Necrosis Identification in Glioblastoma H&E Slides

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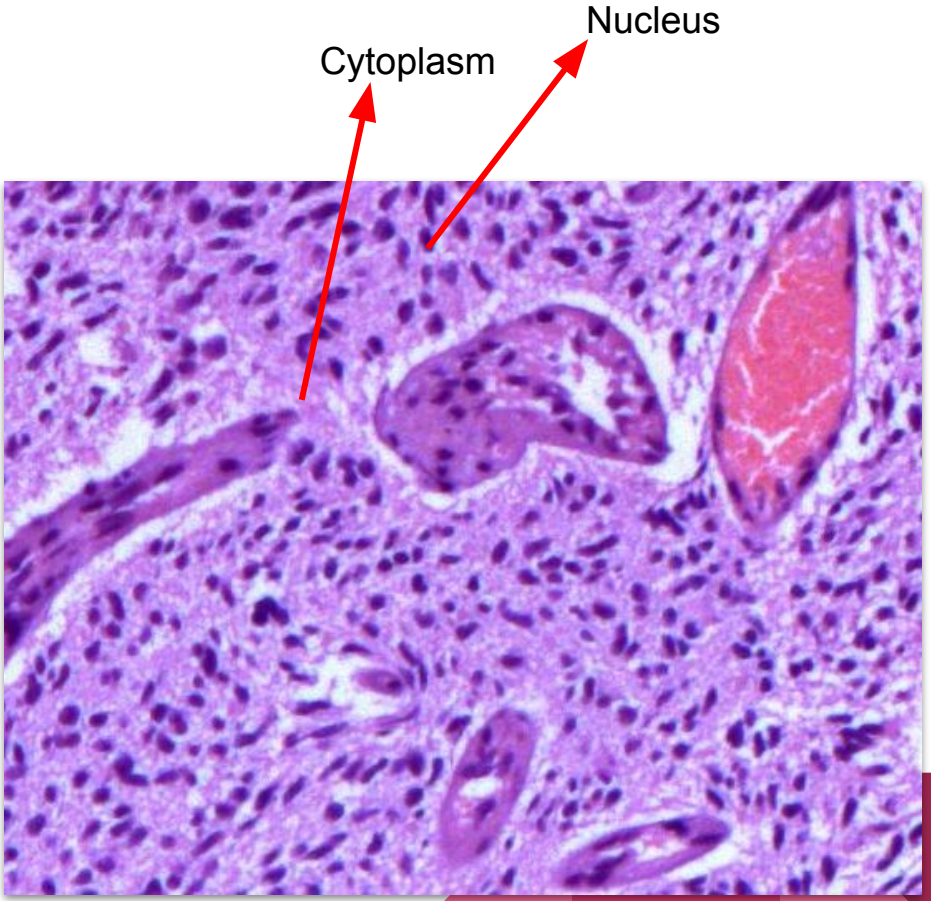
Glioblastoma Multiforme

- Grade IV Brain Tumour - Aggressive and Infiltrating
- The actual Pathological Diagnosis has to be made at the time of surgery. (Tissue is removed and examined by a Neuropathologist).
- Presence of Necrosis - Important Diagnostic Feature.
- Glioblastomas are not surgically curable, but there is good evidence that the more the tumor that can be removed, the better the prognosis.
- Greater degree of Necrosis - Prognosis worsens.

H&E Staining

- Haematoxylin - Purplish Blue / Violet Stain - Basic dye.
- Eosin - Pink stain - acidic dye
- Nucleic acids attach to basic dye and proteins and other cytoplasm are acidophilic.
- Nucleus and parts of cytoplasm containing the RNA gets stained as Purple. Proteins and rest of cytoplasm, cell walls get stained with Pink.



Dataset & Preparation

- GBM H&E slide images in .tiff Format
- Extracted Level 1 image of size 16000x18000 from above mentioned .tiff images
- Annotated the level 1 image & Cropped patches of necrosis of min size 200x150 and max size 500x900

Training Data: 30 of these patches from each classes were used.

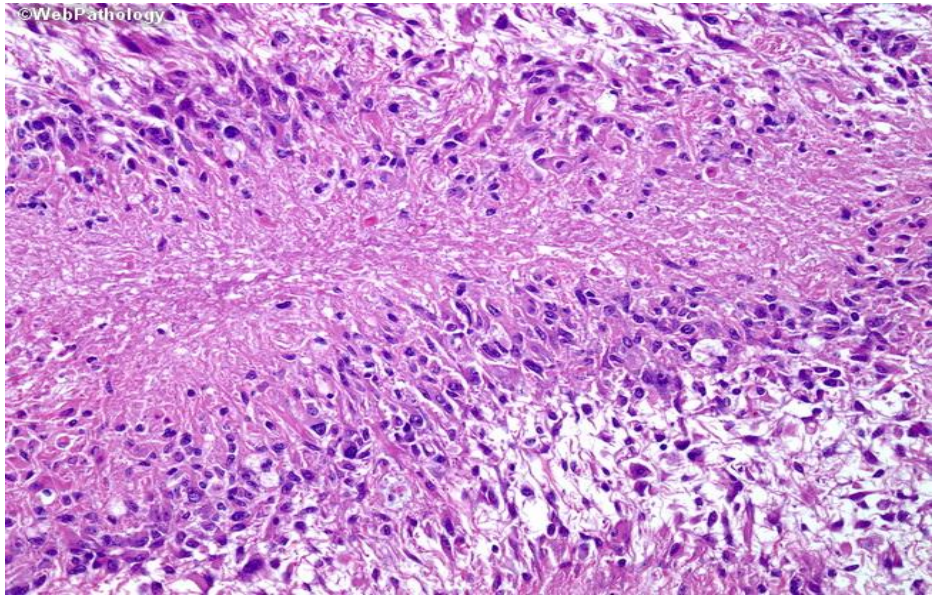
Testing Data: 10 of these patches from each class were used.

Necrosis

Necrosis regions in H&E slides of glioblastoma are identified by:

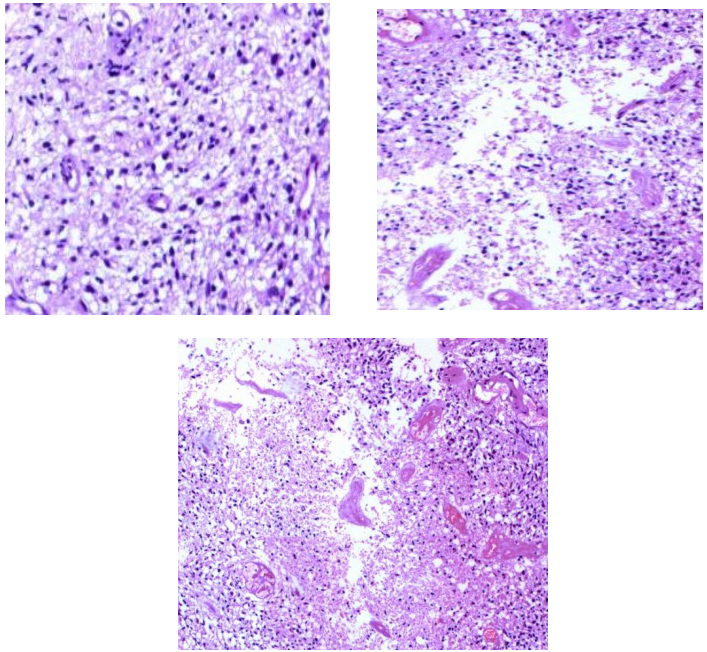
- Less density of nuclei and/or region of dead cells.
- Lack of cytoplasm which remain white in H&E staining.
- Region generally has high mitotic activity around it.
- Typically, we also observe pseudo-palisades around necrotic region which give a distinctive texture to the necrotic region.

Necrosis (contd.)

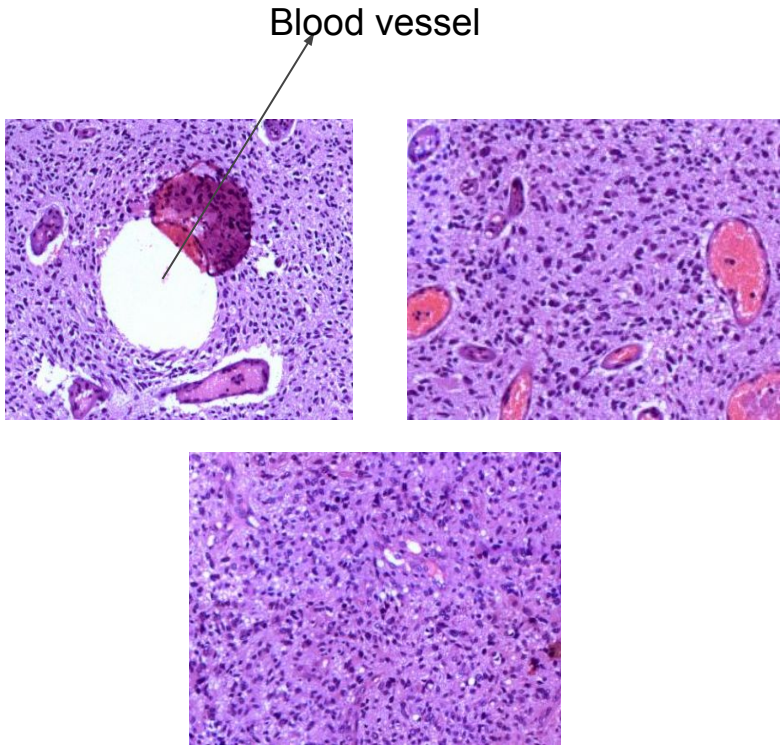


Higher magnification showing nuclear pseudopalisading - aggregation of tumor cells around the periphery of the necrotic areas. Image Source: http://www.webpathology.com/image.asp?case=738&n=13

Necrosis (contd.)



Various Scenarios of Necrotic regions

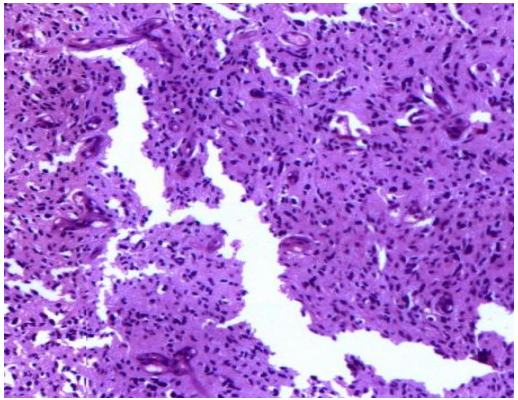


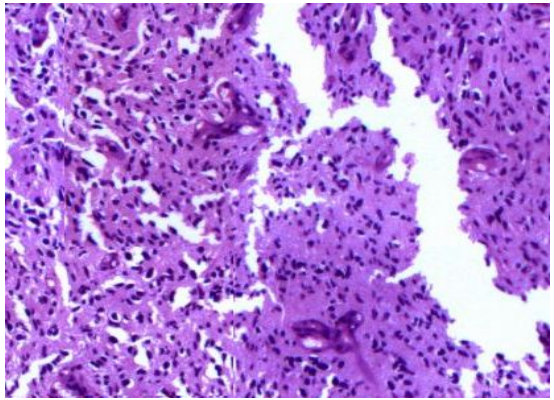
Various Scenarios of Non-Necrotic Regions

Challenges:

Necrosis vs Non Necrosis had many confusing cases like:

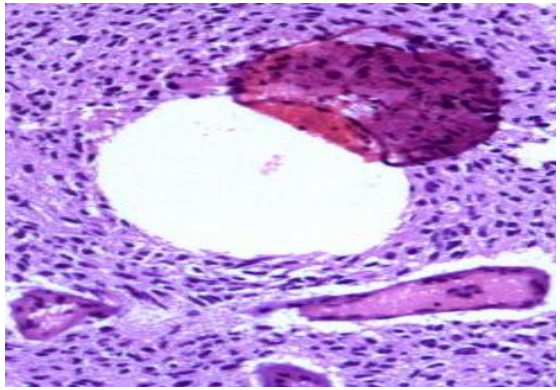
- Tears - clear boundary of the tissue, no cytoplasm or low density nucleus in white region





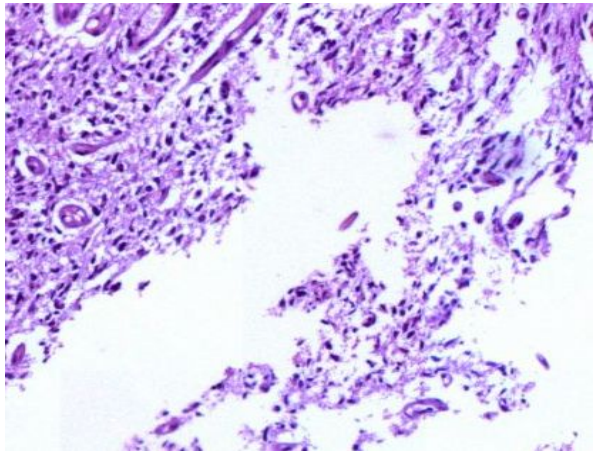
Challenges (contd.)

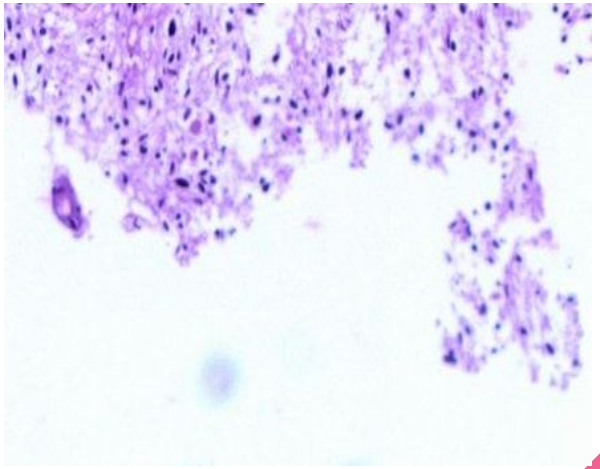
Blood Vessels - definite white boundary usually along with blood cells in the surrounding region. Identified by definite boundary and normal density of nucleus and cytoplasm.



Challenges(contd.)

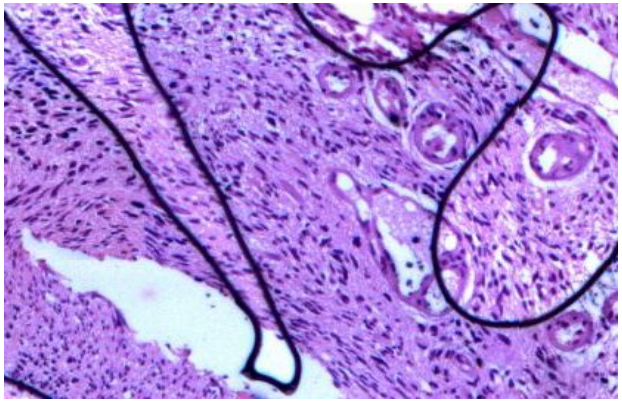
Tissue Ends - may seem necrotic but also tend to have some boundary

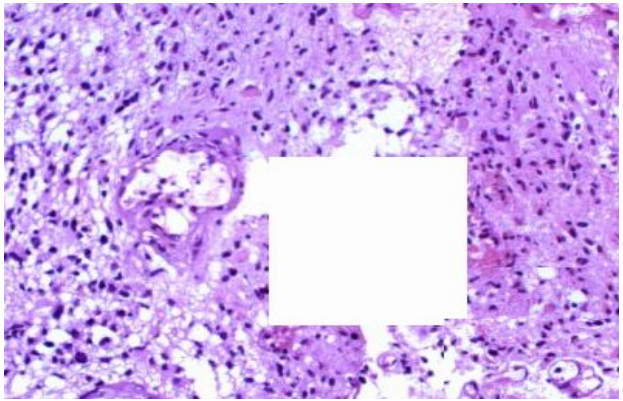




Challenges(contd.)

Artefacts - patches, overlaps, etc during digital scanning of the slides.





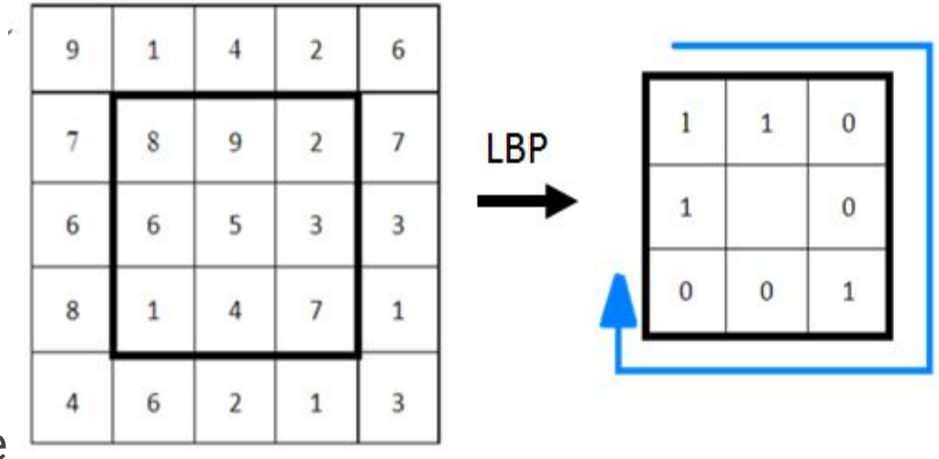
Proof of Concept

- Sand v/s Gravel classification
- Texture features : LBP features -> Histogram of LBP features -> Energy, Entropy, Mean, Variance and Skewness
- 0.9 accuracy - 1 misclassification of gravel to sand

Local Binary Pattern

It is a type of visual descriptor used for classification. It works like:

- Convert the input color image to grayscale,
- Divide the examined window into cells
- For each pixel in a cell, compare the pixel to each of its 8 neighbors. Follow the pixels along a circle, i.e. clockwise or counter-clockwise.



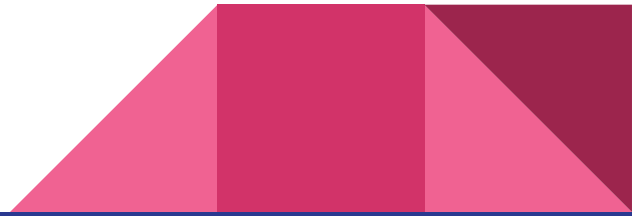
Local Binary Pattern(contd.)

- Where the center pixel's value is greater than the neighbor's value, write "0". Otherwise, write "1". This gives an 8-digit binary number (which is usually converted to decimal for convenience).

Image Source: http://hanzratech.in/2015/05/30/local-binary-patterns.html

Local Binary Pattern(contd.)

- Compute the histogram, over the cell, of the frequency of each "number" occurring.This histogram can be seen as a 256-dimensional feature vector.
- Normalize the histogram.
- Concatenate (normalized) histograms of all cells. This gives a feature vector for the entire window.

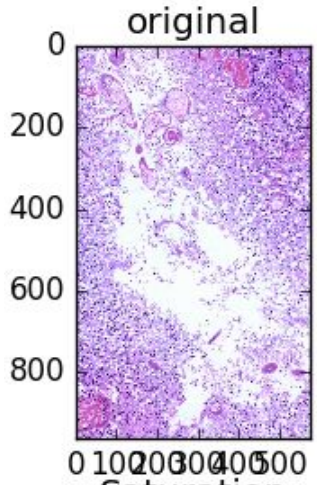


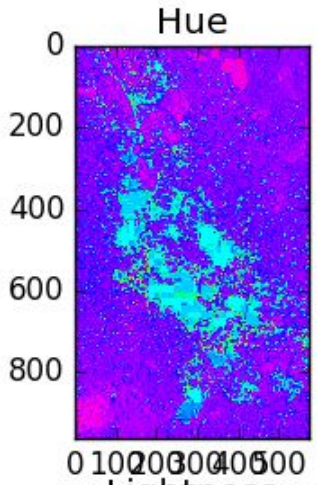
Features

- Used LBP with various radii values {1,3,5,7,9}.
- Extracted the normalised histogram for each image.
- **Texture Features :** 5 Statistical Features are then extracted from the LBP Histogram and used as features i.e. Energy, Entropy, Mean, Variance & Skewness
- **Color Feature :** Proportion of white pixels in Saturation and Lightness axis of HSL Colorspace is also added to the above feature vector.

$$proportion = \frac{\#pixelwithvalue = 255}{n}$$

Where, n = Total number of pixels in the image


original
0
200
400
600
800
0 100 200 300 400 500


Hue
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200
400
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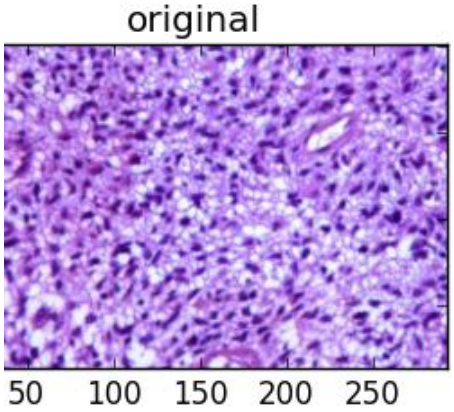
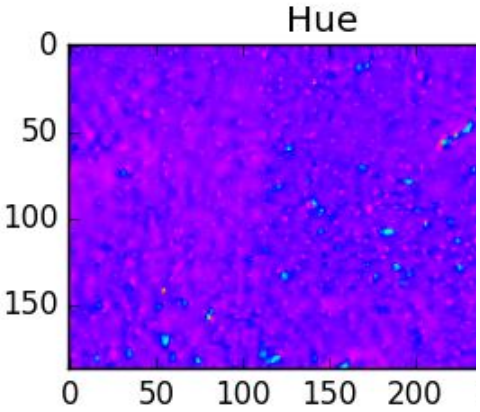
0 100 200 300 400 500


original
50
100
150
200
250


Hue
0
50
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0
50
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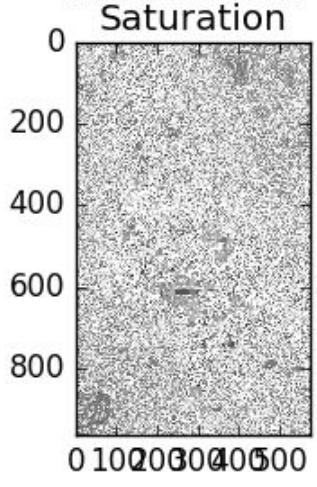
200


Saturation
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200
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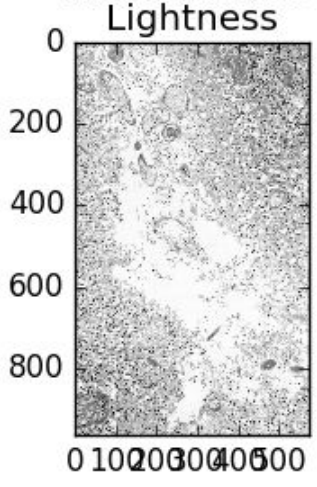
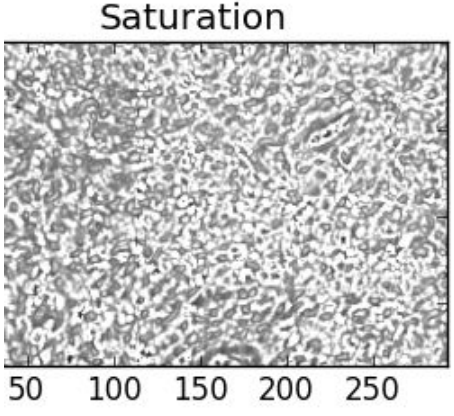
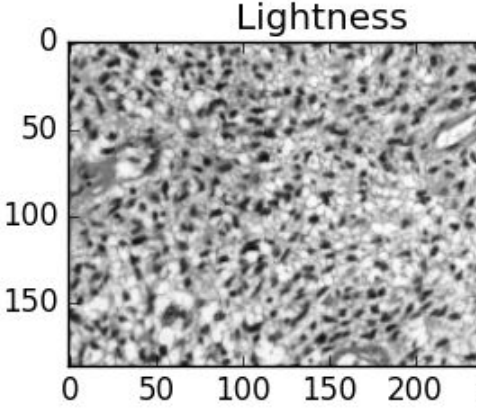
0 100 200 300 400 500


Lightness
0
200
400
600
800
0 100 200 300 400 500

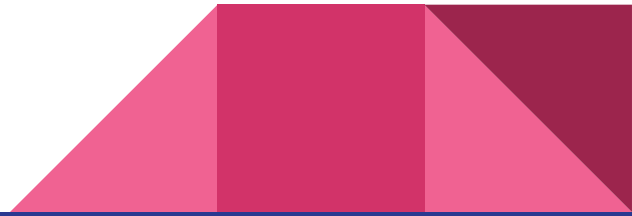

Saturation
50
100
150
200
250


Lightness
0
50
100
150
0
50
100
150
200

Classifier

We used the above obtained classifier and then used it classify the test images using:

- Support Vector Machines (SVM)
- k-Nearest Neighbour (k-NN) (k=9, 15, 19)
- Decision Trees



Training & Testing

- Trained over 60 images of varying images sizes obtained from the H&E slides. 30 images belonged to Necrosis and 30 to Non-necrotic.
- Test set consist of 10 images from each category of varying image sizes.

Results

Confusion Matrices

	Necrosis	Non-Necrosis
Necrosis	0.8	0.2
Non-Necrosis	0.3	0.7

SVM

Accuracy : 0.75

	Necrosis	Non-Necrosis
Necrosis	1.0	0.0
Non-Necrosis	0.4	0.6

k-NN (k = 9)

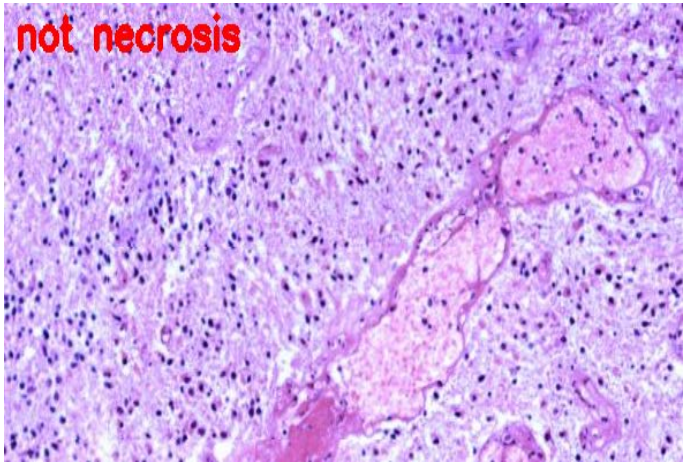
Accuracy : 0.8

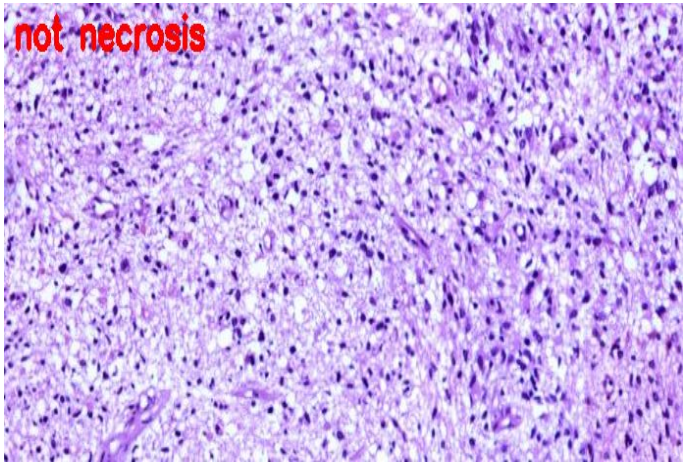
	Necrosis	Non-Necrosis
Necrosis	0.8	0.2
Non-Necrosis	0.0	1.0

Decision Trees

Accuracy : 0.9

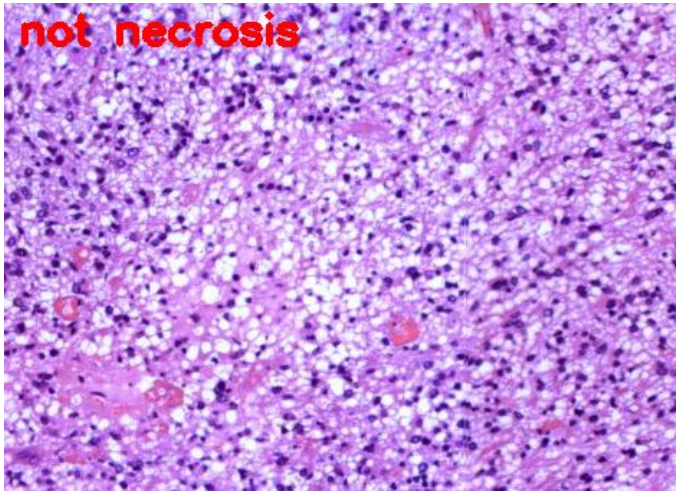
Results

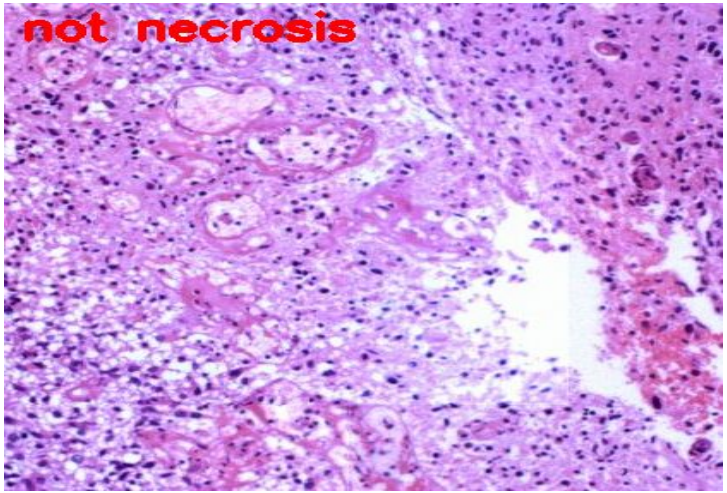




Necrosis Misclassifications - possible lack of white region leads to misclassification

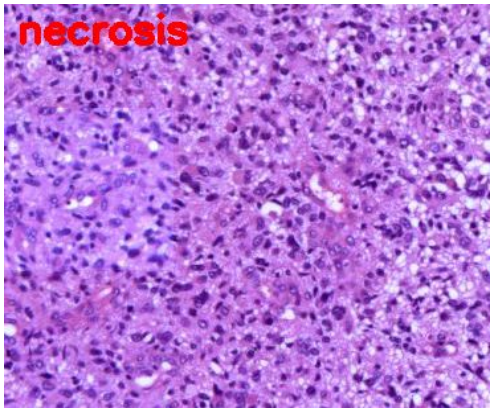
Results (Contd.)

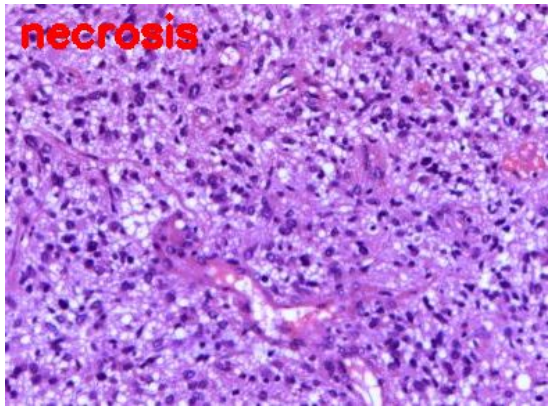


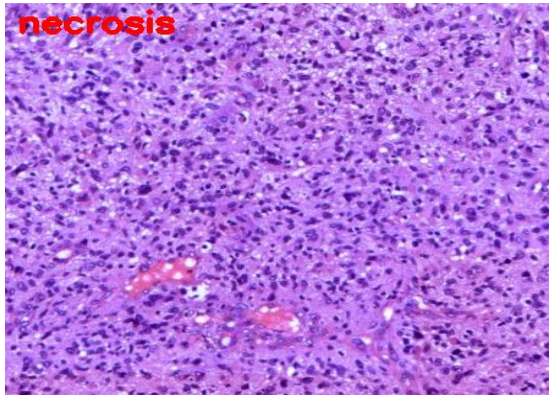


Misclassification for necrosis class - possible different texture in cytoplasm

Results (Contd.)



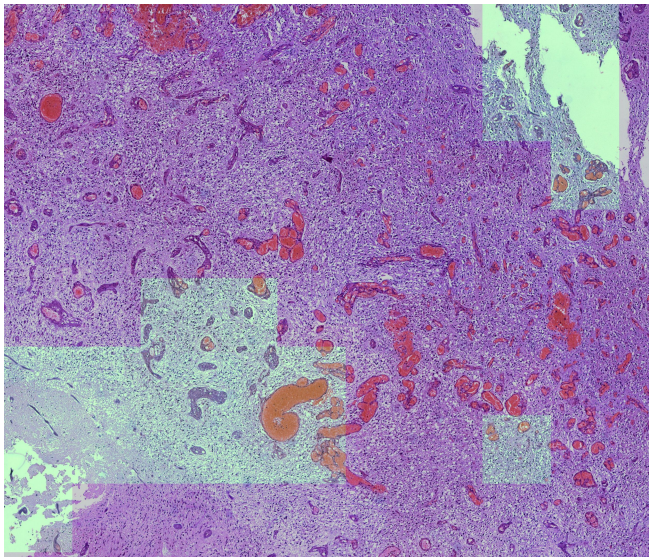




Non necrosis misclassification - possibly due to the white spaces

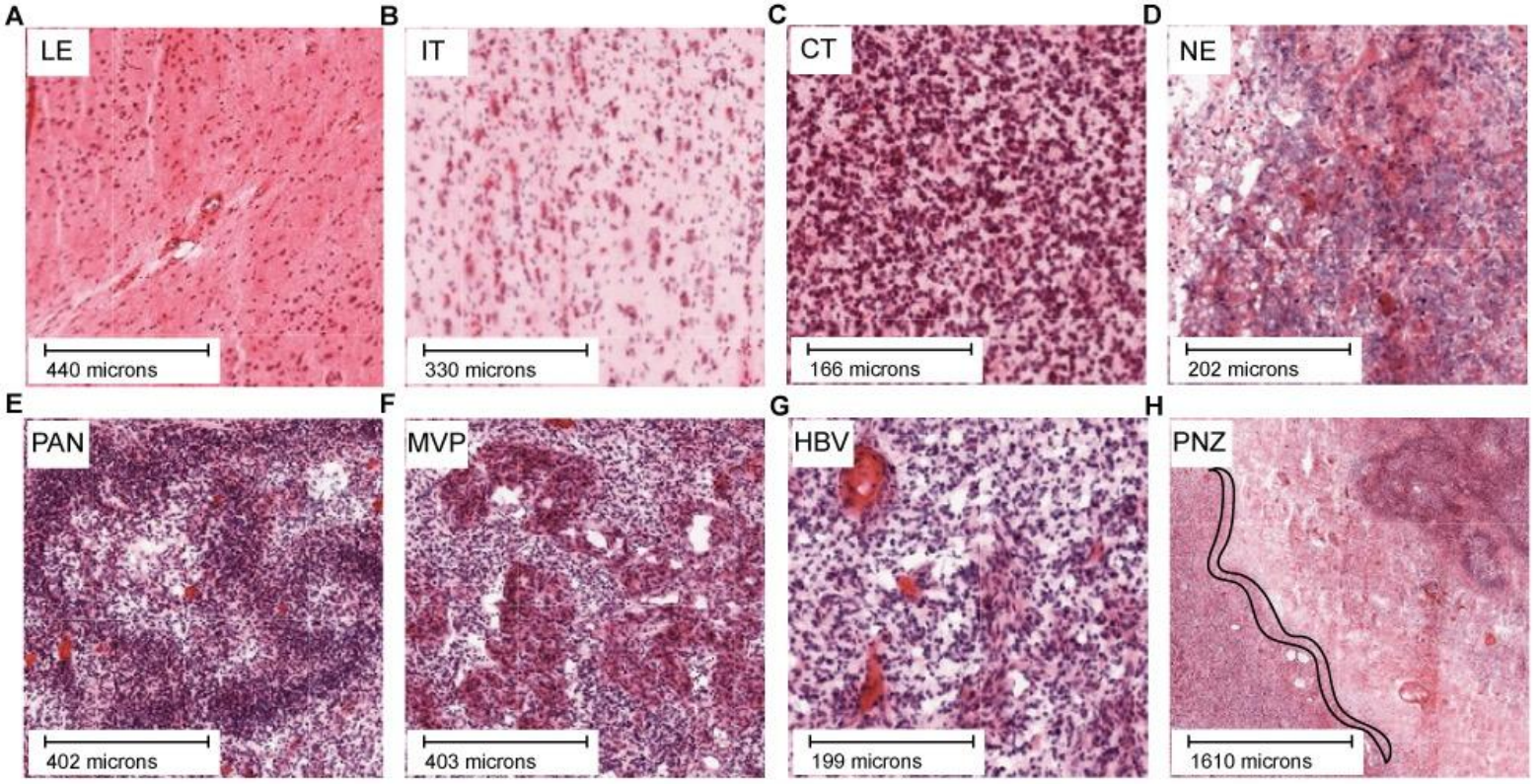
Future Work

- 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.
- Detection of other histological features like PNZ, MVP, PAN etc can be done along with the necrosis.



Necrotic region detection in a given tissue region - not a patch

Other Histological features in GBM



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