Project Report

Necrosis Identification in Glioblastoma H&E Slides

Advised by: Dr. Neelam Sinha

Nishant Oli - MT2016096

Suparna Ghanvatkar - MT2016138

Introduction

The project aims to identify regions of necrosis in Glioblastoma H&E stained slides. There are multiple grades of gliomas - grade II, grade III, with grade IV being the most malignant. Glioblastoma is Grade IV Brain tumour which is aggressive and infiltrating - they spread into other parts of brain quickly. The presence of necrosis is an important distinguishing feature for identification of Glioblastoma Multiforme.

The H&E(Hematoxylin and Eosin) staining is used for the tissue to do histological analysis. The Hematoxylin(purplish-blue) is a basic dye while the Eosin(pink) is acidic. Nucleic acids attach to basic dye and proteins and other cytoplasm are acidophilic. Thus, nucleus and parts of cytoplasm containing the RNA gets stained as Purple. Proteins and rest of cytoplasm, cell walls get stained with Pink.

Motivation and Relevance

Glioblastoma Multiforme is the most severe of brain tumours with a very low patient survival rate and very fast infiltration. The diagnosis includes CT scan and MR imaging, but the actual pathological analysis has to be made at the time of the surgery. For the pathological analysis, the tissue is removed and analysed by neuropathologists. Glioblastomas are not surgically curable, but there is good evidence that the more the tumor that can be removed, the better the prognosis. The radiation and chemotherapy are designed to target the infiltrative component of the glioblastoma and delay the tumour progression.

In Glioblastoma, we see large amount of vascular hypoxia, which leads to cells dying out of oxygen. This region of dead cells forms necrosis. The key histological features for identification of Glioblastoma Multiforme is necrosis and endothelial proliferation. We will focus on necrosis identification as it helps improve the identification and prognosis to a huge extent.

Literature Survey

1. M. Milagro Fernández-Carrobles , Ismael Serrano, Gloria Bueno, Oscar Déniz, *"Bagging Tree Classifier and Texture Features for Tumor Identification in Histological Images"*, International Conference On Medical Imaging Understanding and Analysis 2016, MIUA 2016

   The goal of the paper was to evaluate new and existing algorithms for automated detection of metastases in H&E stained whole-slide images of lymph node sections. The 1st order & 2nd order statistical descriptors are based on the image histogram, such as mean, variance or percentiles, Grey Level Co-occurrence Matrix (GLCM) of the image. GLCM are 2nd order histograms that represent the spatial dependence of the image pixels.

2. Fabio A. Spanhol , Paulo R. Cavalin y, Luiz S. Oliveira , Caroline Petitjean z, and Laurent Heutte, *"Deep Features for Breast Cancer Histopathological Image Classification"*,

   This paper uses deep learning(CNN) for feature engineering. Different CNNs are combined using rules like sum, max, product. The model used is a modification of AlexNet. It is interesting to note that the model learnt vertical and horizontal edges and also ones resembling Gabor filter, which emphasizes the importance of texture analysis for the problem.

3. Albarqouni, Shadi & Baur, Christoph & Achilles, Felix & Belagiannis, Vasileios & Demirci, Stefanie & Navab, Nassir. (2016). "*AggNet: Deep Learning From Crowds for Mitosis Detection in Breast Cancer Histology Images"*. IEEE Transactions on Medical Imaging. 35. 1313-1321. 10.1109/TMI.2016.2528120.

   The paper uses crowdsourcing for evaluating trustworthiness of participants and integrate this knowledge to further process of annotations. Though, the paper deals with mitosis detection, it is similar to our problem as the mitosis activity can be used to identify regions of infiltrating tumour which generally further proceeds to form necrotic region. A multiscale CNN architecture is used for mitosis detection with 3 convolutional layers and 2 fully connected layers before softmax.

4. Yan Xu, Tao Mo, Qiwei Feng, Peilin Zhong, Maode Lai, Eric I-Chao Chang, *"DEEP LEARNING OF FEATURE REPRESENTATION WITH MULTIPLE INSTANCE LEARNING FOR MEDICAL IMAGE ANALYSIS"*, 2014 IEEE International Conference on Acoustic, Speech and Signal Processing (ICASSP)

This paper also explores the deep learning approach to feature learning. In medical image analysis, objects like cells are characterized by significant clinical features. Deep learning features are generated using DNN with and without using PCA. Multiple instance learning is used for classification of these features generated. Unsupervised features are also generated by single-layer network of centroids derived from K-means clustering algorithm. The features generated using automatic(DNN) and unsupervised are contrasted with manual features(SIF, L*a*b* and LBP). The automatic features have been found to outperform all.

Data set

- The GBM brain tissues (5 micron thick) are formalin fixed and H&E staining of the tissues is done to obtain the slides. The digital imaging of these slides if done to generate whole slide images in TIFF format.
- The highest resolution image cannot be opened on a local machine. So a first level downsampled image available in TIFF image is extracted using the “openslide” library in python. The image size of this *level 1* image is 16000x18000.
- This image is opened and annotated with necrotic regions in the image. These regions are then cropped out to generate training images of minimum size 200x150 and maximum size of 500x900. Similarly, non-necrotic regions are also cropped out. The cropped out images do not have too much context but mostly cover the necrosis only.
- The total images cropped out were 40 necrotic and 40 non-necrotic. This was used to create the dataset:
    - 30 necrosis and 30 non-necrosis images used to create training data - total of 60 images
    - 10 necrosis and 10 non-necrosis images used to create testing data - total of 20 images.

Proposed Methodology

Necrosis regions in H&E slides of glioblastoma are identified by less density of nuclei and/or region of dead cells with lack of cytoplasm which remain white in H&E staining. This region generally has high mitotic activity around it. Typically, we also observe pseudo-palisades around necrotic region which give a distinctive texture to the necrotic region.

Proof of Concept

For proof of concept we picked up task of differentiating between Sand and Gravel. A dataset is generated by compilation of images from internet. To identify and classify sand v/s gravel, we used texture analysis. We used three different different texture analysis:

1. Gray Level Co-occurrence Matrix (GLCM): We got an accuracy of 0.7 on the sand v/s gravel with 3 misclassifications for sand using SVM classifier.
2. Maximum Response Filter (MR8) Bank: The filter bank by VGG has 38 filters but uses 8 filter responses by collapsing to get only maximum response in each orientation. Using this filter bank responses, we got 0.5 accuracy.
3. Local Binary Patterns (LBP): Using uniform LBP histogram as the feature along with SVM classifier, we got 0.9 accuracy with 1 misclassification for sand.

We decided to proceed with LBP texture as the feature. Then, we decided to use statistical features from the normalized histogram - energy, entropy, mean, variance, skewness. To use these features for texture classification, we again performed sand v/s gravel classification with SVM classifier. This gave us encouraging results of one misclassification of gravel(true label) to sand(label).

Classification of cropped patches of necrosis

The data set generated for necrosis v/s non-necrosis classification has cropped patches of various sizes. The features proposed to be used for classification are:

1. Texture features: The statistical features of the LBP texture as verified in proof of concept can be used as the texture features.
2. Color features: The image was converted into HSL color space and the proportion of white pixels in saturation and lightness is used as a feature. This feature is used to account for the white in the necrotic regions. It helps to balance for the intuition that

necrotic regions have white pixels in a certain proportion such that very low proportion implies non-necrotic regions and very high proportion could be a tear.

$$proportion\ of\ white\ pixel\ = \frac{number\ of\ white\ pixels\ (pixels = 255)}{total\ number\ of\ pixels}$$

Thus, a feature vector of 7 features is generated - energy, entropy, mean, variance, skewness, proportion of white pixel in saturation, proportion of white pixel in lightness.

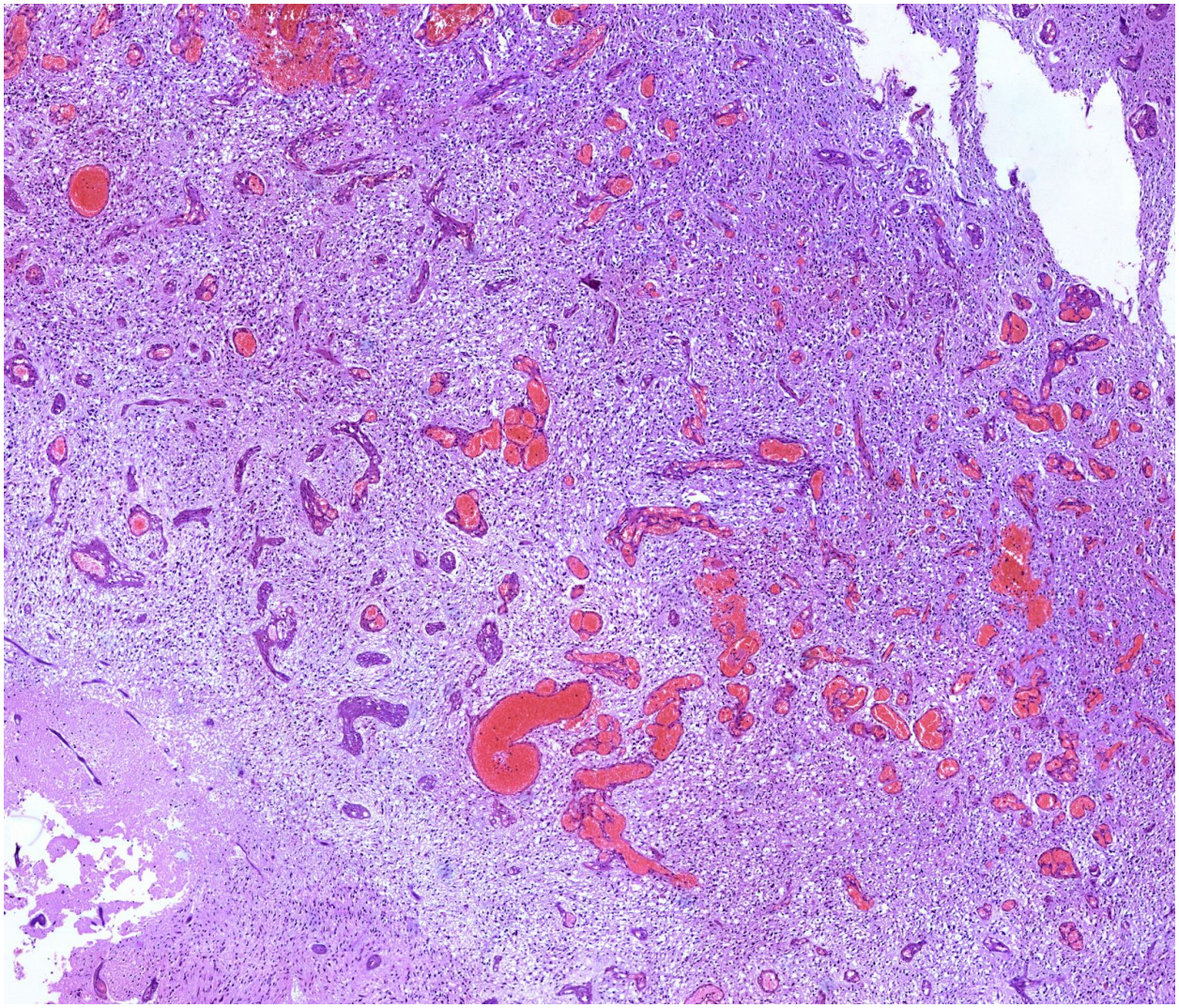
The classification is performed using 3 different classifiers: Support Vector Machines(SVM), K-Nearest Neighbour, Decision Trees.

Identification of necrotic region in tissue

A test image of size 2831 x 2421 is cropped from the level 1 image. This test image has both necrotic and non-necrotic regions. Our aim was to generate a mask on the image for all the necrotic regions.

- Features: For this task we used uniform LBP features and created a normalized histogram out of this.
- Training: We resized the images to same size for consistency to 100x100. The histogram of this resized image was used to train classifier.
- Testing: For testing, we had to identify necrotic patches in the tissue image which is a bit different task from training where we have patches having only necrosis. To mitigate this issue, we used the principle of image pyramid and sliding window.
    - Image pyramid: To consider the tissue image at various scales, we generated a image pyramid with scaling factor 1.5.
    - Sliding window: We considered windows(patch size) of various size from an image in the image pyramid and resized it 100x100 and fed it to the classifier to predict if the patch is necrotic or not. In case of identifying the patch as necrotic, we update the mask. This window is sliding through the image with stride size 32.
- An alternative to the resizing approach was using the same sliding window technique to training images rather than resizing, thus also augmenting the data.

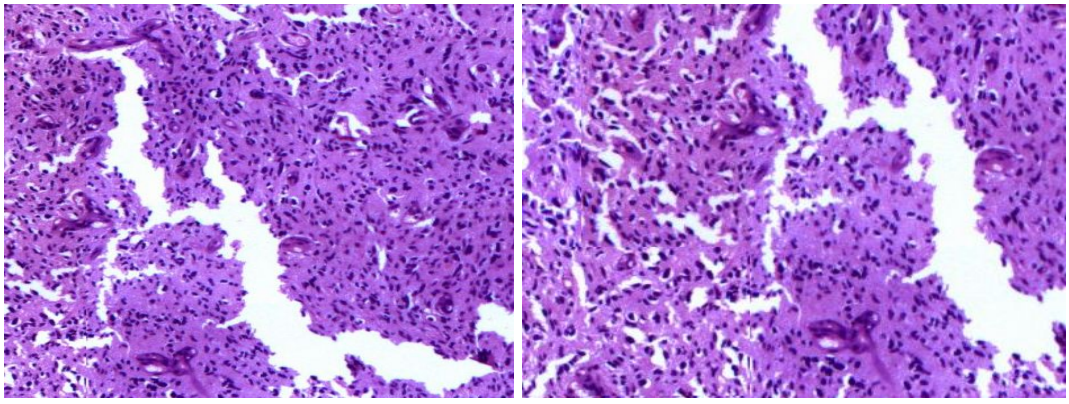
This methodology helped us identify regions in the tissue image which were necrotic - which is close to our original aim. But, the results also classify tears as necrosis and also the methodology requires a lot of consideration to combine the results obtained at various scales from image pyramid to effectively generate the final annotation. The test image used is attached below:



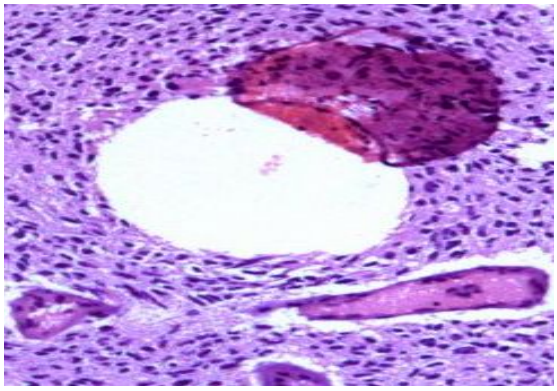
Challenges

Identification of necrosis is a tough task. Necrosis can be confused with the following which prove to be a huge challenge for identification of necrosis:

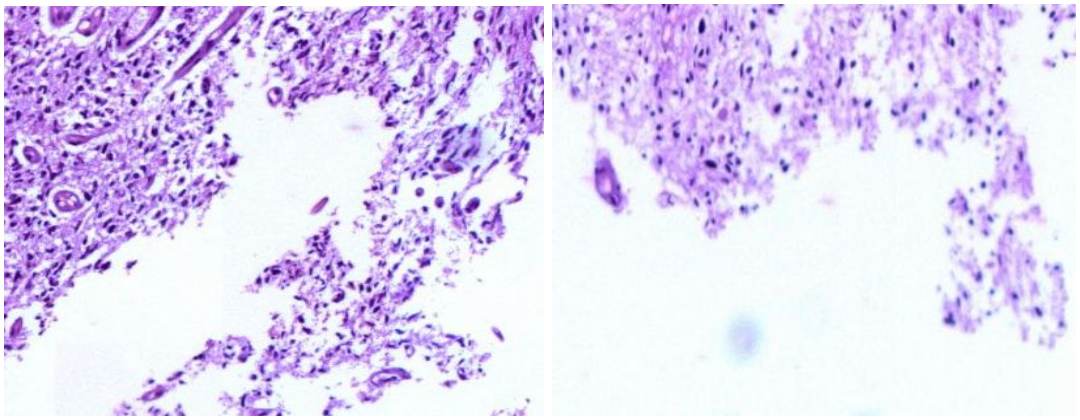
1. Tissue tears - It has a clear boundary of the tissue and no cytoplasm or low density nucleus in the white portion.



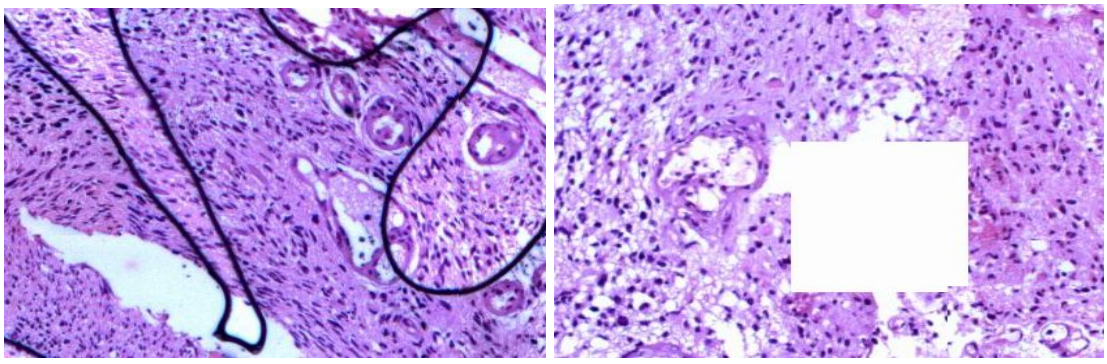
2. Blood vessels - It has definite white boundary usually along with blood cells in the surrounding region. It is identified by definite boundary and normal density of nucleus and cytoplasm.



3. Tissue ends - These may seem necrotic but also have some boundary to them.

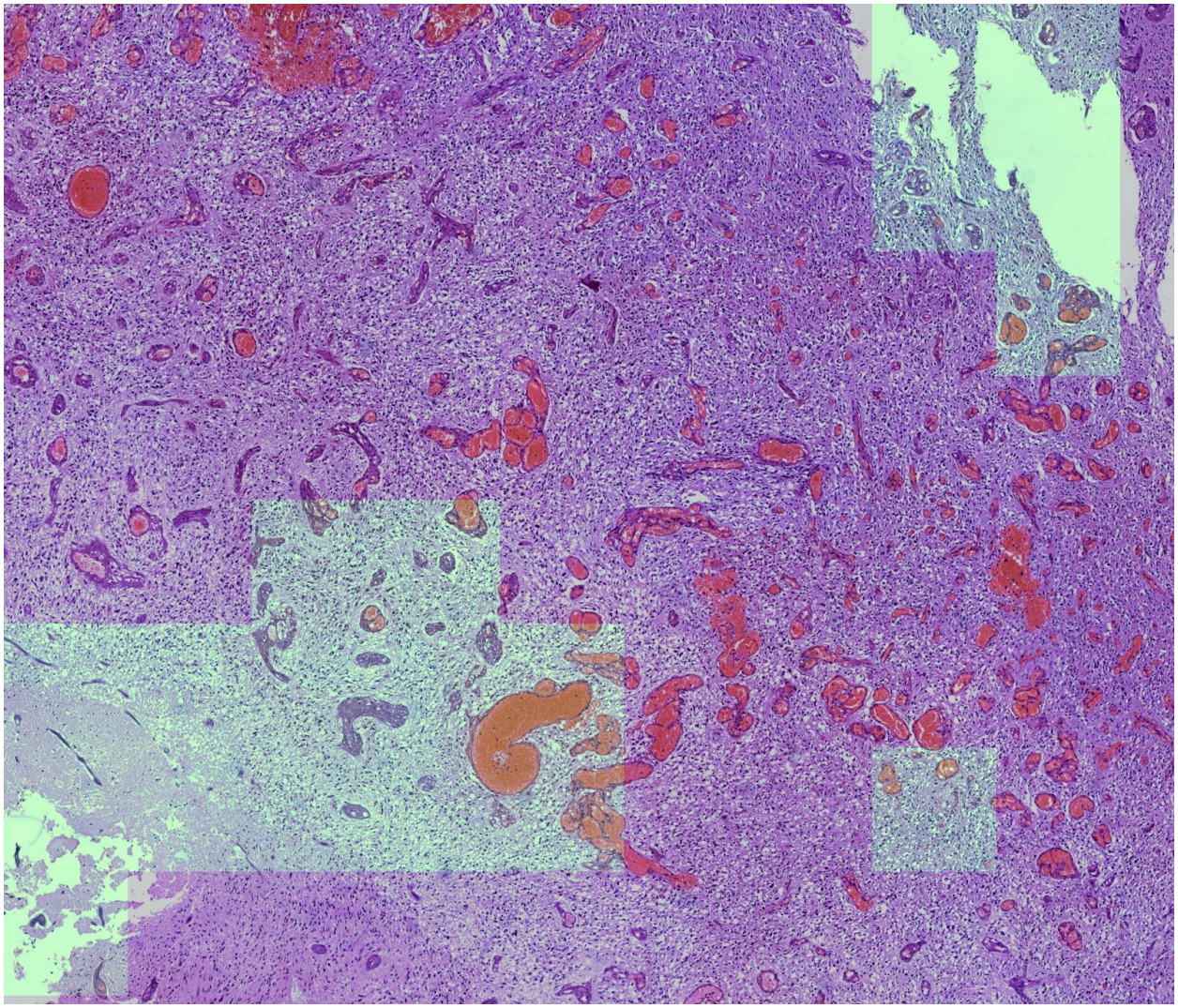


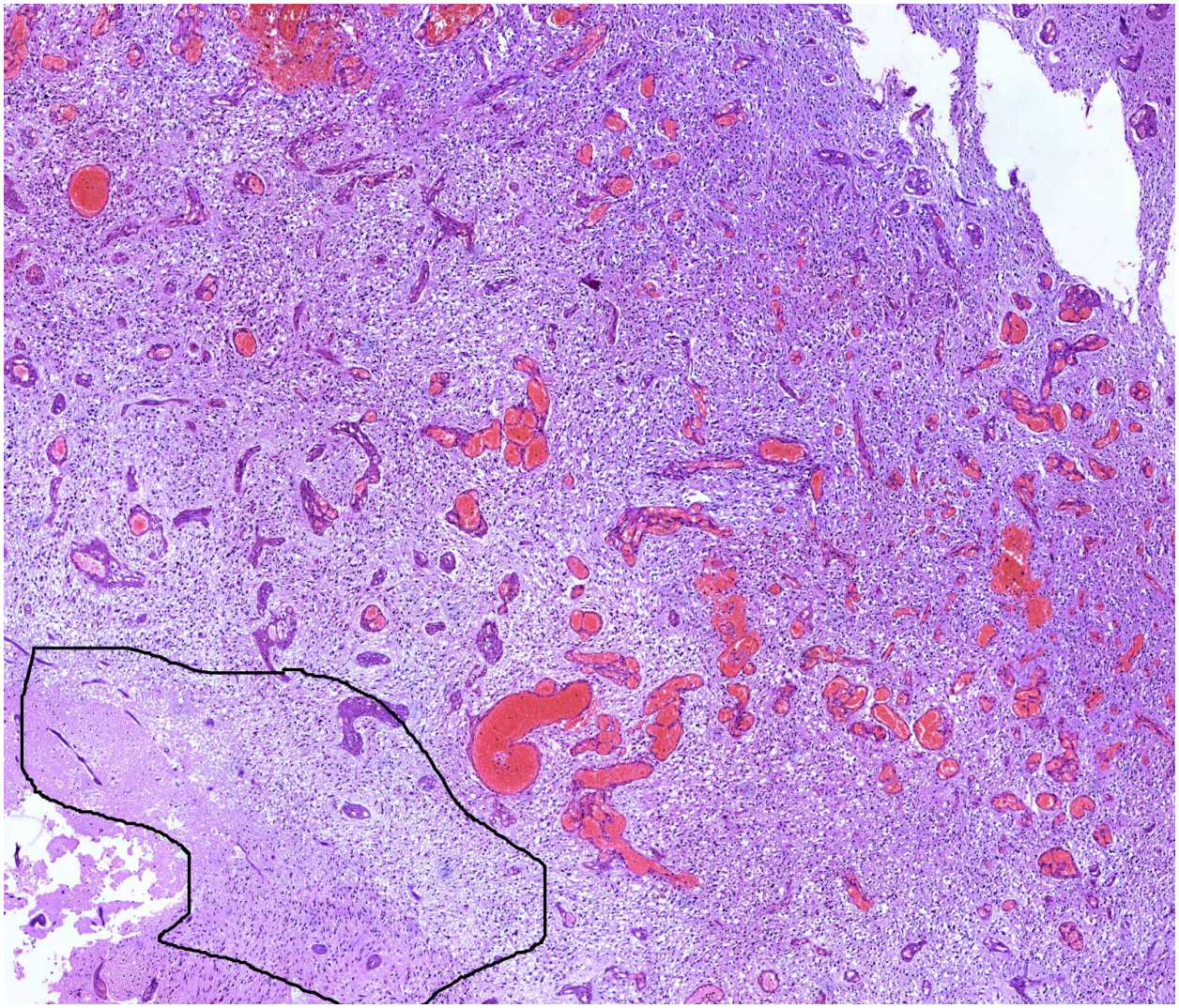
4. Artefacts - These are patches, overlaps, etc generated during digital imaging of the slides.



Results and Comparison

We experimented with various patch size for necrotic region identification in the tissue images. The patch sizes we experimented with were 100x100, 200x200, 300x300, 400x400, 500x500. Overall we found the 300*300 to perform best. The result for sliding window size of 300x300 is attached below. This image is result for sliding window based training and testing without resizing. The ground truth is also attached below.





The smaller problem of proper classification of necrosis v/s non-necrosis was then chosen to be perfected and kept as scope of project. For this task, we used the training set of 60 images and test set of 20 images as explained in dataset section.

We experimented with various values of radius in LBP(uniform).

LBP Radii	Classification Results with SVM
1	0.5
3	0.75

5	0.6
7	0.55

Then we choose the radii = 3, as it gave better performance than other values.

We got the following results using SVM, k-NN and Decision Trees. The results for the necrotic vs not all classification were as follows:

SVM:

	Necrosis	Not Necrosis
Necrosis	0.8	0.2
Non-Necrosis	0.3	0.7

Accuracy: 0.75

k-NN (k = 19):

	Necrosis	Not Necrosis
Necrosis	0.8	0.2
Non-Necrosis	0.2	0.8

Accuracy: 0.8

k-NN (k = 9):

	Necrosis	Not Necrosis
Necrosis	1.0	0.0
Non-Necrosis	0.4	0.6

Accuracy: 0.8

k-NN (k = 15):

	Necrosis	Not Necrosis

Necrosis	0.5	0.5
Non-Necrosis	0.3	0.7

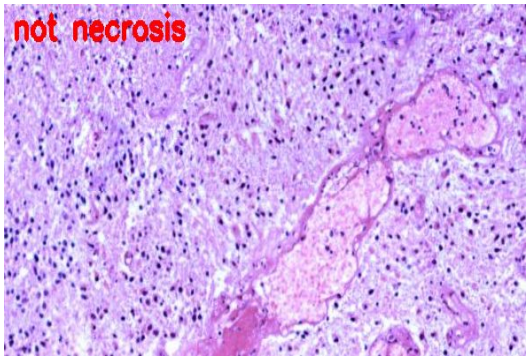
Accuracy: 0.6

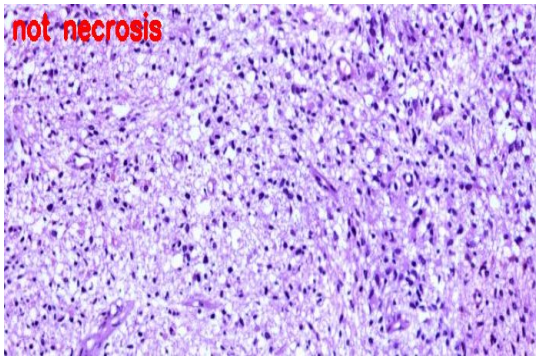
Decision Trees:

	Necrosis	Not Necrosis
Necrosis	0.8	0.2
Non-Necrosis	0.0	1.0

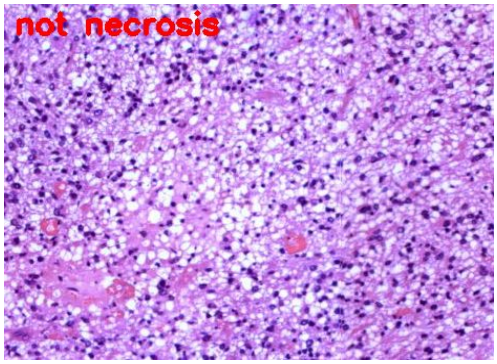
Accuracy: 0.9

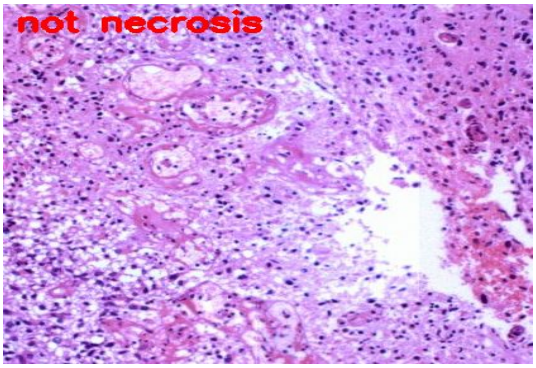
Some of the cases of misclassifications were:



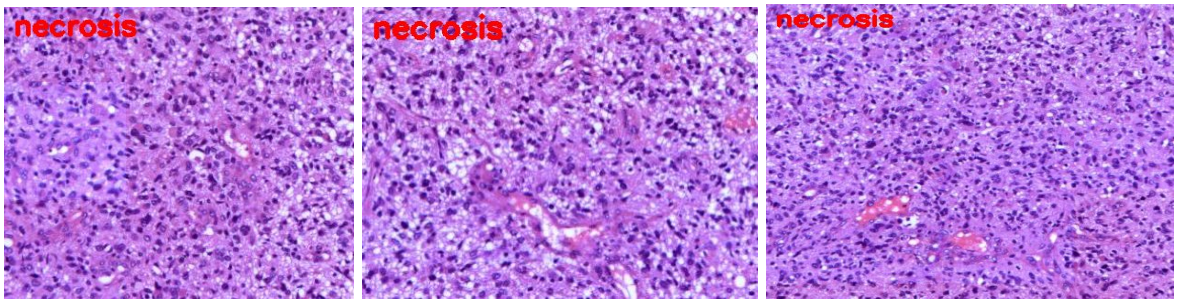


Here, possible reason of misclassification seems lack of white region.



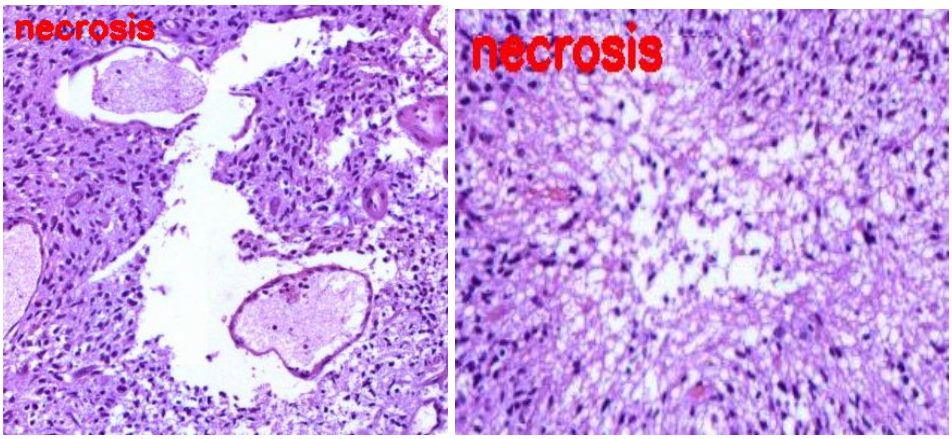


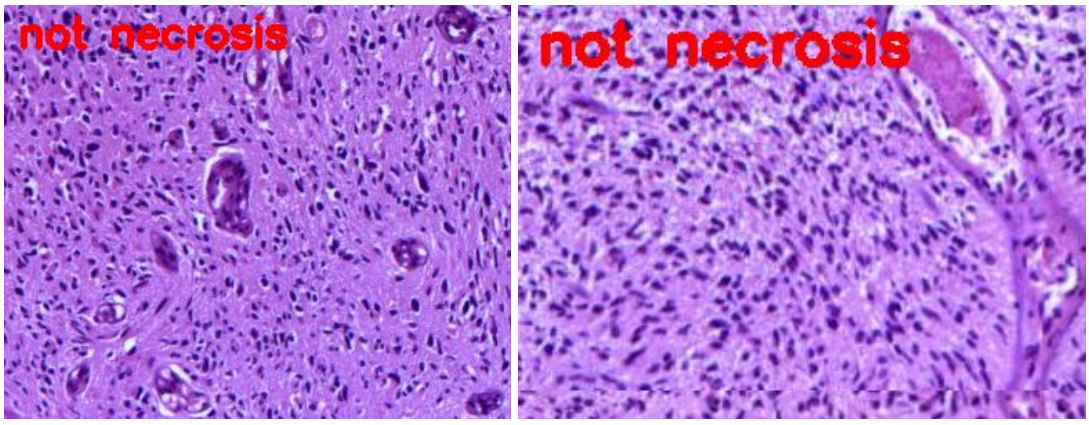
Here, possible reason of misclassification for necrosis class is possible different texture in cytoplasm.



Here the reason of non necrosis misclassification is possibly due to the white spaces.

Many cases were identified properly as shown below.





*All the examples listed in document are combination of typical classification results, to check exact results, please view the drive link.

The dataset along with results for all experiments present at: Drive link

Limitations & Future Work

The limitation of the work is that although it is able to classify a given image as necrotic and non necrotic. It is not able to label a certain patch from the whole slide image as necrotic and then mark the boundaries of the necrotic region. There are certain other histological features other than necrosis which will require all some other texture analysis to get classified.

The following can be carried along as future work to the project:

1. We took the patches of the image and then classified them as necrotic & non-necrotic; actual necrosis segmentation & boundary detection can be done in a large image.
2. Detection of other histological features like PNZ, MVP, PAN etc can be done along with the necrosis.

References

1. http://hanzratech.in/2015/05/30/local-binary-patterns.html
2. http://www.webpathology.com/case.asp?case=738
3. https://www.ncbi.nlm.nih.gov/pubmed/21356829
4. https://www.mdanderson.org/publications/cancerwise/2013/04/understanding-glioblastoma.html
5. http://www.aans.org/Patients/Neurosurgical-Conditions-and-Treatments/Glioblastoma-Multiforme