7-plex Immunofluorescence and One Object Classifier to Rule Them All?

Aoife McArdle[1], Victoria Bingham[1], Stephanie Craig[1], Manuel Salto-Tellez[1,2]

1. Precision Medicine Centre of Excellence, Patrick G. Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, Northern Ireland
2. Belfast Health and Social Care Trust, Belfast, Northern Ireland

AIM To assess the extent image artefacts have on the creation of object (cell) classifiers for automated image analysis.

KEY FINDINGS

- Due to the presence of artefacts, a "one size fits all" approach is not possible with object classifiers developed using machine learning.
- When these types of classifiers are trained on images without any of the previously stated artefacts present, accuracy of the classifier is reduced when artefacts are then present.
- A tiered approach i.e. a high, standard and low classifier, for each biomarker may be more effective for accurate cell classification.
  This would enable appropriate detection of true fluorescent staining for cell classification.
- In some cases, the impact of artefacts are too severe to be counteracted by wide category classifiers. The area of concern can be annotated out to ensure the unaffected tissue can still undergo cell classification using the tiered approach. Individual classifiers could be created however this is not suitable for large scale cohorts.

INTRODUCTION

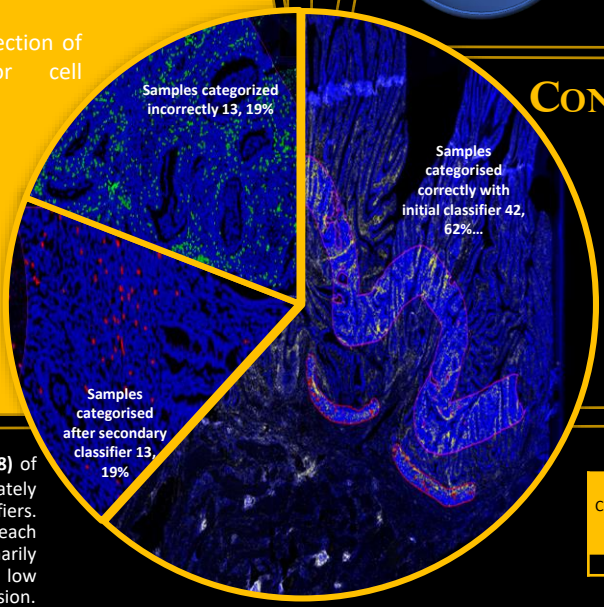
Non-specific staining, edge effect, haemorrhage, and tissue thickness increase the complexity for digital quantitative analysis of tissues labelled by multiplex immunofluorescence. This study aims to assess the degree to which image artefacts may impact the creation of object (cell) classifiers for automated image analysis.

METHOD

STEP 1
An object classifier was created for each biomarker in a digital cohort using machine learning.

STEP 2
All samples underwent rigorous quality control (QC) checks to approve tissue processing, multiplex immunofluorescent staining, spectral unmixing, and the annotated digital images with classified cells produced.

STEP 3
Additional object classifiers were utilised where required based on non-specific digital artefacts identified during QC.



CONTACT

AoifeMcA_Sci1
AoifeMcA_Sci
Aoife McArdle
aoife.mcardle@qub.ac.uk
PRECISION MEDICINE CENTRE OF EXCELLENCE

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RESULTS

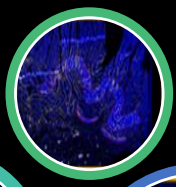
It was found that **62% (42/68)** of samples included in the study were accurately categorised using the initial object classifiers. Additional classifiers, were created for each biomarker. High classifiers were created primarily to account for excessive expression and low classifiers to increase detection of lower expression.

These were applied to the misclassified images only. This resulted in an additional **19% (13/68)** of samples being correctly categorised, resulting in **81% (55/68)** correctly classified overall. Low classifiers were applied predominately to increase the cell count, as a result of suboptimal staining.

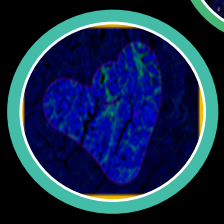
Additional Classifiers used on 1 biomarker per sample	Additional Classifiers used on 2 biomarkers per sample	Additional Classifiers used on 3 biomarkers per sample
4	15	6

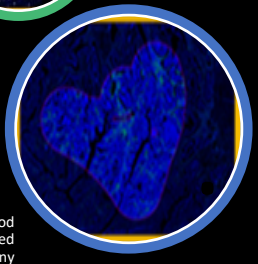
High classifiers were created to attempt to counteract false positive classification caused by edge effect, haemorrhagic tissue, and autofluorescence caused by section thickness, which influenced the ability of the software to register true vs non-specific staining. However, in some cases the impact of these artefacts was too severe and could not be counteracted using classifiers and were therefore annotated out.

High classifiers were used on 24 samples across 1, 2 or all 3 biomarkers. This contributed to the correct categorisation of all 13 samples.

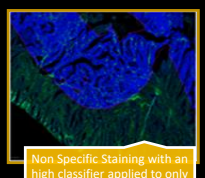


Low classifiers only were required for CD3 and applied to 2 samples. This did not change the categorisation of either sample.

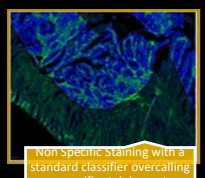




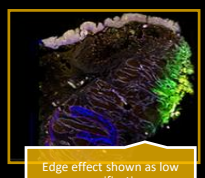
It was noted during this QC period that some artefact required complete removal to achieve any appropriate cell classification.



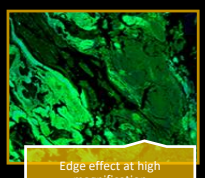
Non Specific Staining with an high classifier applied to only count true signaling.



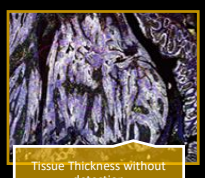
Non Specific Staining with a standard classifier overcalling non-specific staining as true signaling



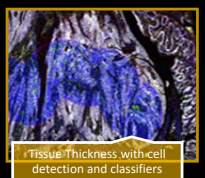
Edge effect shown as low magnification



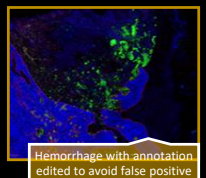
Edge effect at high magnification



Tissue Thickness without detection



Tissue Thickness with cell detection and classifiers applied



Hemorrhage with annotation edited to avoid false positive calls

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