MACHINE LEARNING MEETS MICROSCOPY: CELL EXPLORER TOOL FOR A DIAGNOSTIC LABORATORY

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ABSTRACT

Introduction: Control of tropical diseases has for decades depended on diagnosis by microscopy as the gold standard method of detection. It, however, faces the drawbacks of low sensitivity, operator reliance with user expertise and experience essential to make an accurate diagnosis leading to variable results. [3].

Objective: The purpose of this study was to explore a method that could help improve the process of microscopy via machine learning models for the detection of intra- and intercellular parasites.

Methods: A digital tool known as 'cell explorer' was developed to help in the detection and annotation of microscopic slide images taken from blood smears containing *Leishmania donovani, Plasmodium falciparum* and *Trypanosoma brucei rhodesiense* parasites. Advanced statistical modelling techniques were used including Convolutional Neural Networks, open-source image processing algorithms and clustering algorithms to detect cellular morphology of the parasites. Using Simple Linear Interactive Clustering, the cell explorer also functioned as a cell counter.

Results: The neural network was able to immediately detect cellular morphology and identify the *Leishmania* and *Trypanosome* parasites as well as different stages of the Plasmodium parasite with an average accuracy of ~95% . It was also able to accurately quantify the number of cells presented within each slide image.

Conclusion: The cell explorer presents a fast and accurate computer-aided microscopy tool with the ability to detect cellular morphology, successfully identifying *Leishmania donovani, Plasmodium berghei* and *Trypanosoma brucei rhodesiense* parasites. This work highlights the research potential of machine learning models as disease diagnostic applications effective in improving the microscopy process.

INTRODUCTION

Conventional microscopy is a routine laboratory diagnostic method widely utilised for morphological identification of cells and still remains the standard instrument available for parasite detection [4].

Tropical and infectious diseases such as Malaria, Leishmaniasis and human African trypanosomiasis predominantly affect poor, rural communities with limited access to quality healthcare. Disease control depends on accurate diagnosis and subsequent treatment, however, face the difficult challenge of inadequate tools. Existing diagnostic methods such as serological tests and molecular methods have the disadvantage of requiring well-established laboratories with equipment, cold chain and trained personnel, which are usually lacking.

Parasite visualisation in blood or aspirate material by microscopic examination has been the gold standard of diagnosis as prescribed by the WHO

Microscopy possesses the advantage of being a low-cost tool however it also faces the drawbacks of being labour-intensive and is highly operator-dependent leading to variability and inconsistency in results.

The purpose of this study was to explore an computer-aided method for microscopic detection of intracellular and extracellular parasites that would lead to greater efficiency and diagnostic accuracy.

MATERIALS AND METHODS

Experimental design

A total of 15 BALB/c mice of either sex aged 6-8 weeks were acquired from the Rodent facility, Animal Science Department at the Institute of Primate Research, Karen, Nairobi, Kenya. The mice were housed in cages, at an ambient temperature of 22°C. They were fed on mice pellets (Unga Farm Care, Nairobi, Kenya) and water was provided *ad libitum*. The BALB/c mice were divided into 3 groups as follows: *L. donovani* (n=5; 3 Female ,2 Male), *P. berghei* (n=5; 1 Female,4 Male) and *T. b rhodesiense* (n=5; 5 Male).

Experimental parasites

Leishmania donovani strain NLB-065 was obtained from hamster's spleen by aspirate and cultured in complete Schneider's Insect media (Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany). Promastigotes at the stationary phase were harvested by centrifugation at 280g at 4°C for 15 minutes as described [5]. The pellet was later washed in sterile Phosphate Buffered Saline (PBS) by centrifugation as before. Cryopreserved stocks of *Plasmodium berghei ANKA* (wild type, supplied by Malaria Research and Reference Reagent Resource Center program (MR4)) were retrieved from liquid nitrogen and revived by thawing at 37°C in a water bath followed by three washes in PBS at 250g for 10 minutes.

Thin blood smears were prepared from *Leishmania donovani* strain NLB-065 obtained from hamster's spleen by aspirate and cultured in complete Schneider's Insect media (Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany); *Plasmodium berghei* ANKA (wild type, supplied by Malaria Research and Reference Reagent Resource Center program) and cryopreserved *Trypanosoma brucei rhodesiense* (isolate IPR001) isolated from the

cerebrospinal fluid of a late-stage human African trypanosomiasis patient in Bugiri, Uganda, in 2008).

RELATED WORK

There have been significant advances of application of machine learning techniques in the life sciences. For instance, we are able to predict protein structure with Alpha Fold (*Jumper et al., 2021*) and researchers are able to share, collaborate to create tools to aid in lung disease detection (https://paperswithcode.com/dataset/luna).

Indeed we are living in times when collaboration is key and open science is driving this cause. We are reviewed a few pieces of work in the lines of what we present. Image segmentation on stages of the life cycle using U-Net architecture to detect organelles[7]. This produced fantastic results compared to the ground truth labels.

White blood cell subtype detection and classification was proposed by Praveen et al. Using white blood cell detection with an accuracy of 90% and using the YOLOv3 for the localization of the detected white blood cells. This can also be extended to do counting of particular cells for instance Red blood cells. (https://arxiv.org/pdf/2108.04614v1.pdf)

To overcome class imbalance and the limitation of classification models. Mateus el., 2020, proposed the use of deep belief networks and Restricted Boltzmann machines to use in intestinal parasites classification; They used the Larval, Egg and helminth stages of various parasites. After data augmentation, the RBM and DBN produced 95.09±0.33 & 91.40±0.79 for the balanced accuracy score on Larvae respectively, 92.09±0.68 & 91.06±0.62 on Eggs and 77.84±0.82 & 77.66±1.88 protozoa.

However, we acknowledge that image localization, image classification, hyperparameter tuning and class imbalance are a common problem. We attempt to overcome these using oversampling techniques on *L.donovani* amastigotes, adjusting learning rate using fastai utilities that is, learning rate finder strategies, transfer learning to reduce training time in construction of neural network architecture and using multilabel classification to detect coinfections or other types of cells/parasites in the image in case the thin smear has not been made well. In addition, we present a tool that can be used in the diagnostic laboratory after improvements.

RESULTS AND DISCUSSION

Images were captured using an AMSCOPE Microscope 2000X LED Trinocular compound [9] at 100X oil immersion magnification. Using the attached AMSCOPE 1.3MP camera and software a total of 401 images were captured including: 198 images of *L. donovani* promastigotes, amastigotes, 102 images containing different stages of *T. b rhodesiense* and 101 images of *Plasmodium berghei* as well as other blood cells from stained thin blood smears. The slide pictures were first prepared using an image explorer

https://github.com/Shuyib/cell-explorer-tool: The image explorer uses several open source libraries namely: ipython[10], ipywidgets[11], Pillow[12], numpy[13] and pandas[14] where functionality provided by the mentioned libraries helped create various components such as the dropdowns, radio buttons, checkboxes that enabled the user to adjust image parameters such as position, coordinates and size when the user interacted with them in the application. The study images (training set) were then processed and grouped into independent and dependent variables aided by the data blocks API and data loaders from fastai [15] presented to a pre-trained 18-layer neural network model known as residual network 18 (Resnet 18) [16]. We used the BCEWithLogitLoss function since the images have multiple labels and more than one cell or parasite was present in some images. The Resnet 18 transformed the images in several steps known as the training process, including, convolution and pooling steps responsible for breaking down the image into two dimensional grayscale 2 by 2 matrices. These matrices known as feature maps displayed patterns that were easily identified such as morphology of the parasite or infected cell. The neural network was able to identify cellular morphology with an average accuracy of ~95.0980% and a F1 score of 0.766013 on the validation set: it consists of images not used in training to confirm that the model has "learned" the patterns of interest. The cell explorer also utilised Simple Linear Interactive Clustering [16], an algorithm which works by dividing the image into segments using Kmeans clustering, to count cells in the images. The cell explorer was thus capable of identifying the different parasites and life cycle stages as well as count the number of cells presented to the tool.

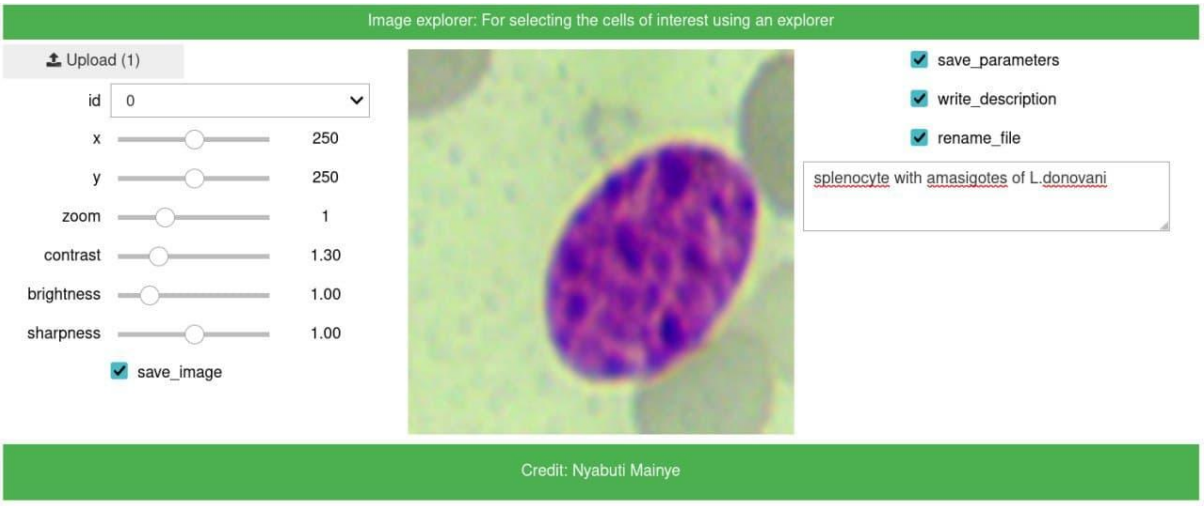

Figure 1: Image explorer. Jupyter[17] app which allows you to upload an image or multiple images and adjust image parameters such as position, coordinates and size.

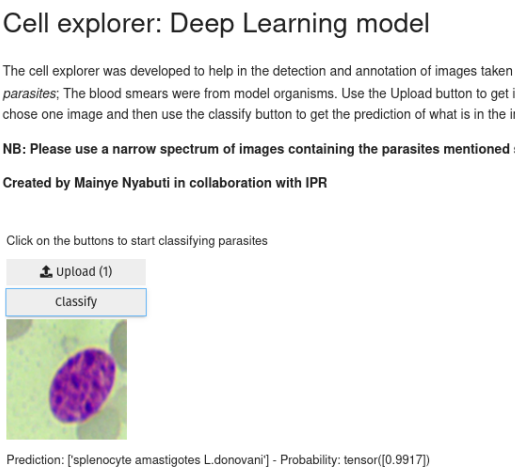

Figure 2: Deep learning model. Gives a prediction of what cell(s) are in the image.It was able to identify the splenocyte with amastigotes of *L. donovani*. It is able to identify more than one type of cell: it is capable of multi-label classification. More source code is available upon request via the corresponding author.

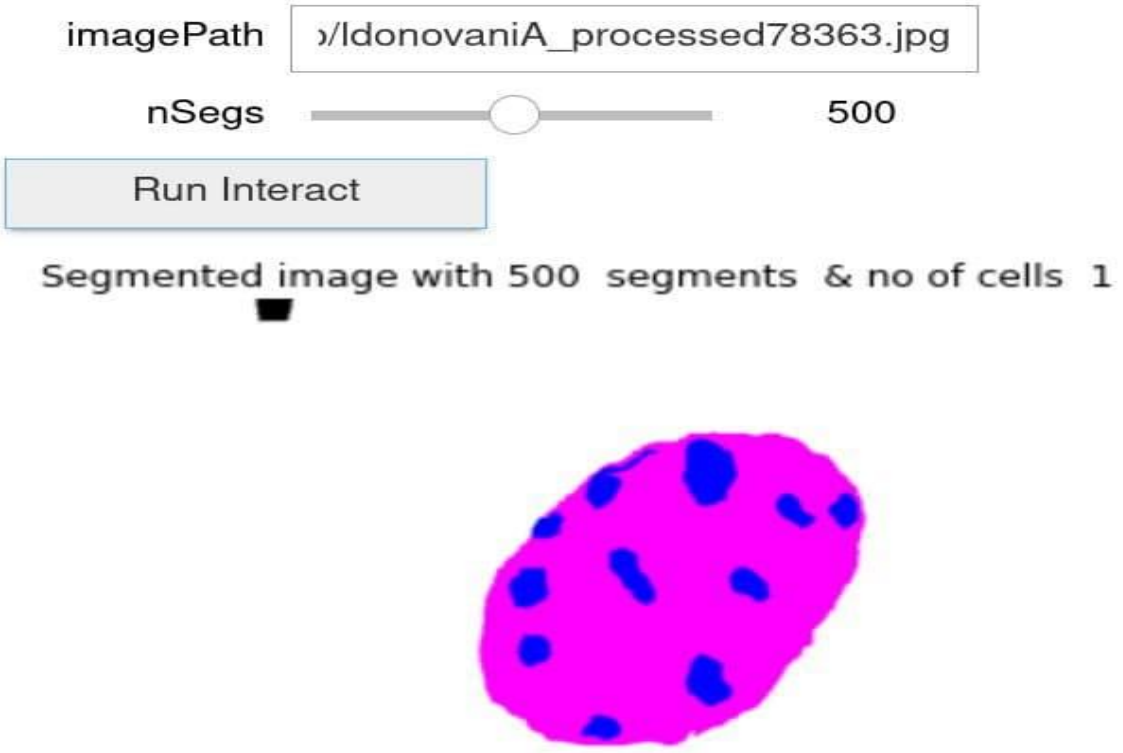

Figure 3: Machine learning model. Uses the simple linear iterative clustering with widgets that allow the user to adjust parameters and count the number of cells in the current image specified in the application. Segmented images of the infected splenocyte.

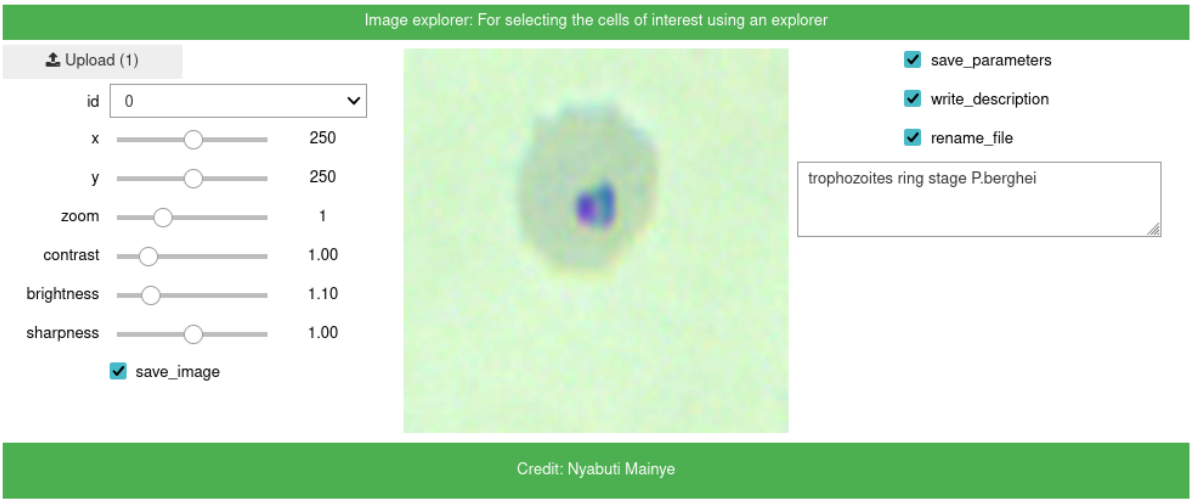

Figure 4: Image explorer. Jupyter app which allows you to upload an image or multiple images and adjust image parameters such as position, coordinates and size.

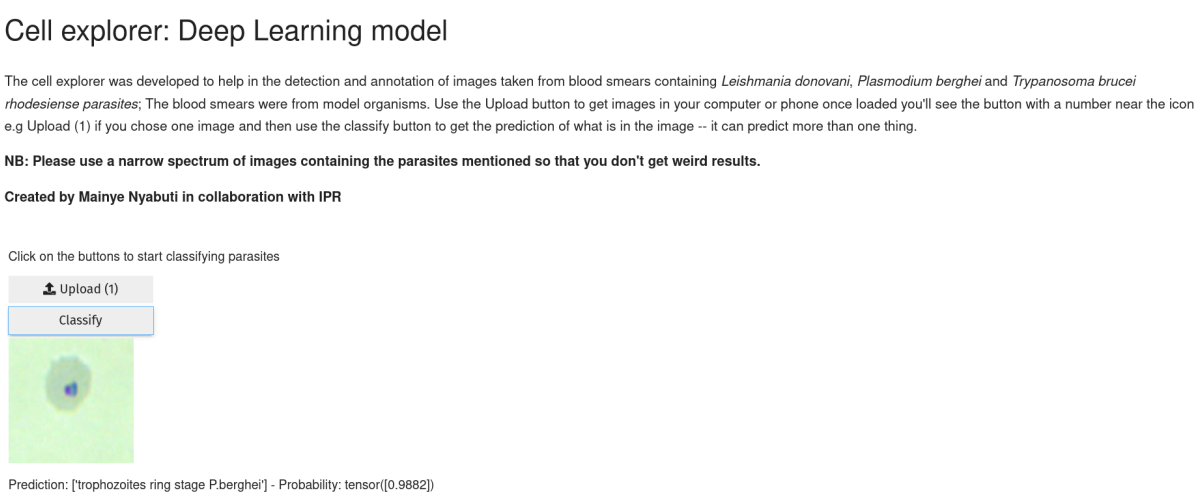

Figure 5: Deep learning model. Gives a prediction of what cell(s) are in the image. It was able to identify the trophozoites ring stage *P.berghei*.

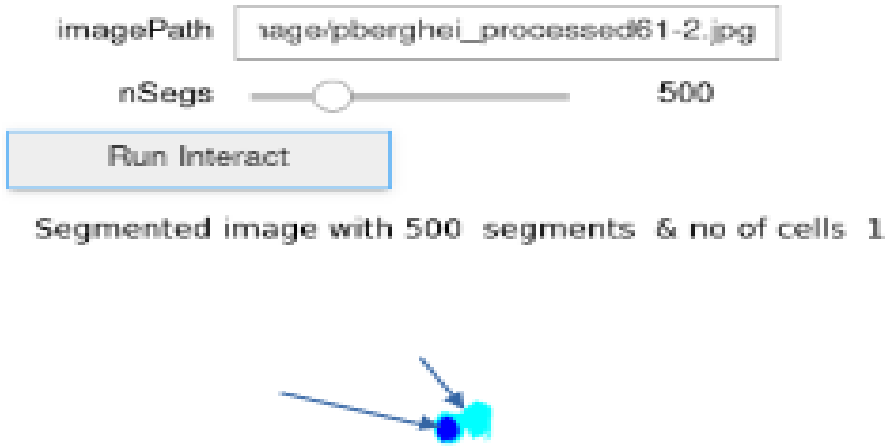

Figure 6: Machine learning model. Uses the simple linear iterative clustering with widgets that allow the user to adjust parameters and count the number of cells in the current image specified in the application. An RBC infected with plasmodium species segmentation reveals the trophozoites. The light and dark blue are segmented regions; the sections have regions with similar pixels indicated by the arrow.

FIGURE LEGENDS

Figure 1: Image explorer is a jupyter app which allows you to upload an image or multiple images and adjust image parameters such as position, coordinates and size.

Figure 2: Is a deep learning model which gives a prediction of what cell(s) are in the image. Here it was able to identify the splenocyte with amastigotes of *L.donovani*.

Figure 3: Machine learning model. Uses the simple linear iterative clustering with widgets that allow the user to adjust parameters and count the number of cells in the current image specified in the application. Segmented images of the infected splenocyte.

Figure 4: Image explorer is a jupyter app which allows you to upload an image or multiple images and adjust image parameters such as position, coordinates and size. (another image)

Figure 5: Is a deep learning model which gives a prediction of what cell(s) are in the image. Here it was able to identify the trophozoites ring stage *P.berghei*.

Figure 6: Is a machine learning model that uses the simple linear iterative clustering with widgets that allow the user to adjust parameters and count the number of cells in the current image specified in the application. An RBC infected with plasmodium species segmentation reveals the trophozoites.

CONCLUSION

The cell explorer presents a fast and accurate computer-assisted microscopy tool with the ability to detect cellular morphology, successfully identifying *Leishmania donovani, Plasmodium berghei* and *Trypanosoma brucei rhodesiense* parasites. This work highlights the research potential of machine learning models as disease diagnostic applications effective in improving the microscopy process as well as minimising operator error and/or bias useful in low resource settings.

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