Quantifying DNA Damage in Comet Assay images using Neural Networks

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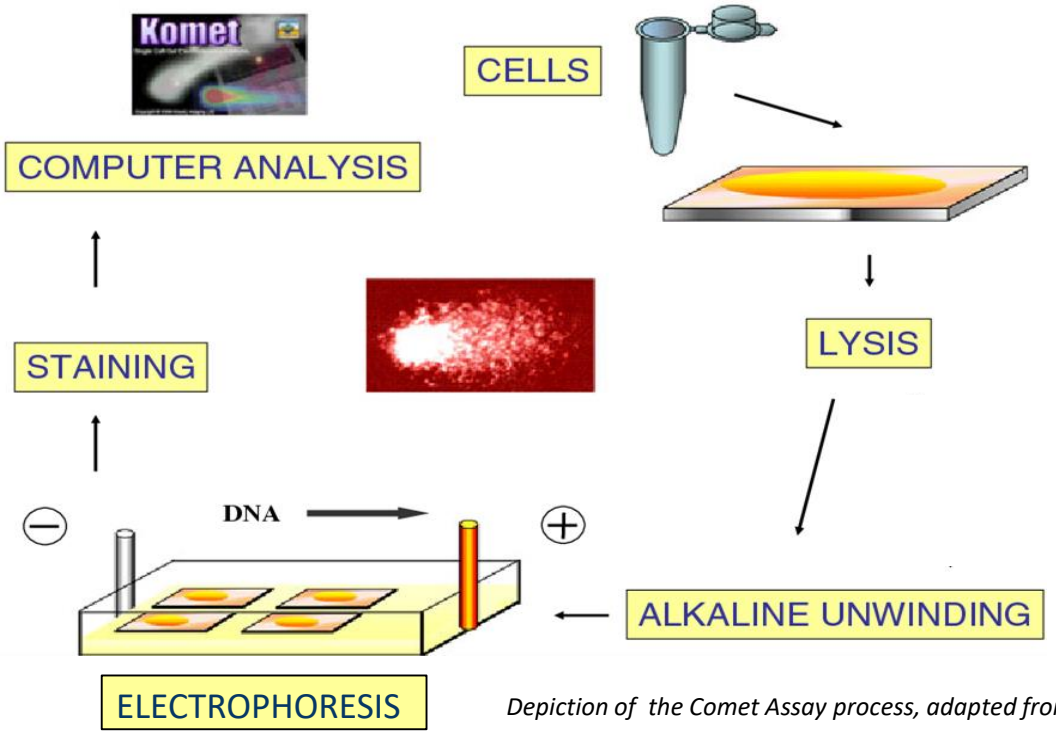
QUASAR GROUP

Overview

- Comet Assay
  - Measure DNA damage
- Program to automatically quantify damage
  - Incorporating NN for instance segmentation
  - Issues data available
- MC simulation of comet assay images

Comet Assay

Procedure for obtaining
DNA damage levels



Depiction of the Comet Assay process, adapted from [1].

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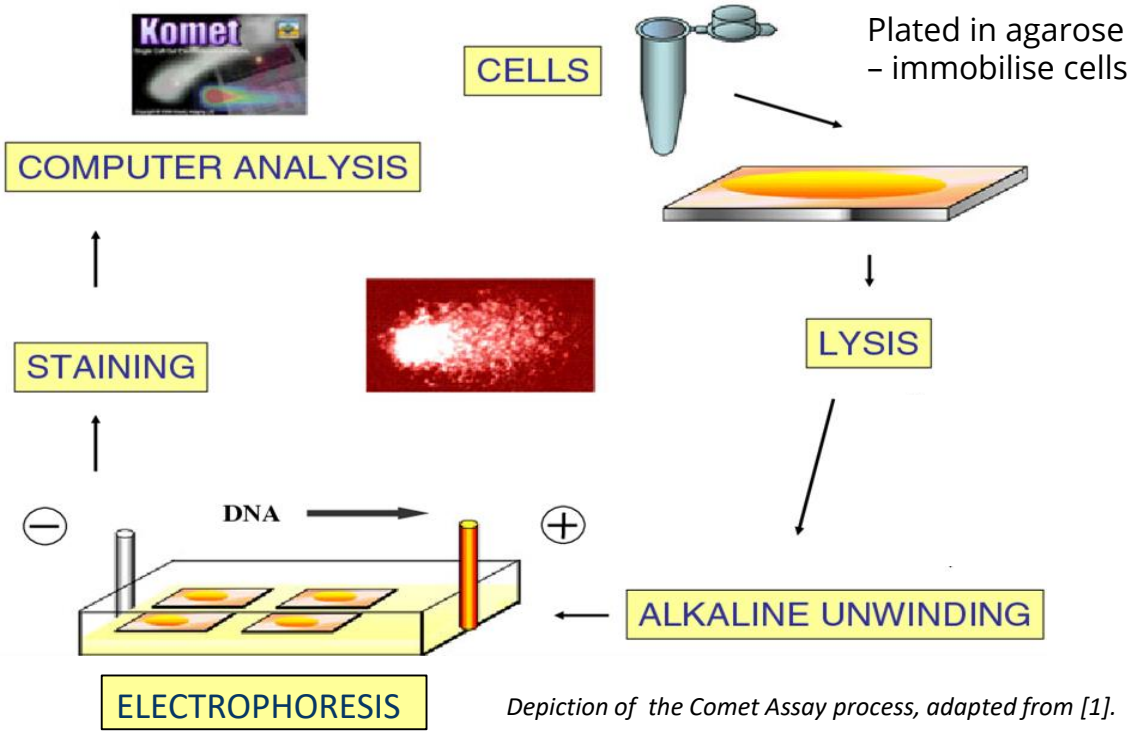

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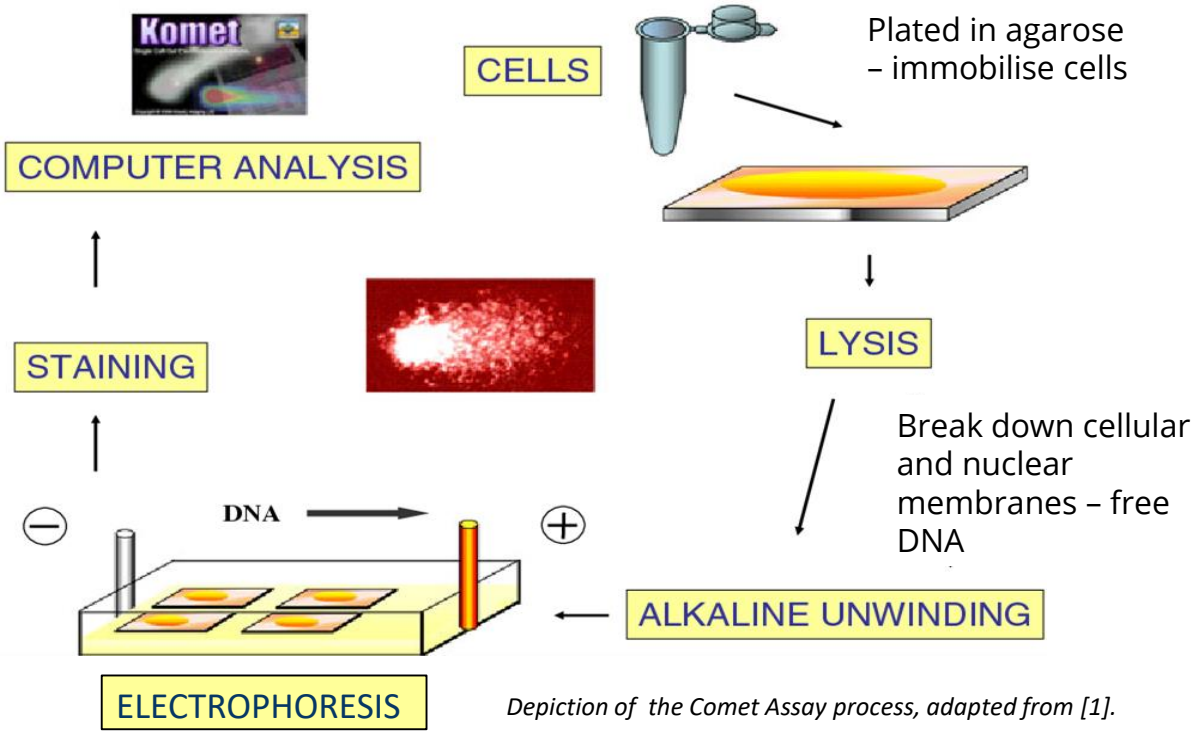

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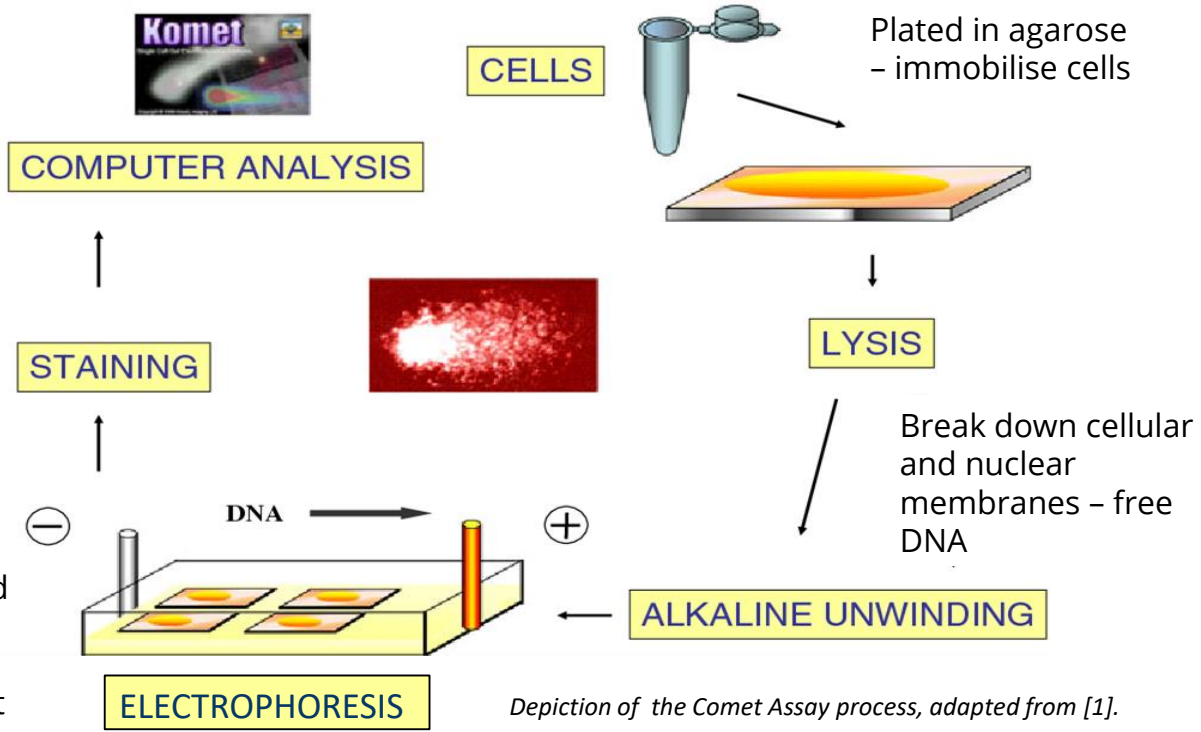

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Comet Assay Images

More fluorescent and elongated tail indicative of more strand breaks

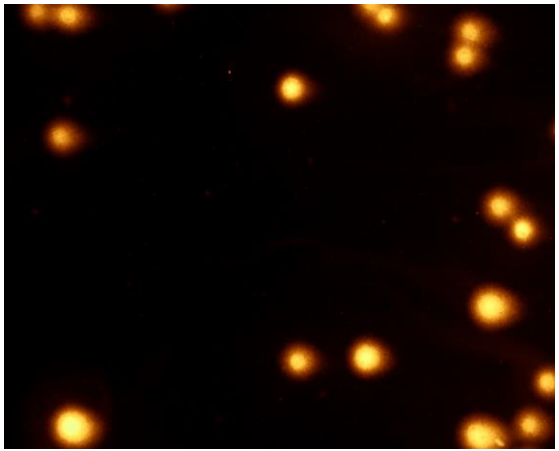

Image of control cells (no irradiation) after a comet assay

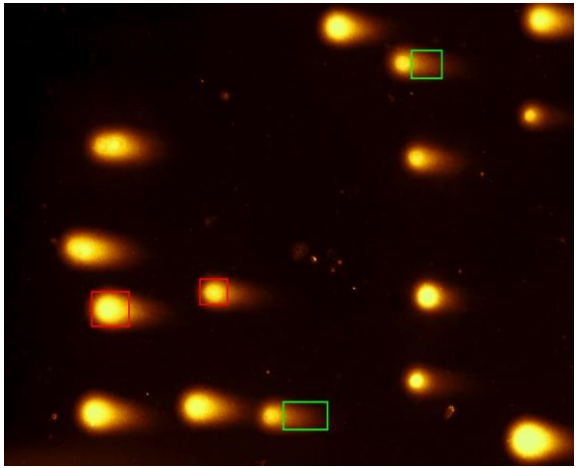

Image of irradiated cells that have formed comets following a comet assay. Comet heads are outlined in red, tails in green.

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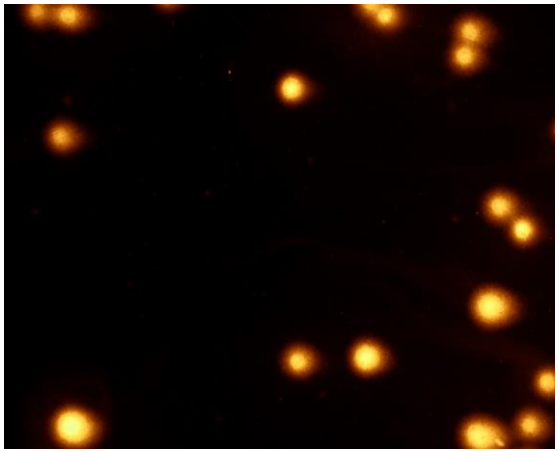

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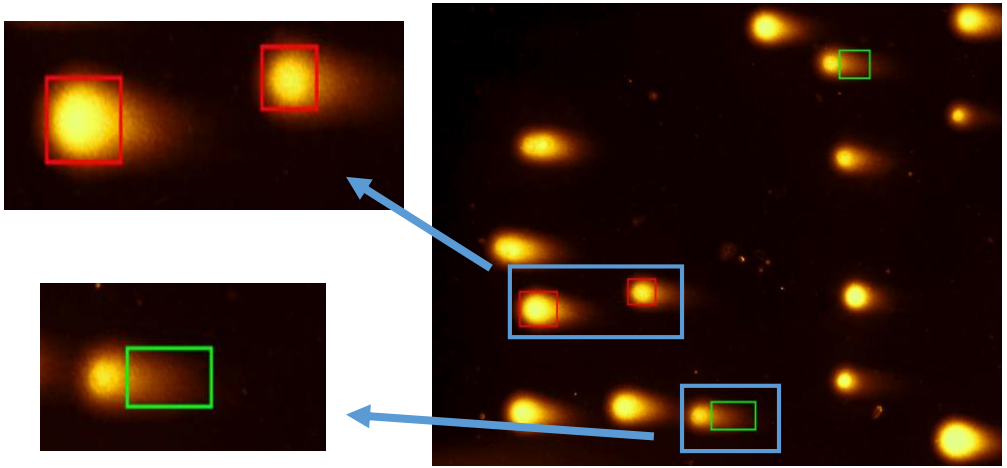

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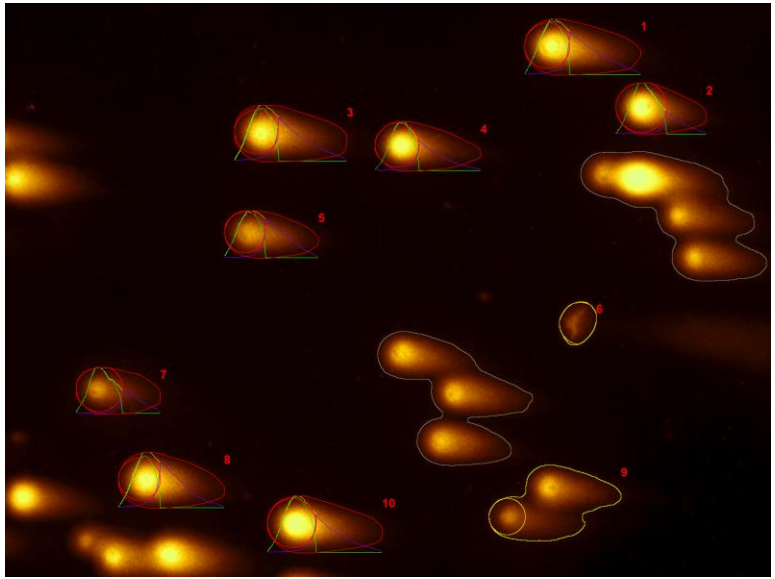
Image Analysis

- Methods of analysis vary a lot
  - By eye - relative & carries lots of variability - no numeric results
  - Semi-automated - user defines comet regions and comet properties are measured
  - Full Automation - quickest, but measurement algorithms still vary
- Developing programme to automate analysis
  - As little user input as possible
  - Written in Python

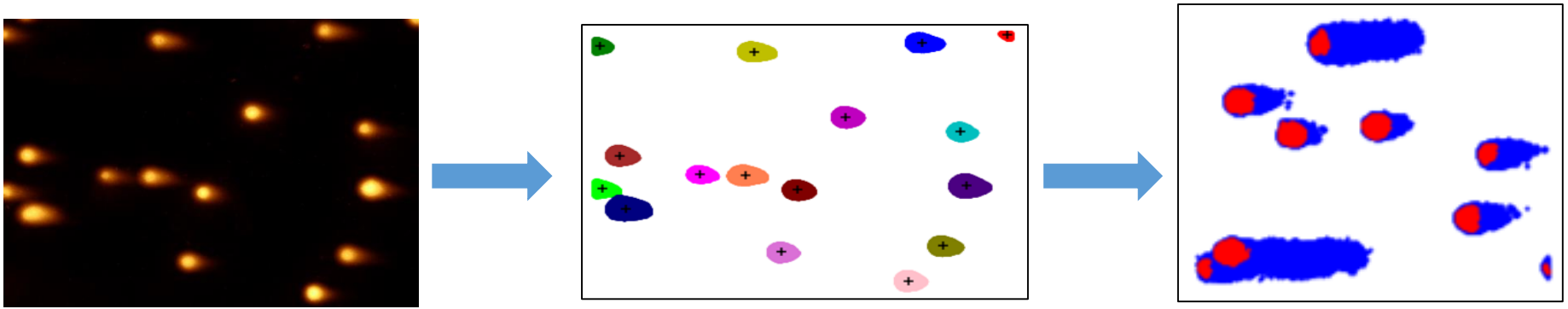


OpenComet
A fully automated free comet assay software

Open Source comet analysis software [1]

Measuring Comets

- Image analysis and thresholding to extract comet body and head
- Widely used measurements: **tail length and tail DNA %**
- Thresholding **loses data** around comet edge



Measuring Comets

$$tail\ DNA = \frac{\sum tail\ pixel\ intensities}{\sum comet\ pixel\ intesnities} * 100$$

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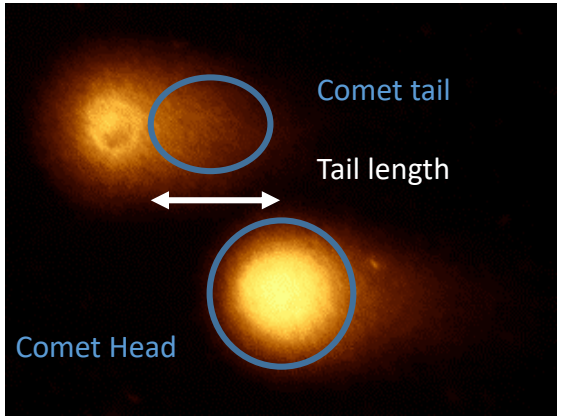
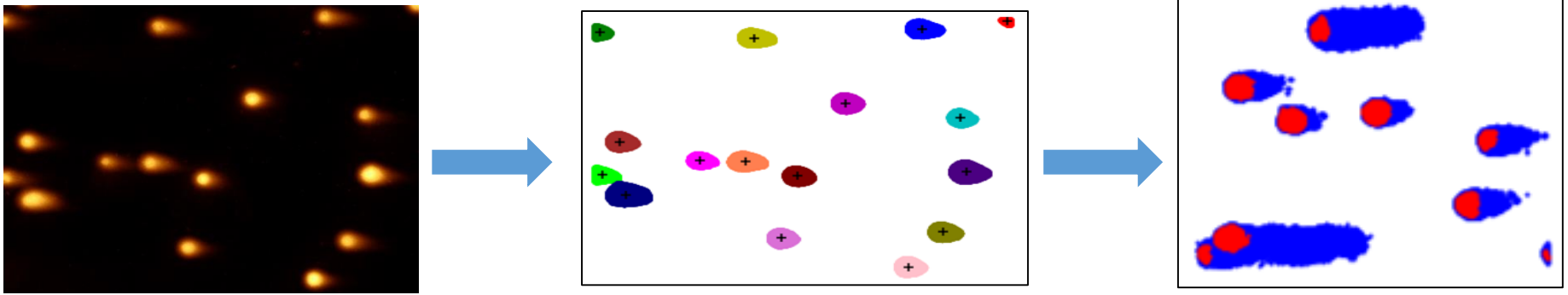
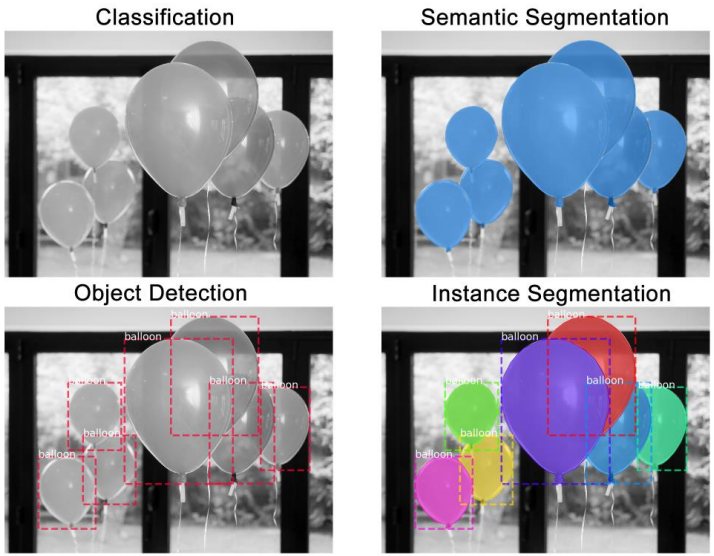




Identifying Comets

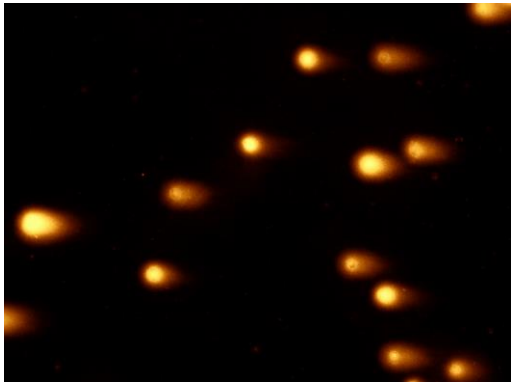
- Train a network to identify comets within images automatically as a first step
- Using **instance segmentation –** identify all instances of comets as separate masks
- Identifications based on overall structure rather than intensity - more robust across different image sets

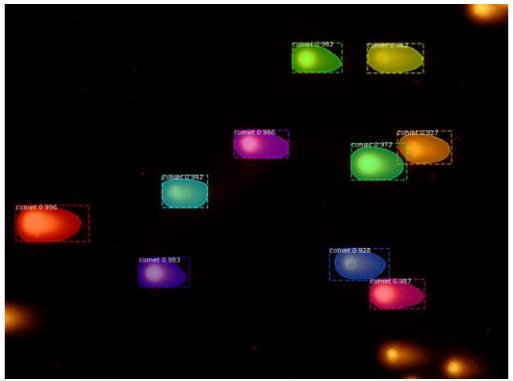


Representation of how the instance segmentation method combines the bounding box and pixel-wise classification methods [3].

Instance Segmentation

- Mask R-CNN combines Faster R-CNN and FCN
  - Faster R-CNN consists of RPN and RoIPool
  - RPN proposes bounding boxes
  - RoIPool extracts features to classify bboxes
- Adding FCN gives additional mask output
  - Total loss is combined from classifications, bounding boxes and masks





Instance Segmentation

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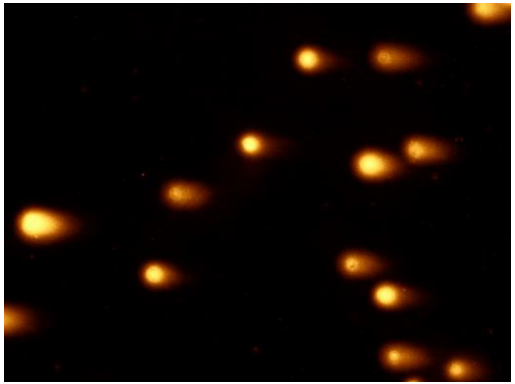
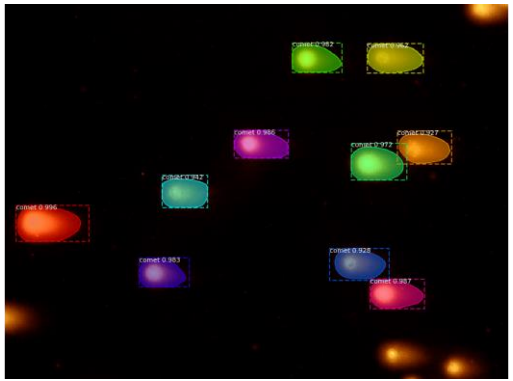
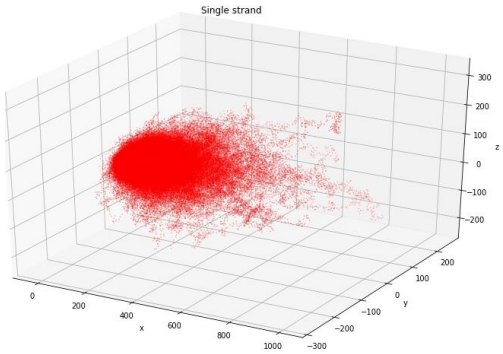
- Adding FCN gives additional mask output
  - Total loss is combined from classifications, bounding boxes and masks
- Transfer learning - use already trained weights (COCO model [5])
  - < 100 images used
  - Vastly quicker train time
  - >90% confidence in masks
- However, **lack of data** available for training/testing

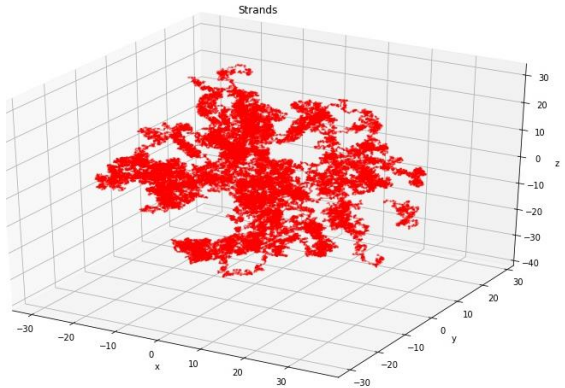


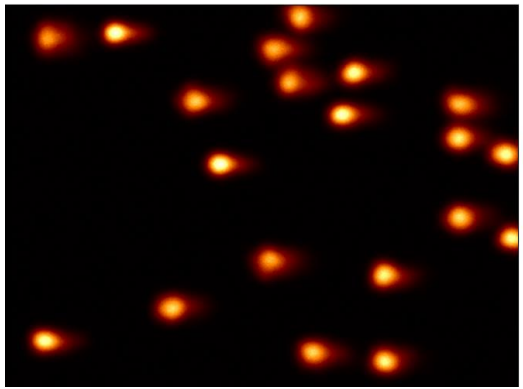


Simulating Images with MC

- Crude modelling of assay to produce simulated images
  - Random breaks on strands
  - Drift broken strands away from cell: E field strength and strand length dependent
- Use to further test neural net performance and train other models







Summary

- Comet assay images need better, consistent quantification of DNA damage levels
- Program proposed automates the process
  - Uses instance segmentation to identify comets
- Monte Carlo
  - Simulate the assay procedure to produce simulated images
  - Provide more data for NN models and further testing of performance

References

[1] H. Karlsson. “The comet assay in nanotoxicology research”. In:Analytical and Bioana-lytical Chemistry398 (2010), pp. 651–666
[2] BM Gyori, G Venkatachalam, PS Thiagarajan, D Hsu and MV Clement. "OpenComet: An automated tool for comet assay image analysis", Redox Biology, 2:457-465, 2014.
[3] https://engineering.matterport.com/splash-of-color-instance-segmentation-with-mask-r-cnn-and-tensorflow-7c761e238b46
[4] Kaiming He et al. “Mask R-CNN”. In:CoRRabs/1703.06870 (2017). arXiv:1703.06870.url:http://arxiv.org/abs/1703.06870
[5] Tsung-Yi Lin et al. “Microsoft COCO: Common Objects in Context”. In:CoRRabs/1405.0312(2014). arXiv:1405.0312.url:http://arxiv.org/abs/1405.0312

Thank you, Questions??