Quantifying DNA Damage in Comet Assay images using Neural Networks

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INTRODUCTION

- Proton therapy is an attractive form of radiotherapy as it deposits maximal energy at the tumour site, very little before the tumour and none after it, **Fig 1.**
- Further study is needed to fully understand the biological effects of proton irradiation on cells.
- These effects can be measured using the Comet Assay; an established technique to reveal the level of damage caused to cellular DNA, **Fig 2.**

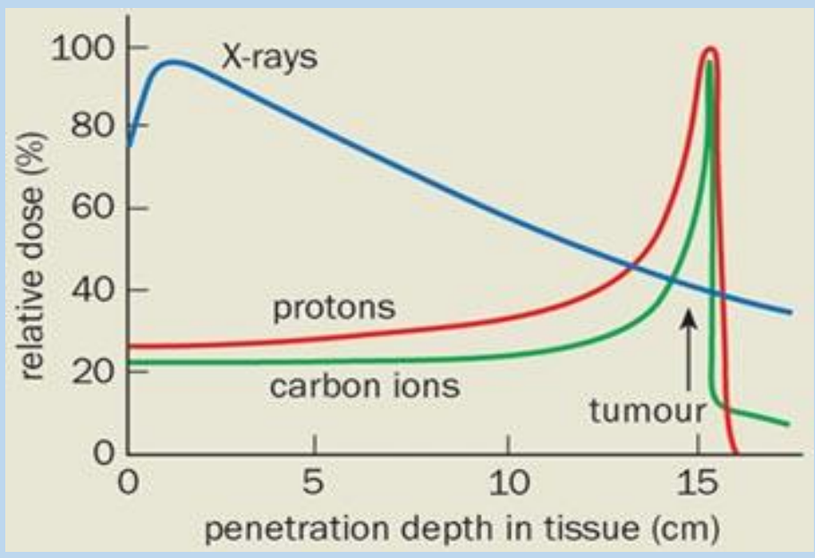


Fig. 1: A plot showing the different energy deposition along the path of protons, x-rays and carbon ions [1].

COMET ASSAY

- Cells are embedded in agarose gel.
- Lysis is performed to break down cellular and nuclear membranes.
- An alkaline environment separates the two DNA strands for investigating single strand breaks, whereas a neutral environment is used to study double strand breaks.
- Electrophoresis causes size dependent motion of the charged DNA fragments, indirectly revealing the number of strand breaks.
- The DNA is stained and imaged.
- Images are then analysed manually or using software.

PROGRAM

- Analysis of Assay images can be time consuming, tedious and potentially subject to bias.
- A program is under development to fully automate the analysis of Comet Assay images.
- The program is designed to take a directory of images and, with minimal user input, perform quantitative measurements.
- It identifies comets in the image via one of two methods and then performs area, tail length and tail DNA % measurements on the comets, **Fig 3a.**
- These data are output in a single spreadsheet for all analysed images, together with an annotated plot.

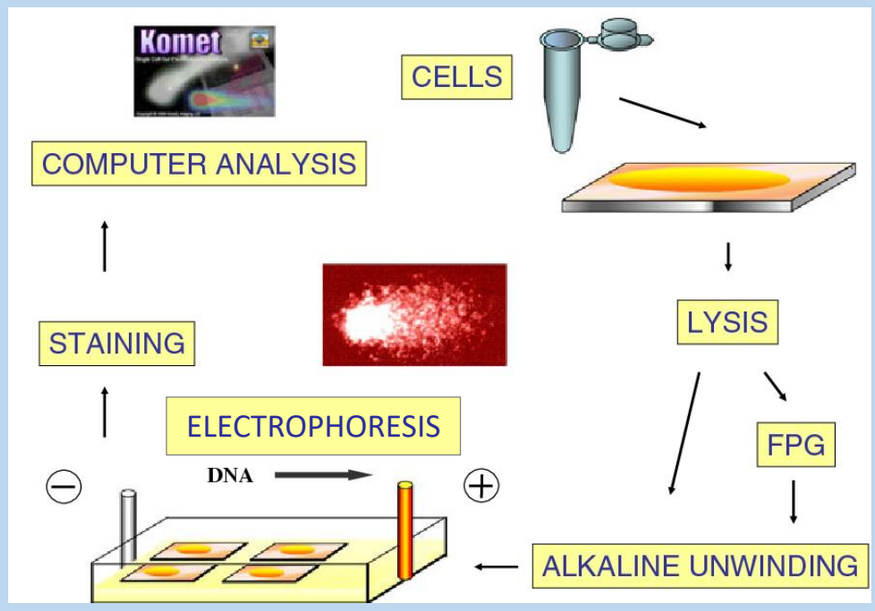


Fig. 2: A depiction of the comet assay process [2].

COMET IDENTIFICATION

METHOD 1

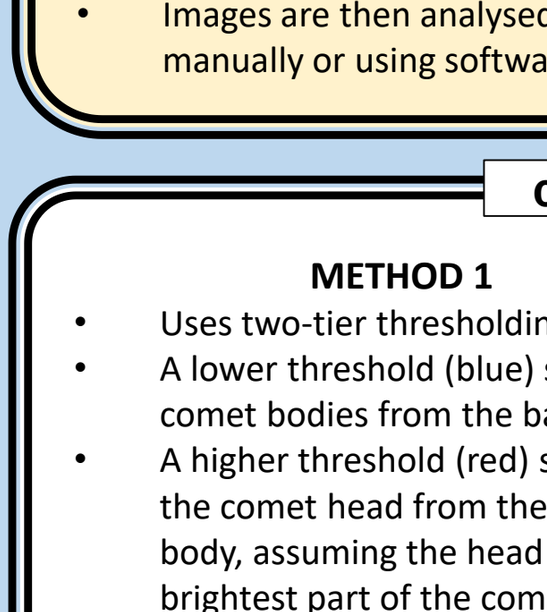
- Uses two-tier thresholding, **Fig 3b.**
- A lower threshold (blue) separates comet bodies from the background.
- A higher threshold (red) separates the comet head from the comet body, assuming the head is the brightest part of the comet.

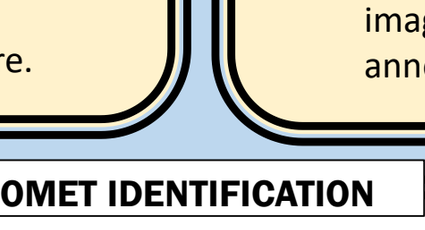
METHOD 2

- Implements instance segmentation using neural network architecture, **Fig 4.**
- Each pixel is classified either background or comet.
- This is combined with object detection to create a mask for each instance of a comet.

NEURAL NETWORK

- Framework is Mask-RCNN's COCO model in Keras and Tensorflow.
- COCO model has been trained on > 200,000 labelled images in 80 object categories.
- Uses transfer learning to minimise training time – utilising COCO's weights
- Allowed model to be trained on < 100 labelled images, annotated using VGG Image Annotator [3].





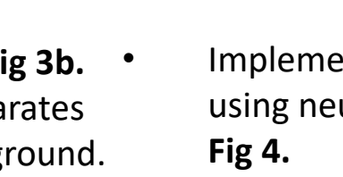
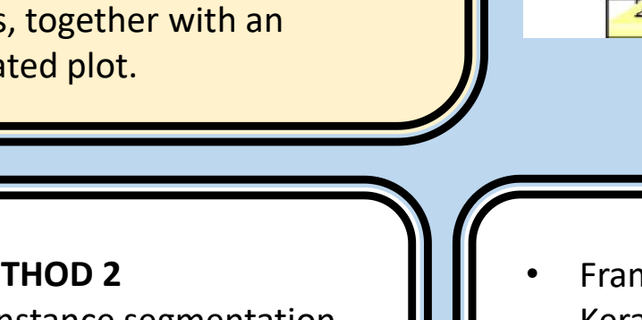


Fig. 3: (a) A depiction of how the comet measurements are performed. **(b)** Applying the two thresholds to identify comet bodies and comet heads. **(c)** An example comet head underestimate shown in darker blue.



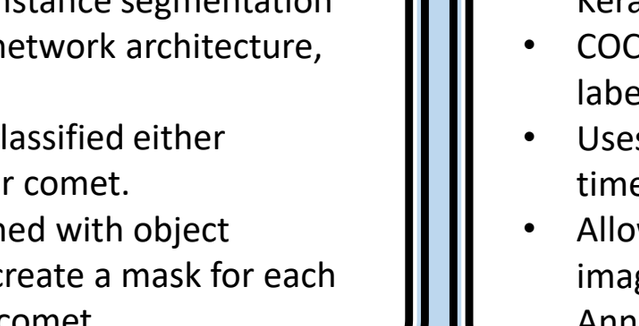


Fig. 4: (Top) an example raw image read in to the program.
(Bottom) The same image after instance segmentation has been performed.

CONCLUSION

- Instance segmentation is the more accurate comet identification method: it keeps more of the identified comets, typically creating a > 90% accurate segmentation mask.
- Instance segmentation requires no modification for different images, whereas thresholding must be optimised image-by-image for best results.
- Further analysis and testing of these techniques will reveal which is the more powerful.

REFERENCES

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