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Classifiers for Lymphocyte Classification from Hoechst Stained Loops

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Introduction

Patients benefit from multiplex immunofluorescence and immunohistochemistry because it allows cancer pathologists to identify proteins expressed on the surface of cells. This allows for cell classification, a better understanding of the tumour microenvironment, and more accurate diagnoses, prognoses, and tailored immunotherapy based on individual patient immune status. However, these methods are costly. They are time-consuming processes that necessitate complex staining and imaging techniques performed by skilled technicians. Hoechst staining is significantly less expensive and easier to perform, but it is rarely used because it binds to DNA rather than the proteins targeted by immunofluorescence techniques. We show in this paper that using deep learning, it is possible to identify an immune cell subtype without using immunofluorescence [1].

Description

Clinical outcomes vary greatly among patients with cancers of the same stage. This is thought to be due in large part to the complex interaction between tumour cells and individual patients' immune responses, as the proportion, location, and sub-type of lymphocytes present in the tissue has been shown to have important implications for patient prognosis. As proposed by Galon et al., there are proprietary methods for assessing immune cell infiltration that formally quantify T cell lymphocytes both in the tumour centre and at the invasive margin. Combining their evaluation with Mlecnik and B-score had significant predictive power for colorectal cancer patient survival [2].

The current study is the first step toward lowering the cost of identifying immune cell subtypes. We demonstrate that deep learning can be used to identify CD3-expressing lymphocytes from a common and inexpensive stain. Hoechst and DAPI staining (popular blue fluorescent, nuclear-specific dyes) are far less expensive and easier to perform, costing pennies and taking only ten minutes per slide. Although DAPI has better photostability, Hoechst is used in this work because of its superior signal-to-noise (genuine DNA stain/autofluorescence) ratio [3].

The novelty of our approach is thus twofold: first, we demonstrate that it is possible to identify CD3 expressing lymphocytes from Hoechst stained tissue; and second, we do so without the use of an intermediary method of virtual staining. We image each tissue section with both Hoechst and immunofluorescence stains; use an intensity-based classifier on the immunofluorescence images to identify which cells express CD3; and use those classifications to label the same cells in the Hoechst-stained images. Then, using only the Hoechst images as input, we train a deep neural network to classify CD3-expressing cells using these Hoechst image/immunofluorescence-classification pairs. We force the network to find patterns in the Hoechst-stained cells that correspond to the correct immunofluorescence labels in this manner.

To understand how the model can identify CD3 expressing lymphocytes, we use Hierarchical Perturbation and standard iterative perturbation in this section. These methods are widely used for deep learning interpretability because they provide intuitive visual interpretations of which input regions were more or less important in determining the model's output. Both work by perturbing regions of the input and then using the change in the model's output as a basis for creating a saliency map. Iterative perturbation accomplishes this sequentially by traversing the input with a perturbation kernel of fixed size. HiPe approaches this in a more dynamic manner, first perturbing large, overlapping regions and examining the relative difference in saliency between those regions [4,5].

Conclusion

We show that using only Hoechst staining, it is possible to identify cells expressing CD3. Furthermore, we demonstrate how, with interpretability techniques, neural networks can be valuable tools for both discovery and automation: we use saliency mapping to visualise which features in the input the model is using to make correct classifications, and we find that these saliency maps highlight the nuclear chromatin within the cells, indicating that the chromatin texture and morphology made visible by Hoechst staining is predictive of CD3 expression. Future work will include investigating semi-supervised and unsupervised classification approaches using clustering to reduce labelling burden when training new models, as well as extending and applying this approach to other cancers and proteins.

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