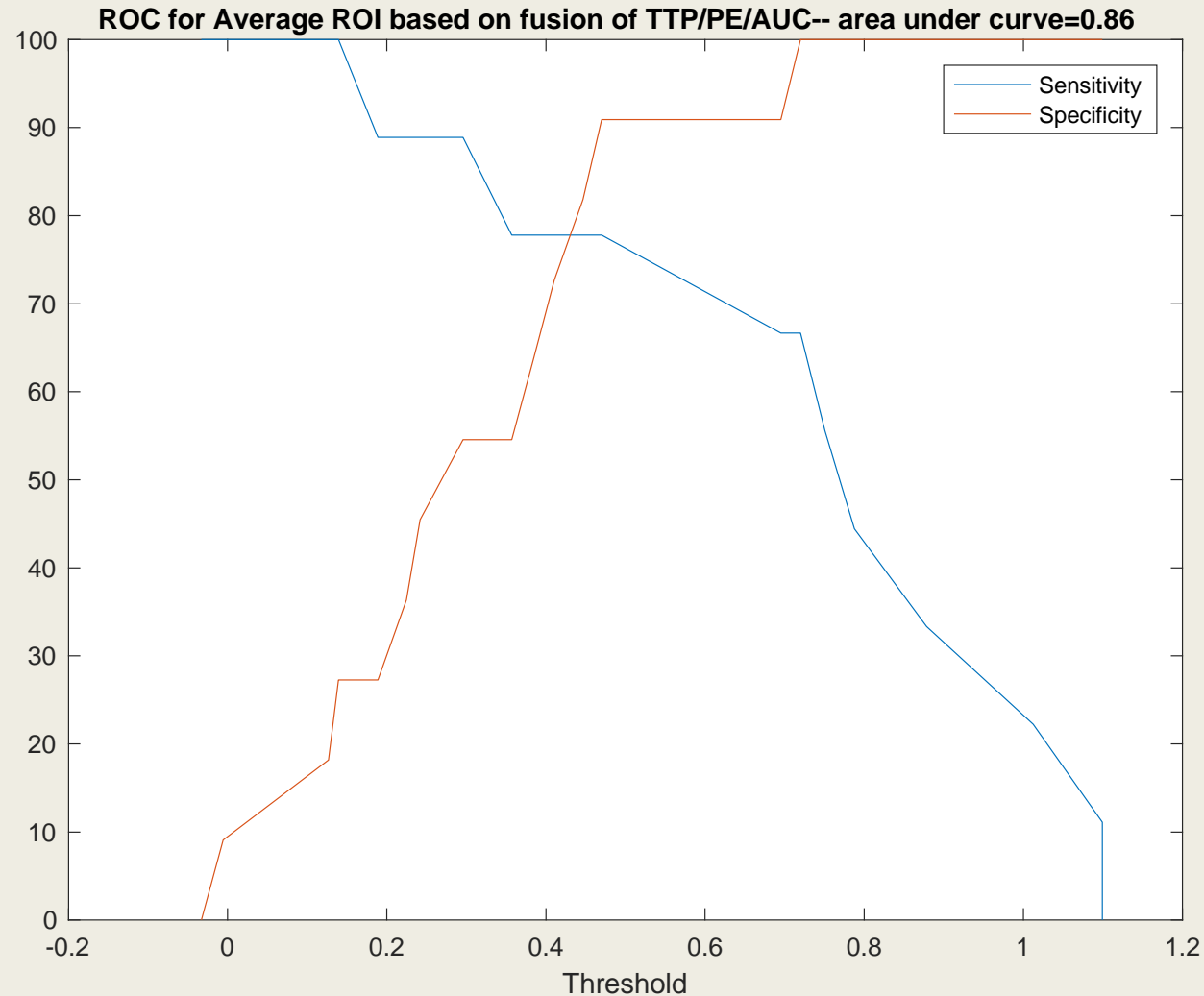
Other times not so much.

Results for the Ovary dataset using the mean value of PE, AUC, & TTP in the ROI over the first 45 seconds to predict pathology.



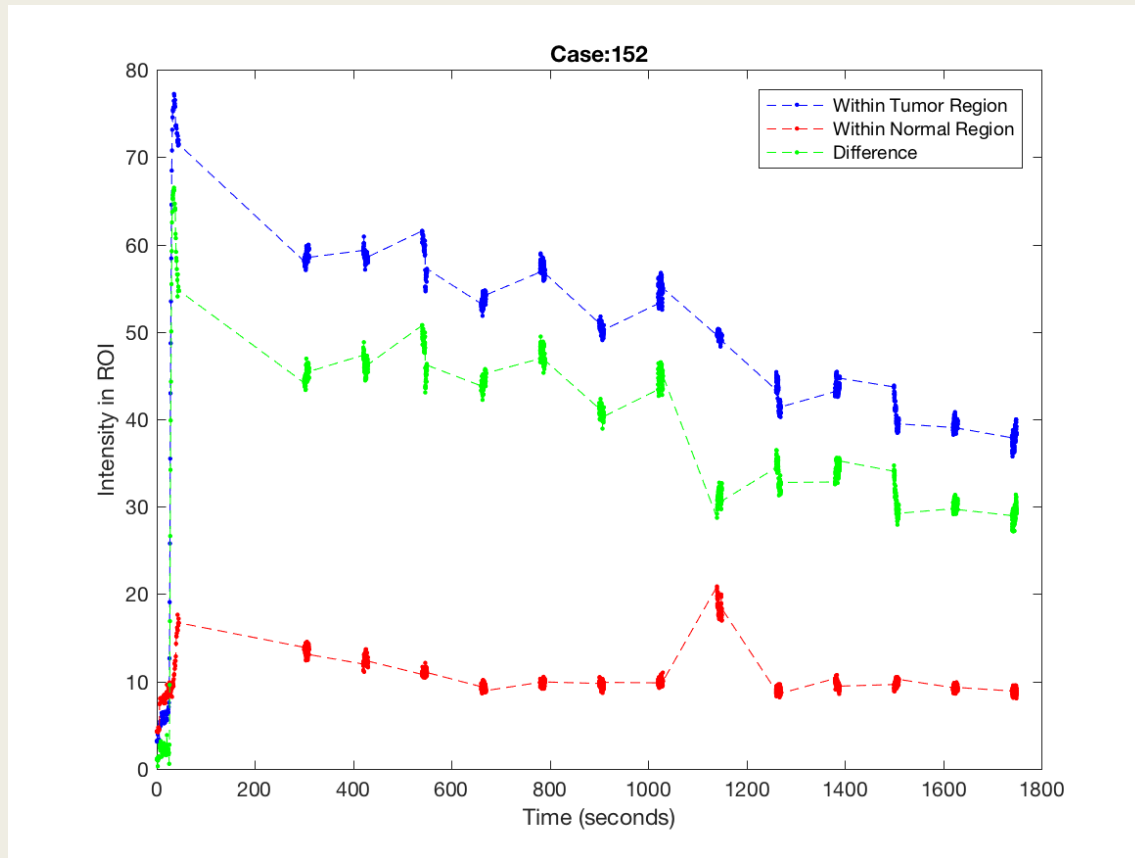
Parameter	ROC AUC
PE	0.58
AUC	0.53
TTP	0.72
Fusion	0.86

Results for the Ovary dataset using the mean value of PE, AUC, & TTP in the ROI over the first 45 seconds to predict pathology.

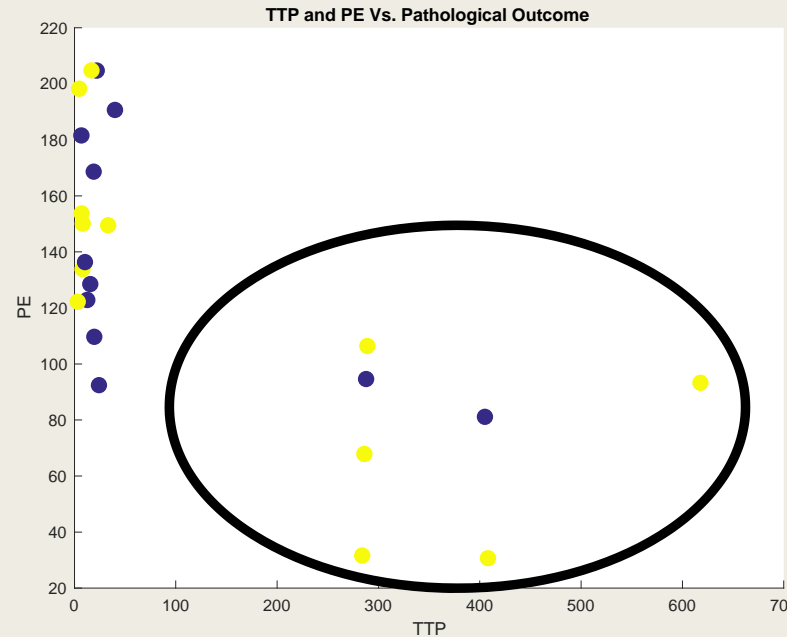
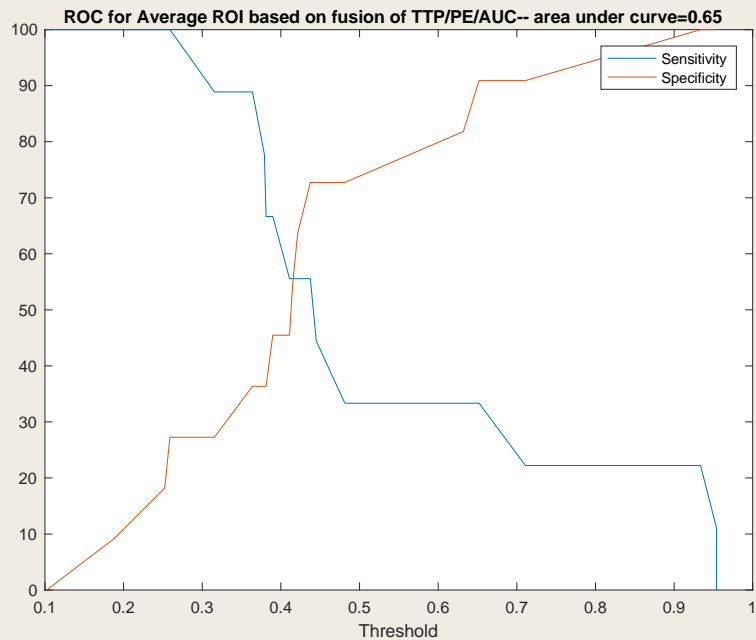


Parameter	ROC AUC
PE	0.58
AUC	0.53
TTP	0.72
Fusion	0.86

Example: It is also possible to look at time intensity curves in designated ROIs over the entire 30 minutes of imaging.



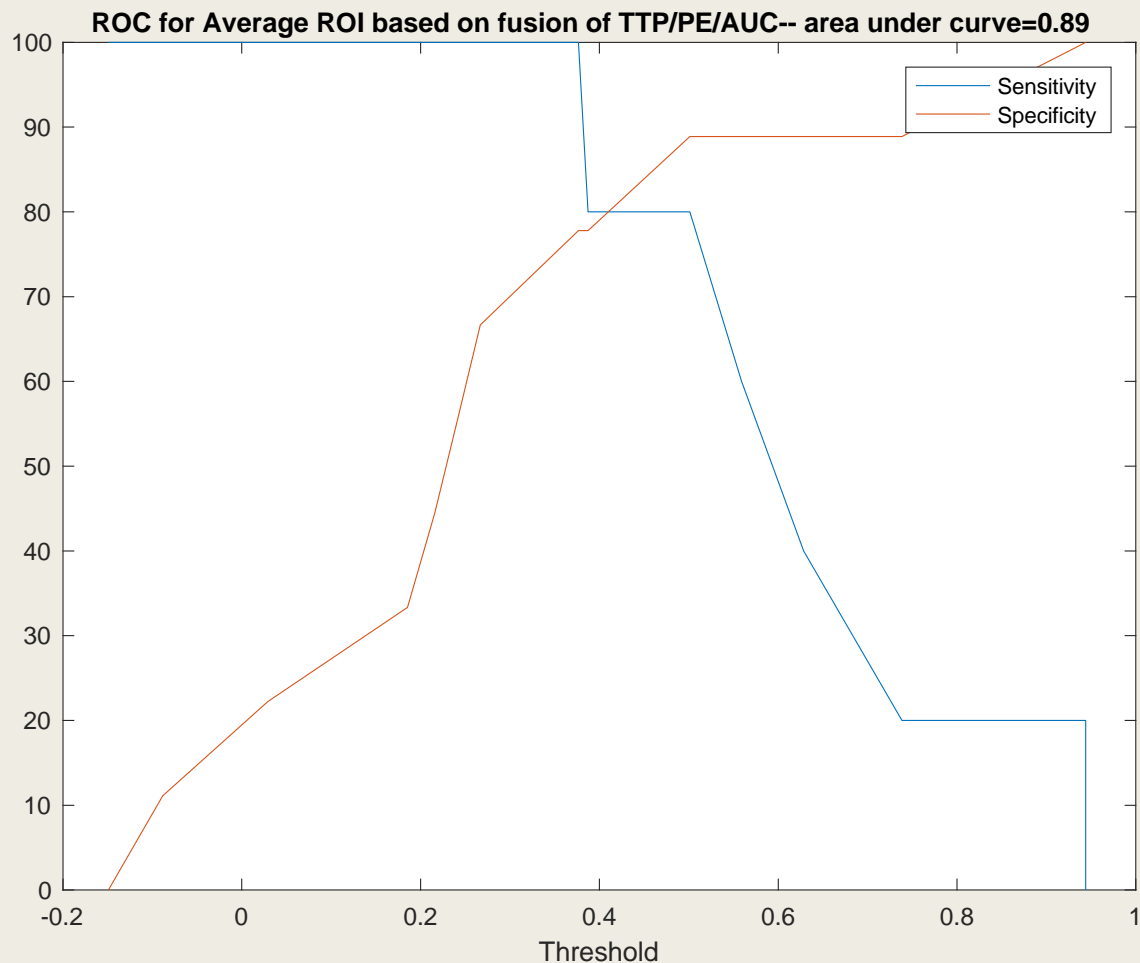
Results for the Ovary dataset using the mean of PE, AUC, & TTP in the ROI over the entire 30 minutes to predict pathology, without a boundary condition for TTP.



Parameter	30 min AUC
PE	0.57
AUC	0.55
TTP	0.47
Fusion	0.65

Keep in mind that the second time-point starts at 5 minutes (300 seconds). Hence, due to the discontinuous nature of the data, if the peak happens after the first 45 seconds, there is significant error in the estimation of time-to-peak.

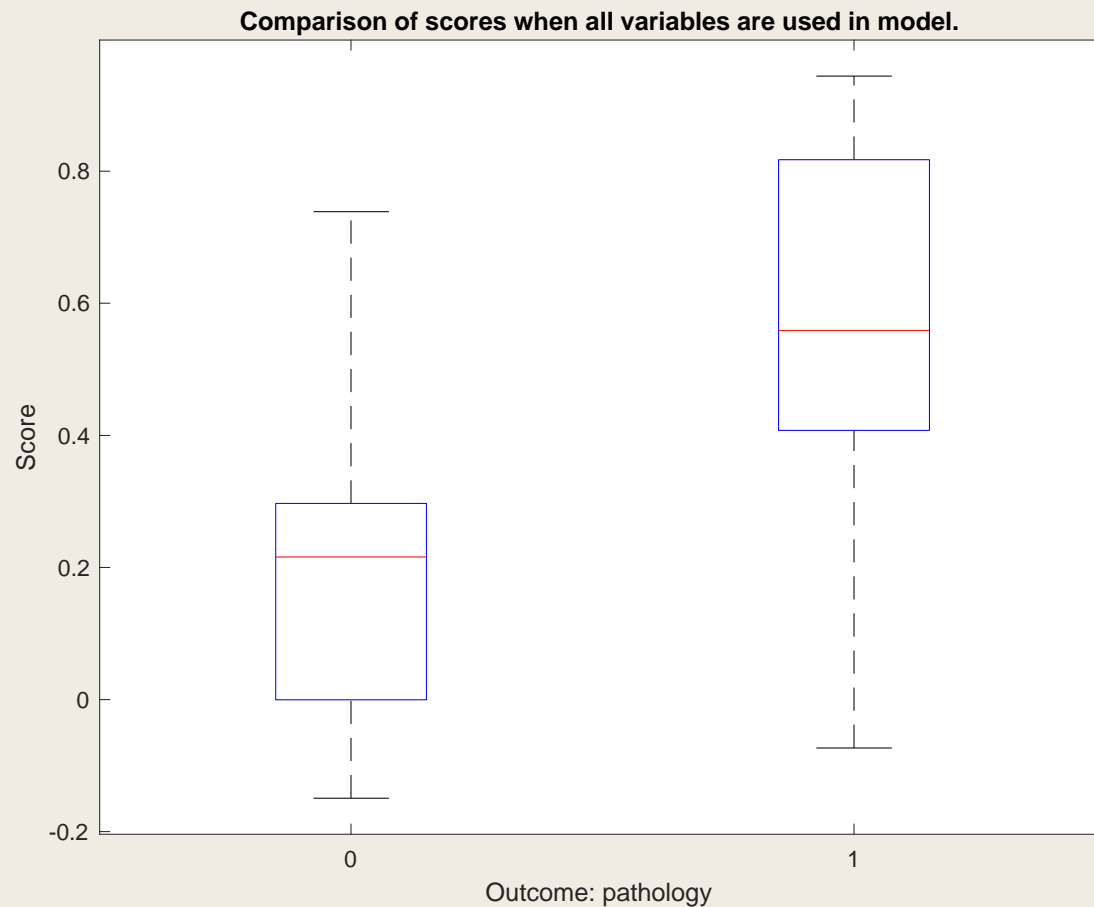
Results for Ovary dataset using mean of PE, AUC, & TTP in the ROI over the entire 30 minutes to predict pathology, **using a boundary condition for TTP (<100 sec)** due to discontinuous data collection.



Parameter	30 min AUC	First 45 sec
PE	0.69	0.58
AUC	0.55	0.53
TTP	0.84	0.72
Fusion	0.89	0.86

Using interpolated curves yields similar performance.

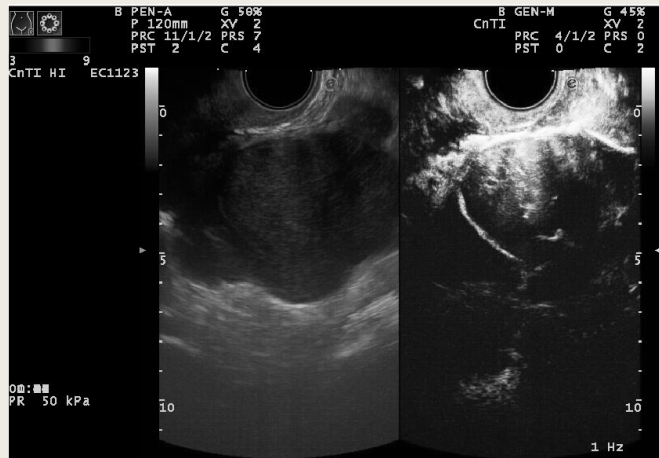
Results for Ovary dataset using mean of PE, AUC, & TTP in the ROI over the entire 30 minutes to predict pathology, **using a boundary condition for TTP (<100 sec)** due to discontinuous data collection.



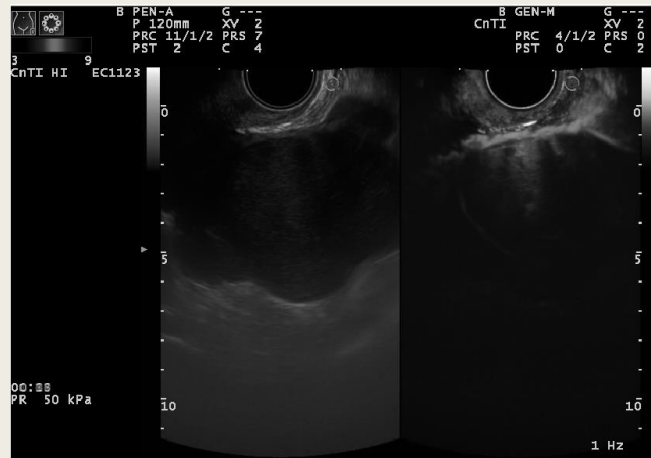
Parameter	30 min AUC	First 45 sec
PE	0.69	0.58
AUC	0.55	0.53
TTP	0.84	0.72
Fusion	0.89	0.86

Using interpolated curves yields similar performance.

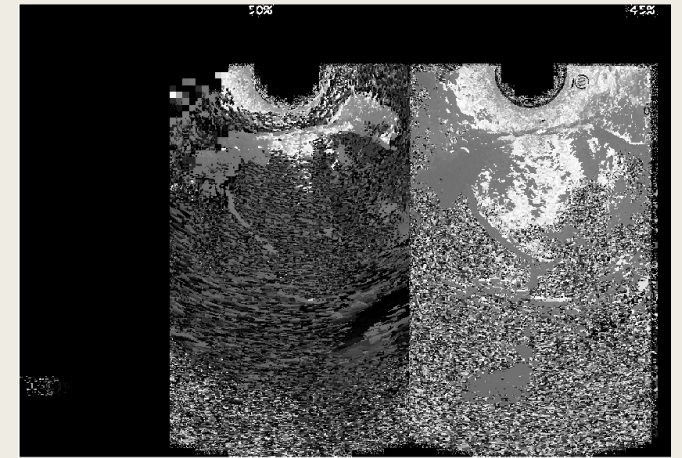
Example parametric maps from the first 45 seconds in Ovary Dataset



PE



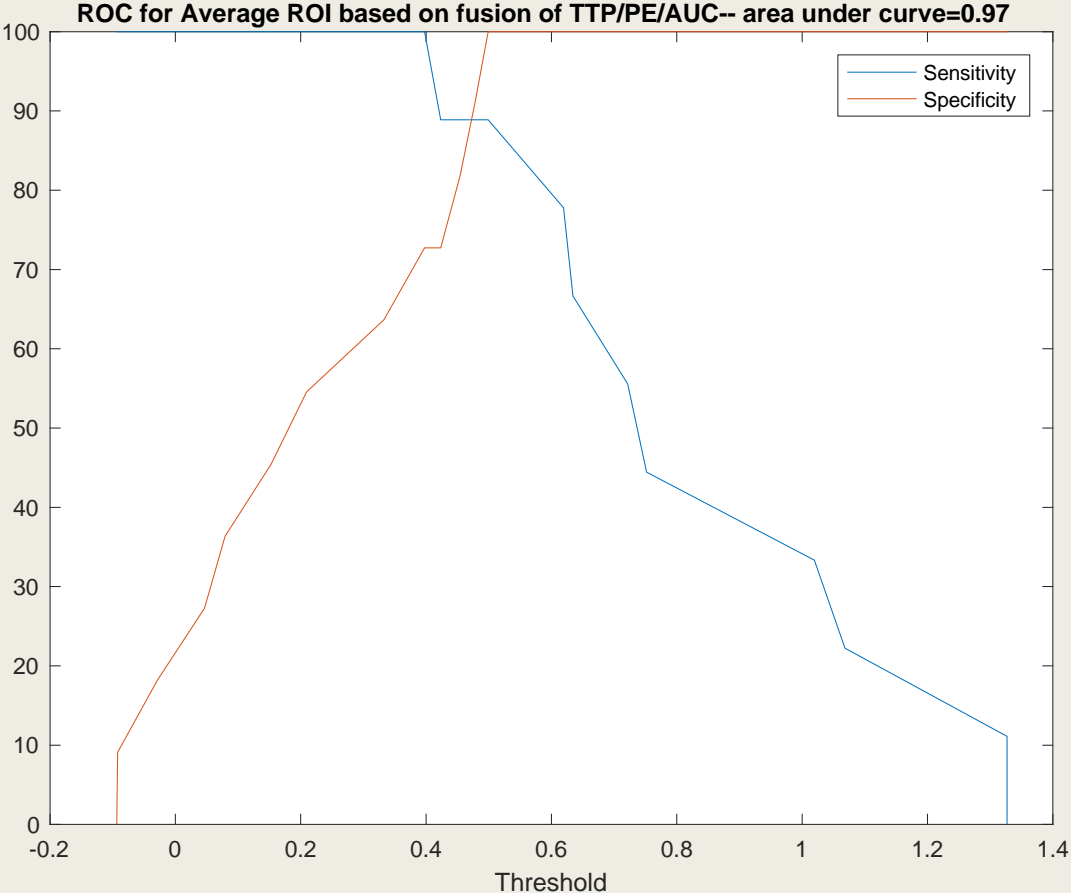
AUC



TTP

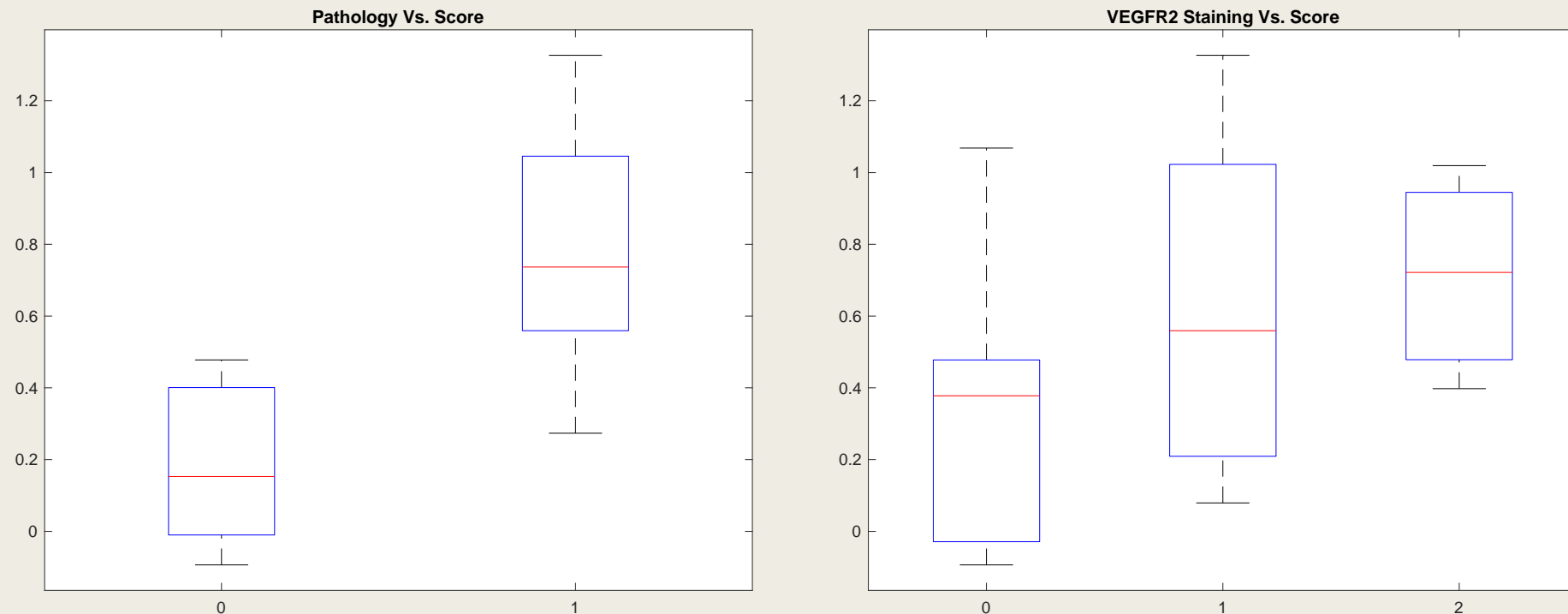
Parametric maps are analyzed within designated Region of Interest.

ROC for the first 45 seconds using parametric maps on the Ovary dataset to predict pathology.



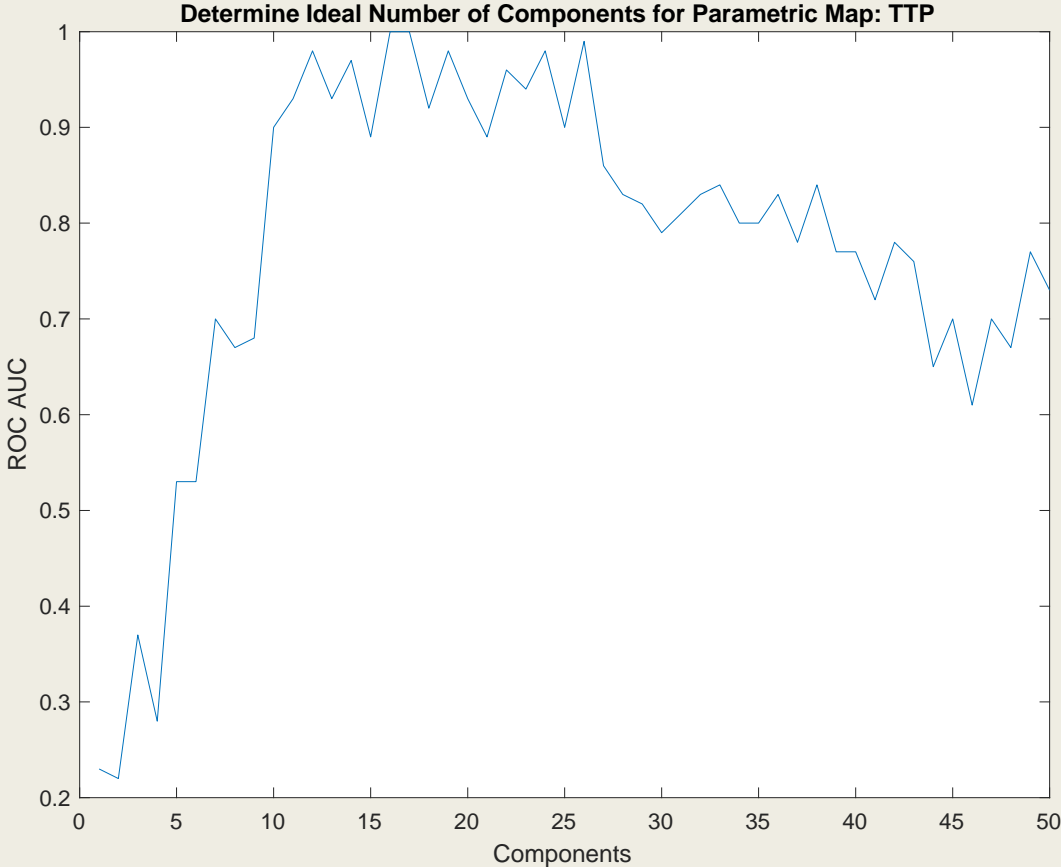
Parameter	AUC
PE	0.99
AUC	0.85
TTP	0.82
Fusion	0.97

Score Vs. Pathology or VEGFR2 Staining in Ovary Dataset

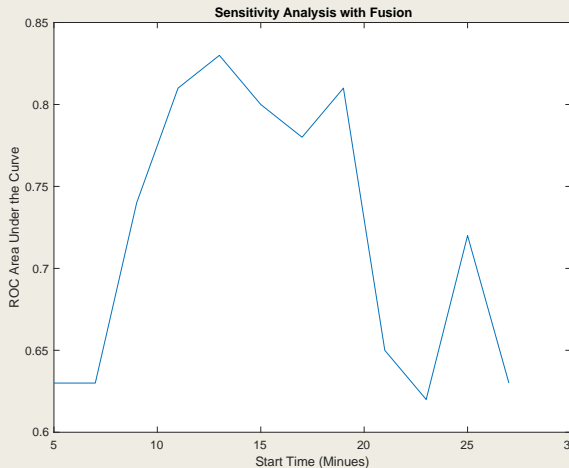
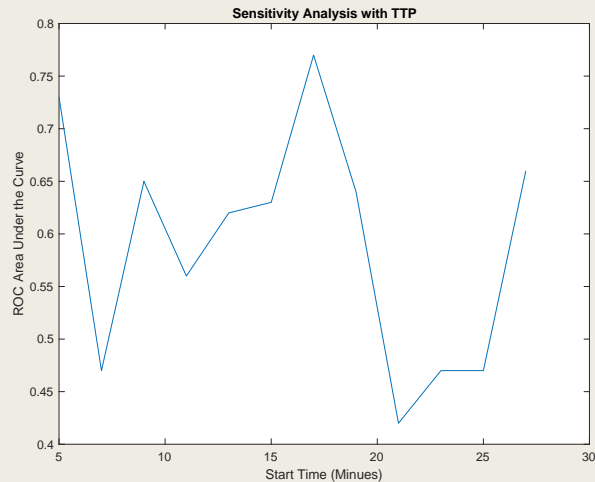
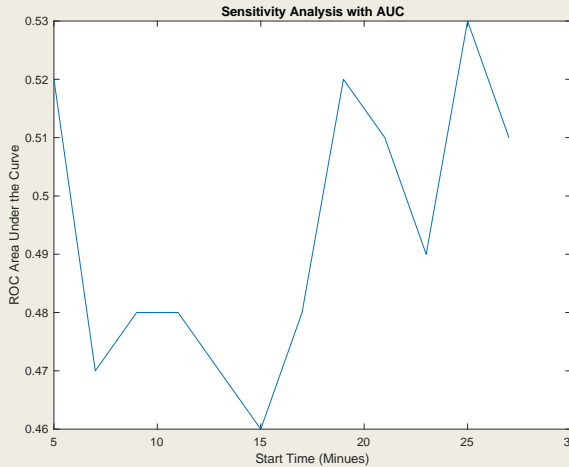
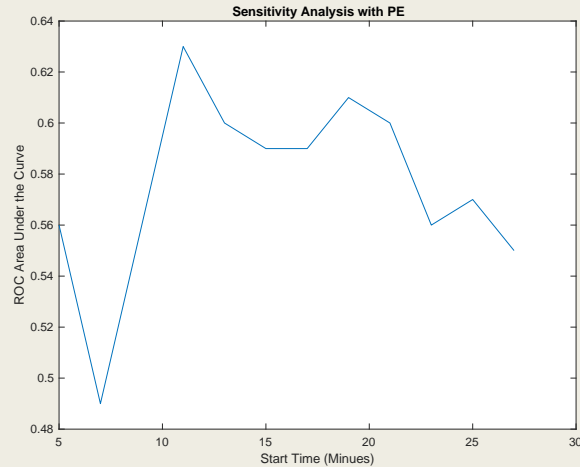


Using support vector machines to predict the binary pathology outcome results in a score. However, the score can also be stratified by the level of KDR expression on immunohistochemistry (low, medium, high).

Sensitivity Analysis: ~15 principal components was ideal.

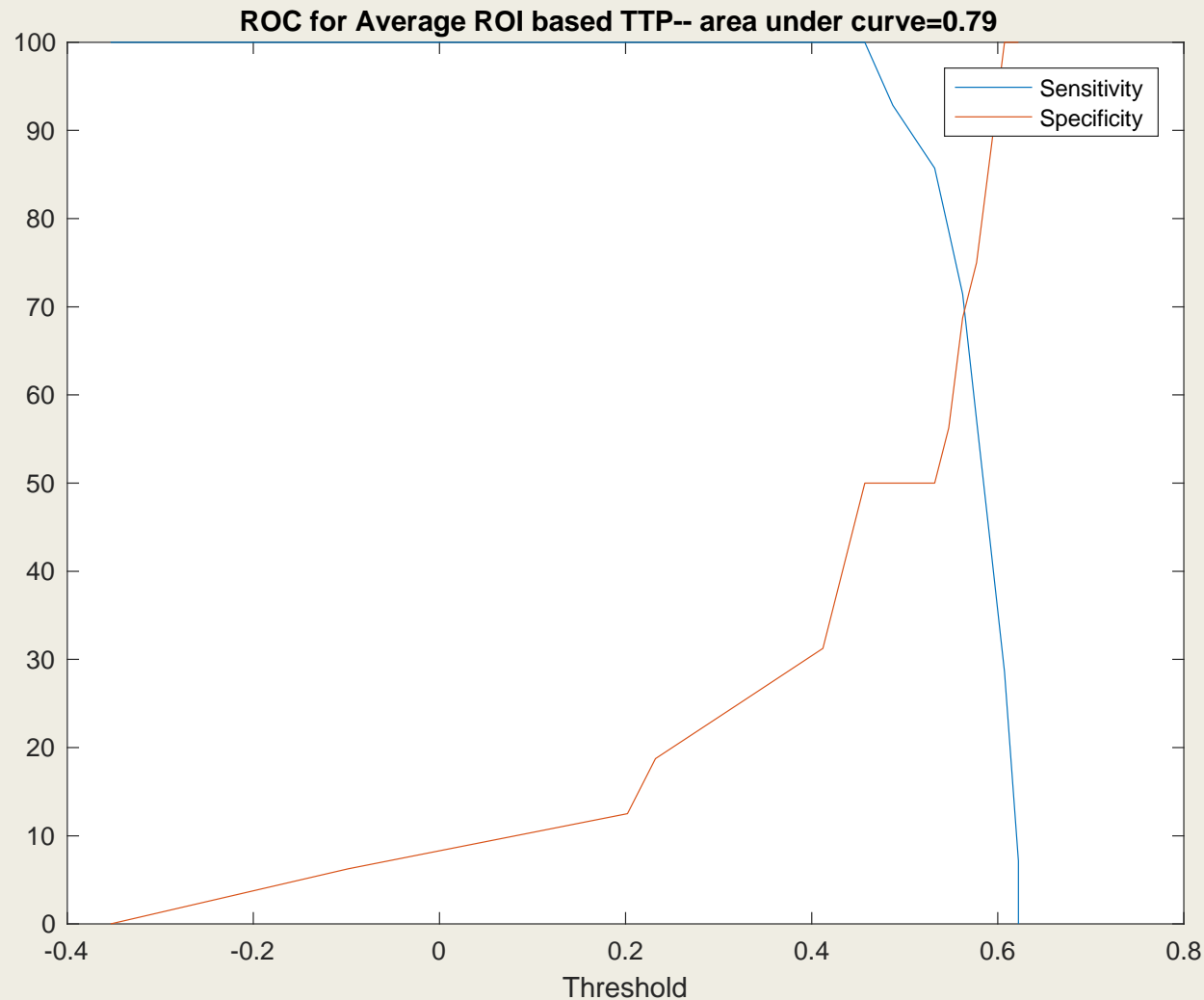


Sensitivity Analysis, Showing Performance of Variable Models on the Ovary dataset as Function of Start Time



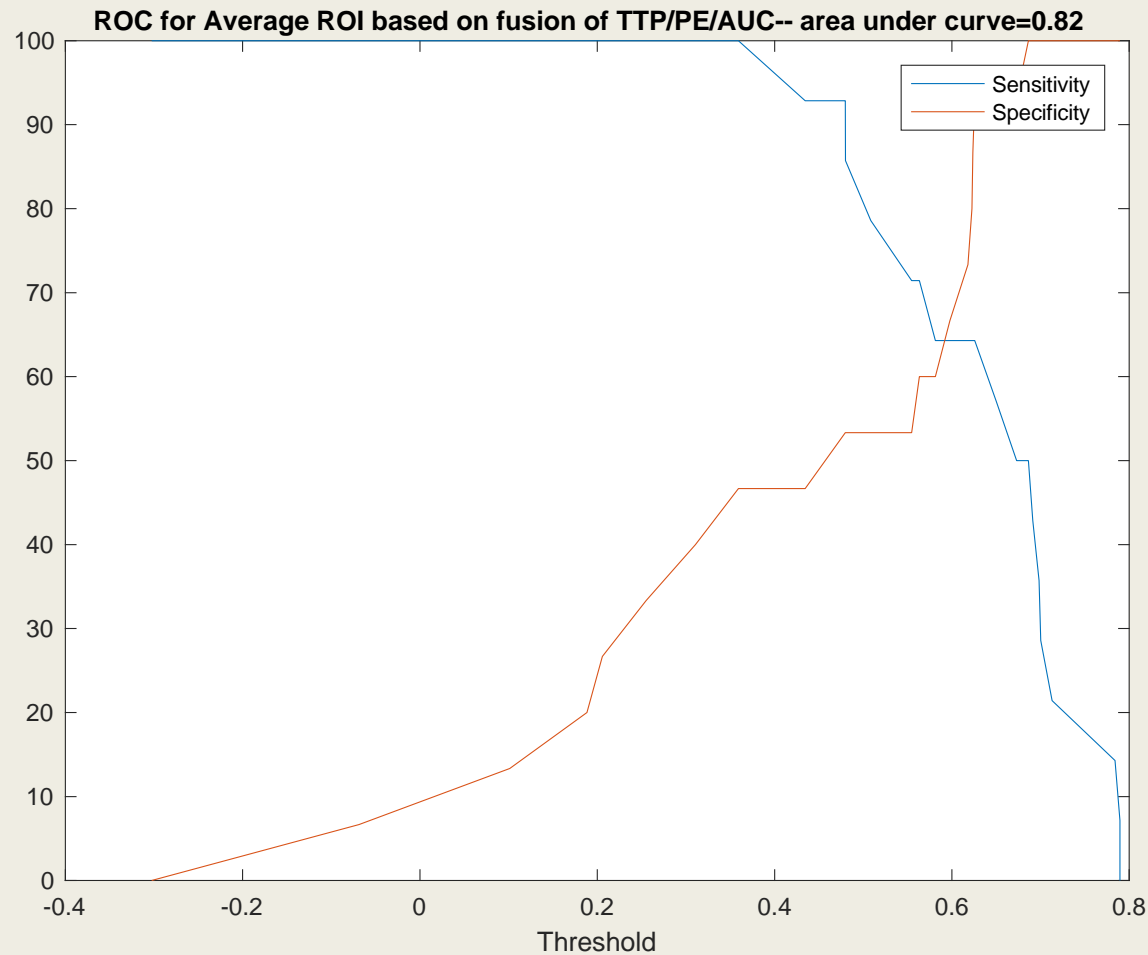
- Overall, the models do not perform as well when the first start time (initial 45 seconds) is omitted (compare to performance on previous slide).
- This makes sense: analyzing perfusion parameters after the initial bolus has peaked would not be expected to perform as well.
- This is in contrast to looking at the texture of the lesion at the later time-point, essentially analyzing the pattern of molecular imaging signal, which performs much better.

Results for the Breast dataset using the mean value of PE, AUC, & TTP in the ROI over the first 45 seconds to predict pathology.



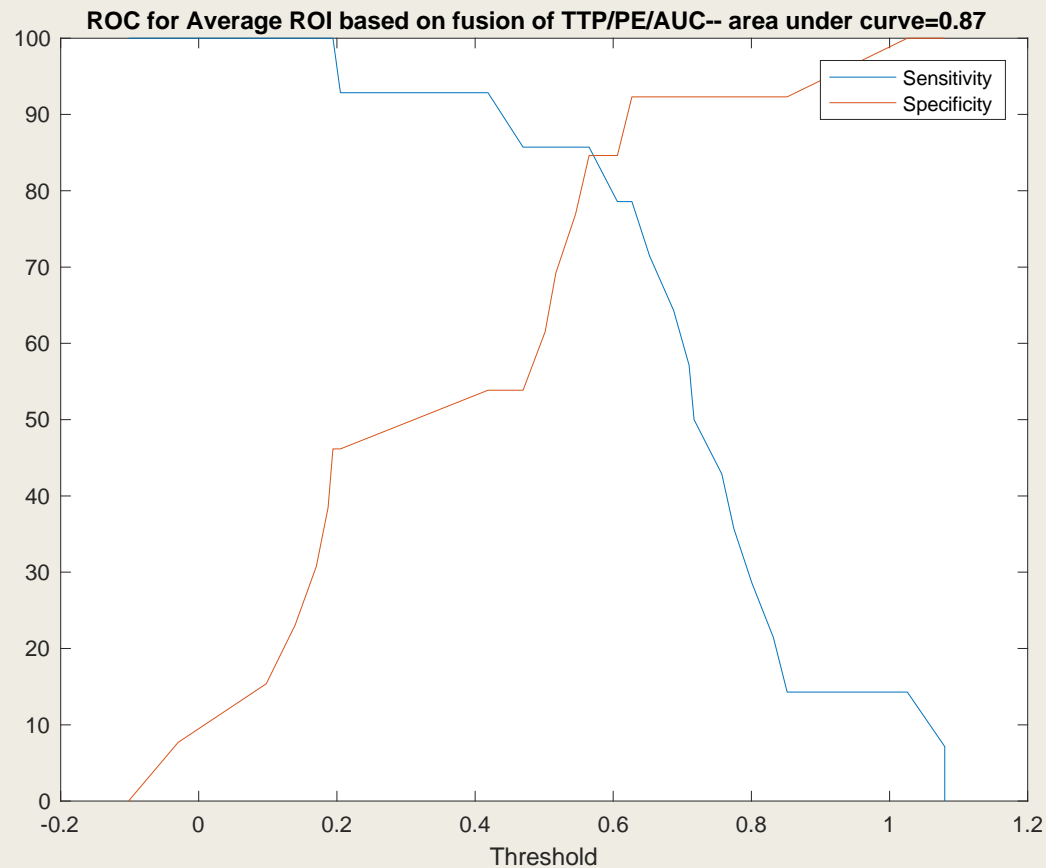
Parameter	ROC AUC
PE	0.76
AUC	0.69
TTP	0.79
Fusion	0.79

Results for Breast dataset using mean of PE, AUC, & TTP in the ROI over the entire 30 minutes to predict pathology.



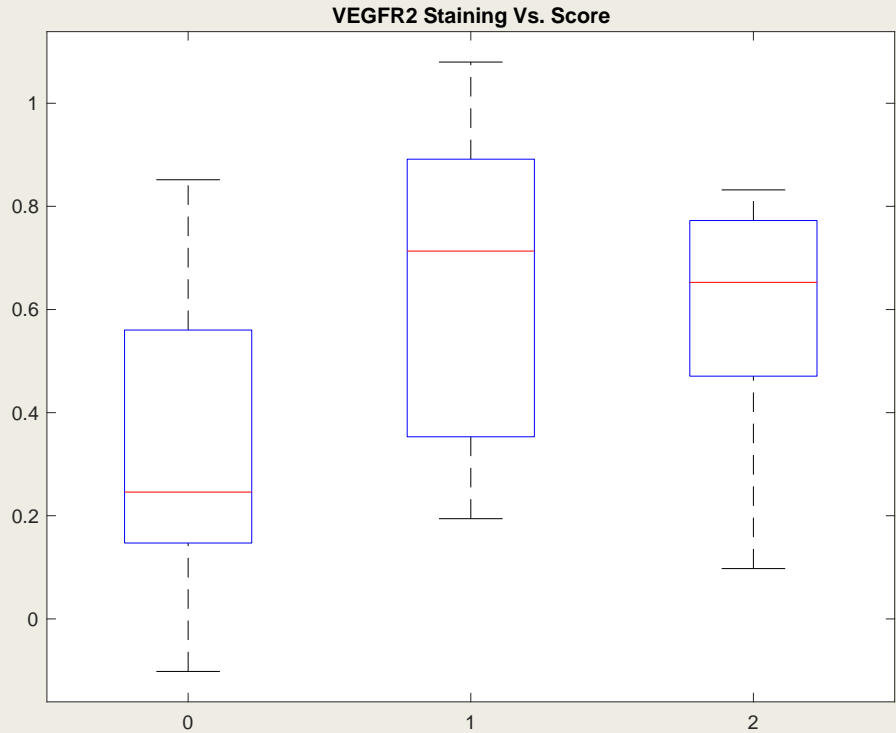
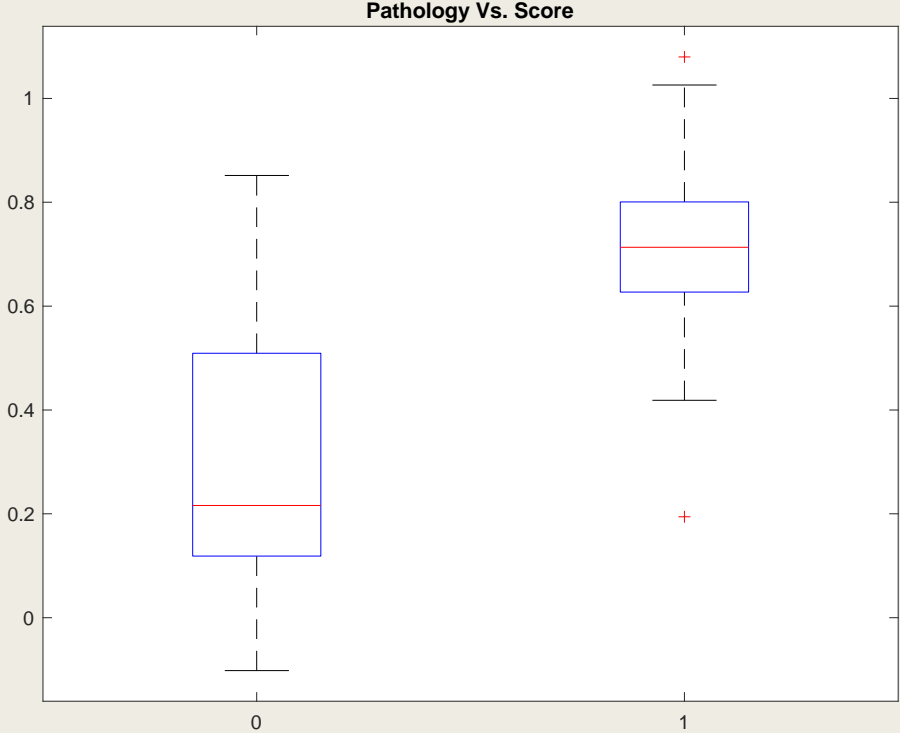
Parameter	30 min AUC	First 45 sec
PE	0.75	0.76
AUC	0.58	0.69
TTP	0.81	0.79
Fusion	0.82	0.79

ROC for the first 45 seconds using parametric maps on the Breast dataset.



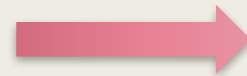
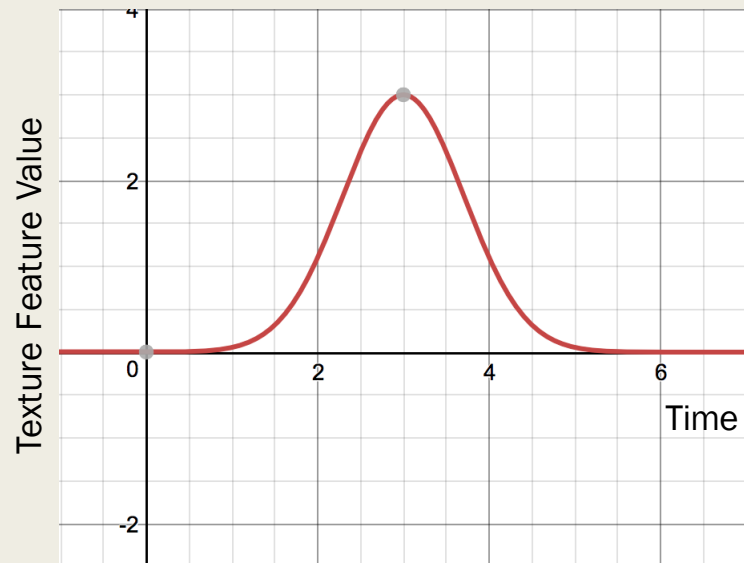
Parameter	AUC
PE	0.81
AUC	0.51
TTP	0.73
Fusion	0.87

Score Vs. Pathology or VEGFR2 Staining in Breast Dataset



Next Steps

- Generate "texture-intensity" curves, which show how textural features vary over time. Discretize the values from these curves and use them as a feature vector for machine learning.

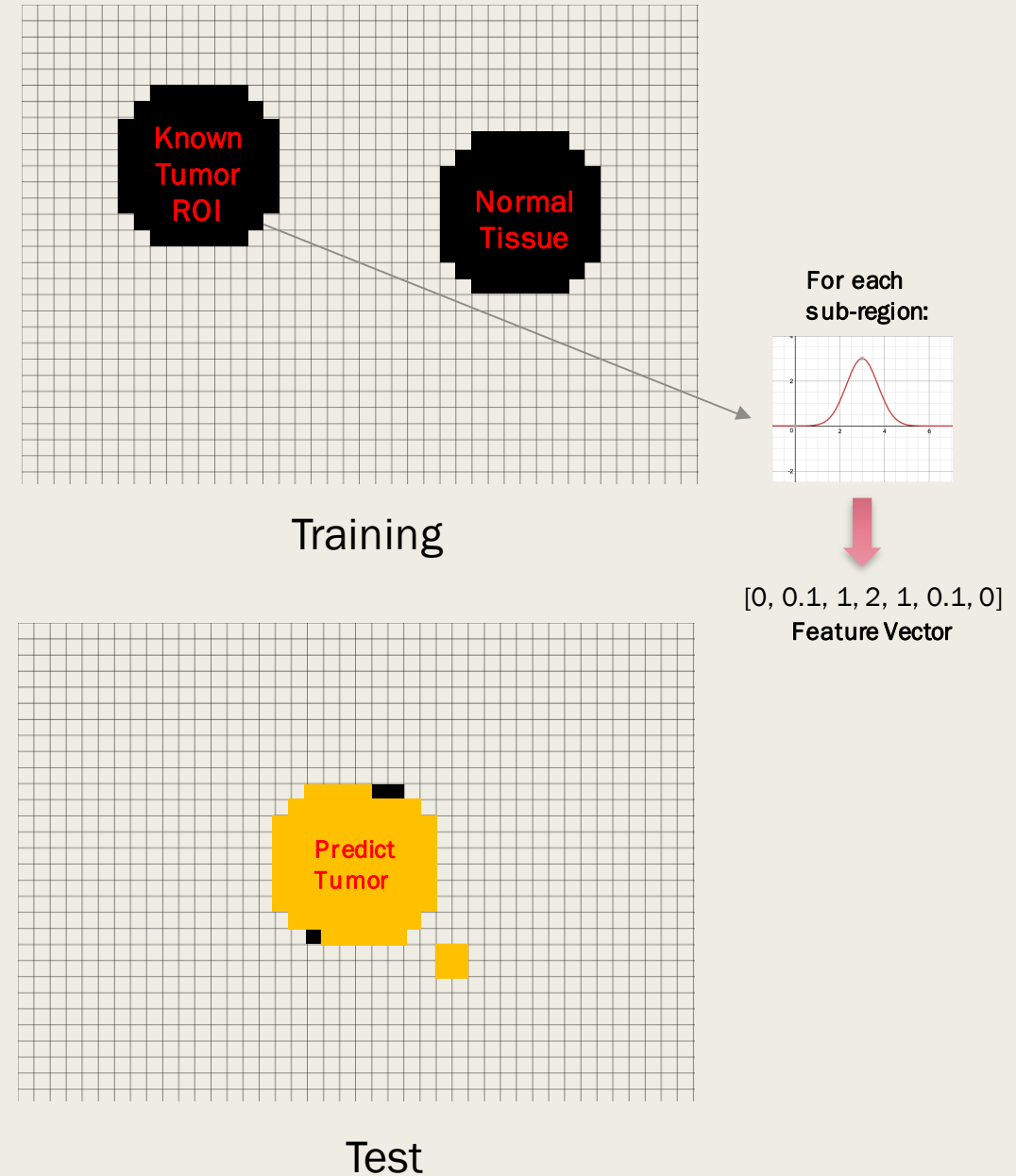


[0, 0.1, 1, 2, 1, 0.1, 0]

Feature Vector

Next Steps

- Divide the entire image into small sub-regions.
- During training, within each sub-region, generate time-intensity curve or texture-intensity curve and get feature vector.
- Use known tumor ROI for classification.
- When a new image is presented, use the time or texture-intensity curve to predict whether each individual sub-region represents either tumor or normal tissue.
- If this works well, it can be used to predict areas of tumor in new images or provide an “auto-segmentation” of sorts.



Future Directions

- It will be important to validate our results on a larger dataset.
- A clinical trial will soon begin in which 60 patients with suspicion for ovarian cancer will undergo imaging with BR55.
 - *Based on our clinical experience at Stanford, 50% will have ovarian cancer and the other will be mostly benign lesions.*
- Clinical outcomes collected as part of this trial include pathological diagnosis, immunohistochemistry to characterize VEGFR2 and CD-34 expression (including pathological scores to quantify endothelial VEGF expression), and a measure of microvessel density.
 - *Overall, this is a much richer and more quantifiable response variable*
- In addition, 50 patients with familial risk for ovarian cancer undergoing risk-reducing salpingo-oophorectomy will also be imaged with BR55.
- Trials will also begin to image other organs with BR55 (pancreas), and these techniques could also be applied to that cohort.

Conclusions

- At the later time points (i.e. 13, 15 minutes, etc), the signal remaining in the lesions should represent true molecular imaging signal. Using the texture from the lesion at these later time points, it is possible to successfully predict pathology with a high level of accuracy in this dataset.
- During the first 45 seconds of imaging, classic parameters from perfusion curves also have predictive ability in this dataset.
 - *Using the parametric maps derived from these parameters performs somewhat better.*
 - *If the whole time period (entire half hour) is used instead, then performance can be hampered if the true contrast peak did not occur in the first timepoint due to discontinuous data collection. If these outliers are accounted for, performance is similar compared to the first time point alone.*
- Nevertheless, it will be crucial to validate these techniques on a larger molecular imaging dataset.

Other Projects

- Using shear-wave elastography to predict RCC vs. AML within a renal dataset.
 - *Using a combination of statistical features both within the lesion and outside the lesion (medulla, cortex) demonstrated higher performance in predicting pathology. Machine learning approaches outperformed the use of median shear wave velocity within the lesion.*
- Using deep learning with 3D convolutional neural networks on 5.5k CT head images and reports in order to predict a number of clinical outcomes (hemorrhage, hydrocephalus, herniation, CVA, mass).
 - *Will compare the performance of “weak labels” derived from the original reports using Snorkel (a Stanford CS-developed tool) compared to radiologist-derived manual labels.*
- Using a 1000 patient, well-curated breast MRI database at Stanford, take advantage of the DCE-MRI curve to predict various pathological and follow-up outcomes with machine learning.

Acknowledgements

- Dr. Daniel Rubin
- Dr. Juergen Willmann
- Dr. Alireza Akhbardeh
- Dr. Ahmed El-Kaffas
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