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.

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.

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].

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

[2] https://ai2-ai2-public.s3.amazonaws.com/figures/2017-08-08/321592ec85a3e0e6652c8c4218f3586dbc203423/3_Figure1.png