Simulated Fluorescent Labeling

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Abstract

The ubiquity of fluorescent labeling in contemporary microscopy research has conferred it a notable reputation for effectively revealing structural information that is typically invisible. Arguably, however, this prized technique is not without its costs. Indeed, confounding effects such as phototoxicity to sample, spectral overlap and chemical fixing impose limits on what can be analysed. With the advent of deep learning, however, unlabeled transmitted light images can be exhaustively interrogated for any latent information they contain. In such a way, the dependency on actual fluorescent labeling can be lifted as the opportunity to artificially simulate its effect arises. This simulated fluorescent labeling (SFL) was the goal of this research project, and its performance was tested against a diverse variety of use-cases, mainly that of single and multi-label prediction from greyscale transmitted light images. The results produced display a very strong correspondence to actual labeling and moreover show a considerable ability to generalise on novel data. Nevertheless, there remains the necessity for a robust metric by which the validity of the inferences can be gauged, should the model ever wish to be implemented for conventional use.

Results

Single-label prediction

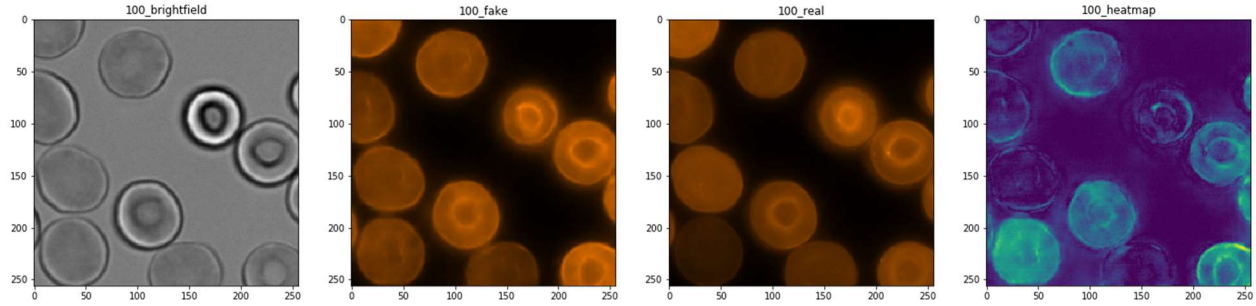

Figure a: Transmitted light image of RBCs (leftmost) from which a membrane label was predicted (2nd) as compared to the ground truth (3rd) via a contrast map (rightmost)

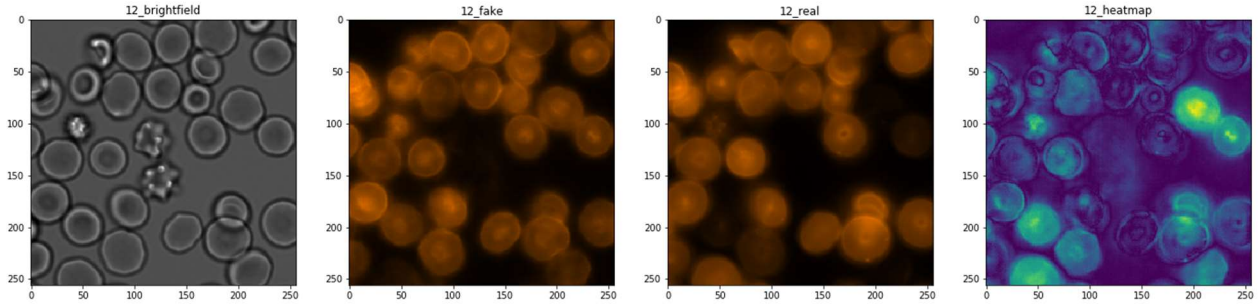

Figure b: Another case of successful selective labeling. Echinocytes (leftmost) were correctly left unlabeled by prediction (2nd), corresponding to the ground truth (3rd)

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Multi-label prediction

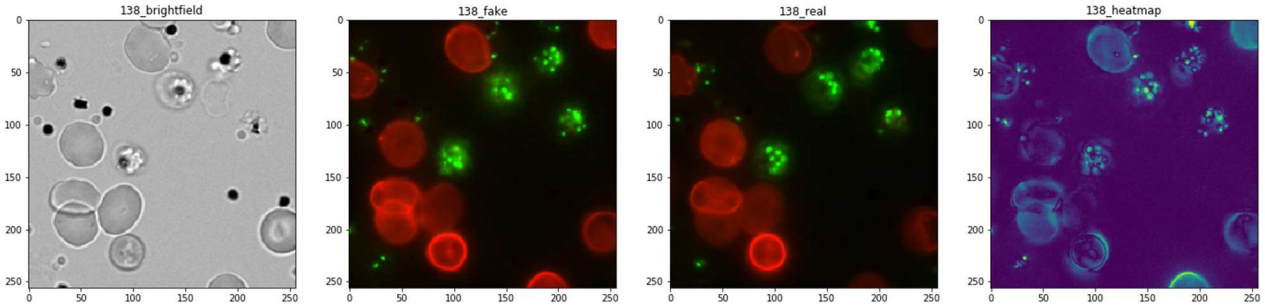

Figure c: Transmitted light image of malaria-infected RBC culture (leftmost) from which uninfected RBCs (red) and parasite (green) were predicted (2nd) as compared to ground truth (3rd)

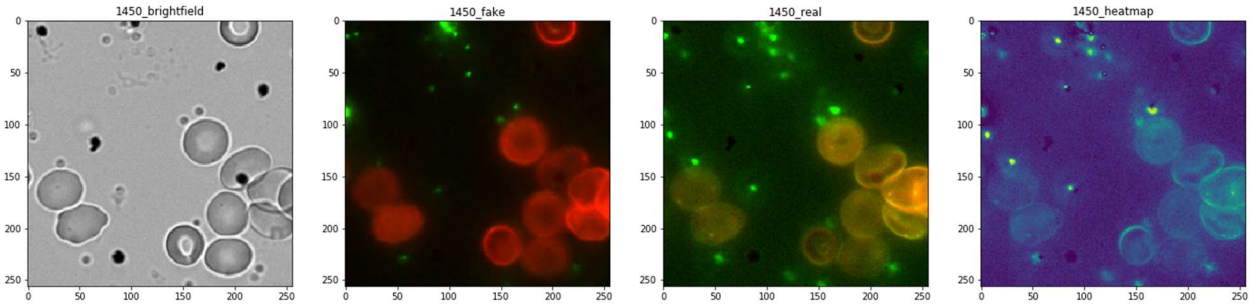

Figure d: Prediction (2nd) is not prone to spectral overlap displayed in ground truth (3rd) between the red and green channels.

Prediction on novel data

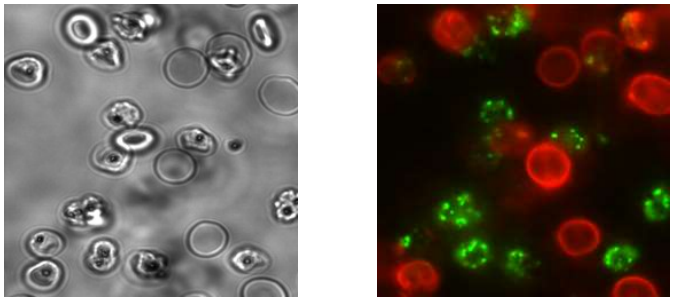

Figure e: prediction of RBCs and parasite labeling (right) from foreign transmitted light image (left) previously unseen

Discussion

A paramount feature of fluorescent labelling that was sought for in the simulated alternative was selectivity. In the single-label trial, the red blood cells in *figure a* are clearly demarcated from the background via a synthetic orange highlight that mimics the effect of real DI-4-ANEPPDHQ labelling of healthy red blood cells. The dimness of the heatmap further indicates the closeness of the two techniques. Moreover, *figure b* shows the correct omission of atypical cells (echinocytes) from this highlight, revealing the high-fidelity results of SFL in accordance to the ground truth. In the multi-label trial, the SFL's selectivity was tested against two different cell *types*, and again, promising results were observed as shown through the generally dim heatmap in *figure c*. *Figure d* provided a unique case of outperformance whereby the simulated labelling had greater spectral clarity compared to the bleed-through of green and red signals observed in the ground truth. Finally, *figure*

e reveals the model's capacity to generalise to unseen data, as shown through the omission of out-of-focus particles in the background. Interestingly, some trophozoites are captured by the SFL as emitting both green and red signals, implying a somewhat accurate understanding of the transitory phases involved in malaria infection.

Conclusion

The remarkable performance of SFL is apparent through its showcased ability to capture the hallmark of real labelling, that is, selectivity. The avoidance of other extraneous effects usually accompanied with traditional labelling makes SFL all the more ideal of a technique to be seriously considered for use. However, with the prospect of implementation comes a greater question of robustness. While heatmaps and other comparative measures provide a good understanding of the reliability of the technology against the ground truth, its performance on novel data is yet to be measured rigorously. This project has achieved the aim of revealing the substantial potential of deep learning in SFL. However, leveraging such technology for use in a scientifically controlled manner becomes a technical imperative which future research be heedful of.