AUTOMATED DETECTION OF FOOD AND WATER-BORNE PARASITES IN LOW COST SMARTPHONE MICROSCOPE IMAGES

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ABSTRACT

Diarrhoeal disease, caused by food and water-borne parasites, is one of the leading causes of death in infants and young children. While brightfield microscopes are commonly used for detecting these parasites, smartphone-based microscopy has been recently proposed as a low cost and portable alternative that does not require well set-up laboratory. However, the need of trained experts in identifying these parasites hinders harnessing the full potential of smartphone microscopy. Herein, we explore a deep learning based approach for automatic detection of the two major diarrhoea causing parasites; *Giardia* and *Cryptosporidium*, in brightfield and sapphire ball lens based low cost smartphone microscopes. A training dataset of microscopic images are prepared using standard reference samples containing the cysts of the two parasites. Similarly, samples prepared from real vegetable samples are imaged using the two microscopes to create a test dataset. We train two well-known deep learning based object detectors, Faster RCNN and RetinaNet on the brightfield and smartphone training set images separately. The trained models are evaluated on the images of real samples. Although the object detectors perform reasonably well on brightfield images of standard samples, their performance are diminished in smartphone images. The models trained on reference samples are not fit for images from real samples having debris. Our results show that a separate model should be trained on real sample images, which may require semi-supervised or one shot learning methods or a larger training dataset of real sample images.

1 INTRODUCTION

Globally, diarrhoeal disease kills around 829,000 people annually in low and middle income countries (WHO, 2019). The diarrhoeal episodes are attributed to biologically contaminated food in more than 70% of the cases(WHO, 2020). *Giardia* and *Cryptosporidium* are considered as the major food and water-borne parasites (Baldursson & Karanis, 2011). These two human pathogens are of great concern in developing countries due to unhygienic lifestyles and poor sanitation (Fricker et al., 2002). Timely and accurate diagnosis of these parasites can help save millions of lives.

Although several methods of detection exists such as immunological assays, cell culture immunofluorescence assays polymerise chain reaction (PCR), quantitative PCR, fluorescence in situ hybridization (Van den Bossche et al., 2015; Adeyemo et al., 2018), these methods are expensive, and require high grade reagents and special expertise. Brightfield microscopy is a standard method that is relatively cheaper and does not require expensive chemicals. Recently, smartphone-based microscopy methods have been developed that could potentially replace other more expensive and less portable methods including brightfield microscopy (Koydemir et al., 2015; Kobori et al., 2016; Kim et al., 2015; Saeed & Jabbar, 2018; Shrestha et al., 2020). Some smartphone-based microscopes use fluorescence which still require expensive chemicals for labelling pathogens and light source with specific wavelength for the visualization of tagged molecules. Shrestha et al. proposed a cheaper and easier sapphire ball lens based smartphone microscopy to detect *Giardia* and *Cryptosporidium*, where the ball lens allows higher magnification of the object at low cost. The microscope is easy to build with a sapphire ball lens, a commercially available light emitting diode (LED) light, and a simple designed microscope stage that can be readily attached to a commercial smartphone (Shrestha

et al., 2020). Despite such an easy and low cost setup, detecting parasites with such microscopic images require trained personnel which are not easily available in many regions of the developing countries. Therefore, development of robust and accurate automated parasites detection methods could help developing countries to truly harness the potential of low-cost portable microscopic devices.

In recent years, several deep learning based algorithms have been reported for automatic detection, segmentation, and classification of various microscopic objects such as cell detection (Xue & Ray, 2017), and live bacteria detection and classification (Wang et al., 2020). Such algorithms have also been used in smartphone-based microscope images for detecting malarial parasite in thick blood smears (Yang et al., 2019), and screening of sickle cells (de Haan et al., 2020). For diarrhoeal parasites, Xu et al. proposed a deep-learning based network ParasNet to detect *Giardia* and *Cryptosporidium* in brightfield microscopic images, and Koydemir et al. proposed a machine learning algorithm to classify *Giardia* from other parasites using features such as area, equivalent diameter and intensity in fluoroscent smarphone-based microscopic images.

In order to develop more robust detection methods in the future, in this work we aim to identify the performance and limitations of the current state-of-the-art deep learning based object detectors for detecting *Giardia* and *Cryptosporidium* in low-cost sapphire ball lens based smartphone microscopes as compared to the more conventional microscopy. Section 2 presents the custom dataset built using samples extracted from standard solutions and vegetables through both brightfield and smartphone microscopes, Section 3 details the implementation and experimental setup with two popular object detectors Faster RCNN and RetinaNet (Ren et al., 2016; Lin et al., 2017), and Section 4 reports the results followed by discussion and conclusion in Section 5.

2 DATASET

The dataset consists of two sets, one for training with images captured of the slides prepared using standard samples and another for test with images captured from the slides prepared with samples extracted from real vegetables. For training, 25 samples were prepared, each from 5 µL standard (oo)cyst suspension (Aqua-Glo G/C direct, Waterborne Inc., USA) mixed with Lugol's iodine. From these 25 standard samples, we captured 830 images (containing *Giardia* and *Cryptosporidium* (or none)) each from two microscopes with the following specifications: i) brightfield with a rectangular field of view of 190 µm X 350 µm and magnification of 400X ii) smartphone with a circular field of view of diameter 200 µm and magnification 200X which was built following the method proposed in (Shrestha et al., 2020). For test set, the spike recovery experiment on real vegetables was performed as proposed on Shrestha et al. to prepare 20 samples, from which we captured 84 images each using the two microscopes.

An expert with more than two years of experience and training annotated these images with bounding boxes and ellipses on all the *Giardia* and *Cryptosporidium* found in these images using VGG annotator (Dutta & Zisserman, 2019). Figure 1 shows some example reference and real vegetable samples and their bounding box annotations.

In the 830X2 reference sample images, the expert annotated 907 *Giardia* and 502 *Cryptosporidium* in the images captured from brightfield microscope samples, and 839 *Giardia* and 534 *Cryptosporidium* in the images captured from smartphone samples. Similarly, in the 84X2 real vegetable sample images, the expert annotated 22 *Giardia* and 45 *Cryptosporidium* in the images captured from brightfield microscope samples, and 75 *Giardia* and 39 *Cryptosporidium* in the images captured from smartphone samples.

3 EXPERIMENTS: TWO OBJECT DETECTORS FOR PARASITES DETECTION

We chose one state-of-the-art object detectors each from the two popular categories of deep learning based object detector frameworks: i) Faster RCNN from two-stage region proposal networks (Ren et al., 2016), and ii) RetinaNet from single shot detectors (Lin et al., 2017). The open source object detection library Detectron2 (Wu et al., 2019) was used with ResNet101 backbone accompanied with feature pyramid network (FPN) for Faster RCNN (Nguyen et al., 2020), and ResNet101 backbone for RetinaNet. Table 1 shows the details of four different models that are trained separately along

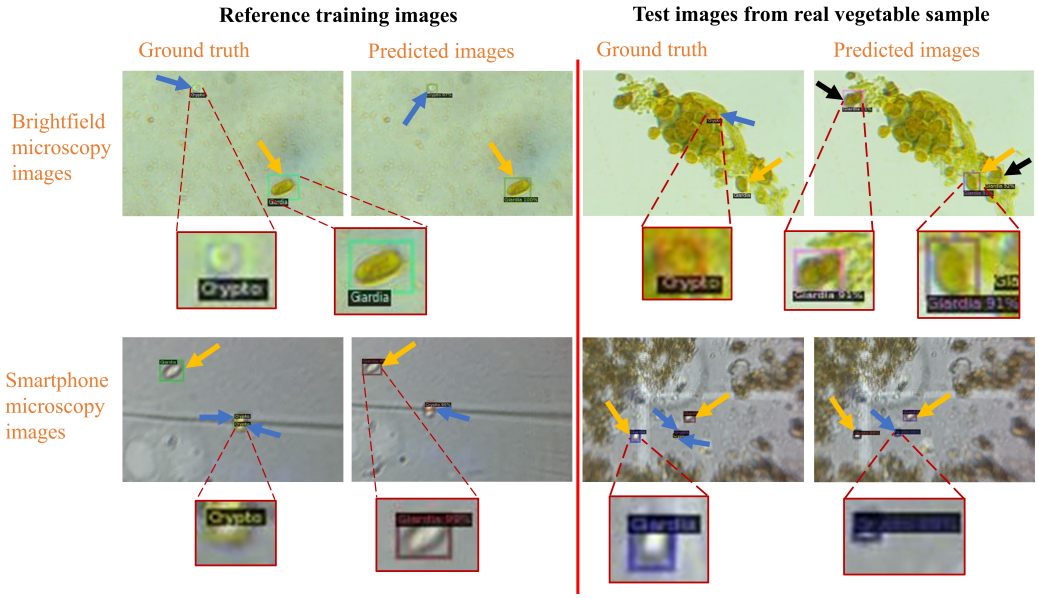

Figure 1: Representative images of training set with reference samples imaged with brightfield and smartphone microscopes, and test set with real vegetable samples. Example Ground Truth annotations of the parasites and predictions from the object detector models. Yellow arrow points *Giardia*, blue arrow points *Cryptosporidium*, and black arrow points debris.

with the hyper parameters modified from the default settings of Detectron2. Resize shortest edge with short edge length of 640, 672, 704, 736, 768, 800 and random horizontal flip with a probability of 0.5 were used as data augmentation at the time of training. The models are trained with 5-fold cross-validation using 830 reference sample images for the two microscopes. Since the goal is to detect the parasites, we evaluate using the four model's precision, recall and F1-score in detecting the two parasites for the two kinds of microscopic images. Finally, 84 real vegetable sample images are then used for evaluating the models on an independent test set.

Table 1: Four detection models and hyper parameter details

Microscope	**Detector**	**Backbone**	**W/N**	**LR**	**Other**
Brightfield	Faster RCNN	ResNet101	1200/1500	0.001	FPN, PPI = 64
	RetinaNet	ResNet101	800/1200	0.001	$\alpha = 0.93, \gamma = 1$
Smartphone	Faster RCNN	ResNet101	1500/2000	0.01	FPN, PPI = 64
	RetinaNet	ResNet101	1200/1500	0.001	$\alpha = 0.99, \gamma = 1.7$

W/N: Warm-up/Maximum Iteration; LR: Learning Rate; FPN: Feature Pyramid Network; PPI: Proposals per image; all other hyper parameters were left as default in Detectron2 implementation.

4 Results

Table 2 shows the average Precision, Recall, and F1-score along with standard deviations in 5-fold cross validation for the images taken from reference samples of the two parasites. The results show that the models perform better in brightfield images compared to smartphone ones. Faster RCNN has better results over RetinaNet with a varied degree of improvement depending on the imaging modality and the parsite being detected. In brightfield images, the Recall and F1 scores for *Giardia* are similar in both the models but these scores are much higher for Faster RCNN compared to RetinaNet for *Cryptosporidium*. Such improvement in sensitivity for Faster RCNN is consistent for both the parasites in smartphone images as well. Precision is similar for both the models in

all cases except for *Giardia* with smartphone images where RetinaNet's precision of 0.63 is much lower compared to Faster RCNN's 0.79.

Table 2: 5-fold cross-validation results on reference sample dataset

Microscope	No. of images	Models	Precision	Recall	F1-score
Cryptosporidium					
Brightfield	830	Faster RCNN	$\mathbf{0.831 \pm 0.036}$	$\mathbf{0.900 \pm 0.051}$	$\mathbf{0.862 \pm 0.018}$
		RetinaNet	0.809 ± 0.103	0.778 ± 0.094	0.784 ± 0.041
Smartphone	830	Faster RCNN	0.637 ± 0.137	0.655 ± 0.068	0.632 ± 0.064
		RetinaNet	0.651 ± 0.164	0.580 ± 0.073	0.592 ± 0.044
Giardia					
Brightfield	830	Faster RCNN	$\mathbf{0.946 \pm 0.024}$	$\mathbf{0.962 \pm 0.017}$	$\mathbf{0.953 \pm 0.010}$
		RetinaNet	0.924 ± 0.035	0.970 ± 0.031	0.946 ± 0.026
Smartphone	830	Faster RCNN	0.791 ± 0.075	0.800 ± 0.139	0.785 ± 0.072
		RetinaNet	0.633 ± 0.110	0.860 ± 0.028	0.724 ± 0.087

Table 3: Results on an independent test set with vegetable samples

Microscope	No. of images	Models	Precision	Recall	F1-score
Cryptosporidium					
Brightfield	84	Faster RCNN	0.443	0.777	0.564
		RetinaNet	0.438	0.555	0.490
Smartphone	84	Faster RCNN	0.439	0.461	0.450
		RetinaNet	0.545	0.461	0.500
Giardia					
Brightfield	84	Faster RCNN	0.640	0.727	0.680
		RetinaNet	0.500	0.590	0.541
Smartphone	84	Faster RCNN	0.792	0.531	0.636
		RetinaNet	0.671	0.600	0.633

Table 3 shows the result of the detection models on the test set prepared using samples extracted from real vegetables. The results show that the performance of the object detectors on real vegetable samples is drastically reduced. Standard dataset consisted of clear images with less to no debris, and no any interfering organisms were present (only the (oo)cyst of *Giardia* and *Cryptosporidium* were present). Whereas, in the test dataset, debris were abundant which was similar to size of *Giardia* and *Cryptosporidium*. Moreover, the images were less clear with other interfering organisms.

5 Discussion and Conclusion

The object detectors have reasonable performance on brightfield images of standard samples, but the models seem to struggle more for smartphone images which have some textured noise compared to the brightfield images. The results show that the models trained on standard images are not fit for testing on real samples. This was expected as the real samples have much more texture, noise and debris that look similar to the parasites. One might explore domain adaptation methods to improve the performance of the real samples. However, as there is a big variation in the image appearance between the standard samples and real samples, and that there are not large number of standard samples already annotated to start with, it would be better to directly increase the number of real samples and build a training dataset of such samples.

In smartphone microscope, the objects were found to be stretched towards the border regions. Because of the use of ball lens, same object had different sizes within the image. In such situation, the models falsely predicted *Giardia* as *Cryptosporidium* and vice versa. The false-negative was mostly observed in blurry images.

The results are evaluated considering annotations from one expert as Ground Truth. However, the expert noted that there is some level of guess work on hard examples suggesting the presence of underlying uncertainty in the annotations. We will very likely see inter-expert variation when another expert annotates the same dataset. Similarly, as our intention is to develop tools that could assist non-experts, it would be interesting to compare the results with non-expert's detection performance as well.

Our future work includes increasing the real sample annotated dataset to use it for training the detection models, annotating with another expert and some non-experts to evaluate inter-operator variability. We plan to release a more complete dataset for the scientific community to have a benchmark dataset, experiment and develop more robust tools. The experts use the size of the object as an important cue in identifying the parasites, although there are some challenges due to similar sized and shaped debris. We will further explore to develop methods that incorporates this prior into the detection models.

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