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Construction and Evaluation of a Low-cost Haematology Slide-scanning Robot

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Abstract

Variation in white cell morphology between avian species and between healthy and diseased individuals, and the fact that avian erythrocytes and thrombocytes are nucleated, are the main challenges to using automatic processes to study avian blood. However manual evaluation of the avian smear can be time consuming for clinicians and technicians and can discourage busy vets and labs from developing their avian haematology skills.

Computer vision has developed considerably over the last three decades and is now employed to perform repetitive tasks such as face recognition at airports, quality control on assembly lines and lane departure monitoring in modern cars. Computer vision has also been used to autonomously guide robots such as drones and self-driving cars. The repetitiveness of tasks such as blood cell counting in haematology has also seen much research into the ability of computers and machine learning algorithms to identify and count blood cell counts from many different species with varying degrees of success. Here, the development and testing of a low-cost robot to evaluate stained avian blood smears will be described. Preliminary data on how well the robot can identify monolayers within a smear and its ability to be 'trained' to identify blood cells in healthy pigeon blood smears will also be discussed.

Introduction

Collection and examination of avian blood is a routine and essential diagnostic tool for the avian clinician. Clinical biochemistry and the haematological examination can provide insight into the function and activity of many of an avian patient's body systems including their immune system and bone marrow, their response to stressors or disease and can provide useful prognostic information which cannot be determined from a physical examination (Harr, 2006; Samour, 2006; Campbell, 2015; Jones, 2015). Many elements of the examination of blood such as measuring the concentration of salts and enzymes in the serum have been automated, however the examination of blood smears and determination of differential white blood cell counts remains a manual operation and requires an experienced haematologist to maximize the amount of clinical information obtained (Campbell, 2015).

Obtaining the avian white cell differential and count manually is typically performed in two different ways (Dein et al., 1994; Walberg, 2001), the first involves the use of specialized stains such as Natt and Herrick's stain (Natt and Herrick, 1952) or phloxine B (Unopette or Eopette kits) (Campbell, 1994), a haemocytometer and a blood smear strained with a Romanowsky stain (Campbell, 2015); the second involves estimating the white cell count from a Romanowsky stained blood smear (Cray and Zaias, 2004; Bickford, 2007). The haemocytometer method is the current gold standard for performing the white blood cell count in avian blood, but is time consuming, dependent on the availability of the necessary stains and expertise, and is typically performed in clinical pathology laboratories and in larger clinics seeing many birds (Walberg, 2001). The slide method is used as a rapid in-clinic test when there is an urgent need for clinical information or when a clinical pathology laboratory is not available to process blood samples. Regardless of the method, useful examination of an avian haemogram requires experience and familiarity with avian blood and awareness of the species differences in cell morphology (Samour, 2006; Mitchell and Johns, 2008), reference ranges, common haematological abnormalities and staining artefacts.

In human and other mammalian species, the process of performing the WBC count and differential has been largely automated (Harvey, 2012). This has benefited diagnostics as it makes it cheaper and quicker to process mammalian blood and get results back to the treating clinician. Automated methods can process larger number of cells quickly to obtain more accurate and reliable blood cell counts (Riley and Idowu, 2003). It also means the haematologist can spend more time on evaluating cell morphology and providing their professional interpretation of the smear rather than counting cells. Additionally, automation has significantly benefited research as it has enabled the processing of large numbers of mammalian blood samples in studies to understand haematological responses to many different diseases and interventions (Macey, 2007).

Automation of blood cell counting is most commonly achieved with an impedance (Coulter) cell counter or a flow cytometer (Harvey, 2012). An impedance counter enumerates cells in an electrically conductive fluid by detecting changes in the resistance of the fluid as cells pass a sensor. The basic principle of flow cytometry is to dilute the blood sample to allow individual cells to be streamed past a laser beam and photodiodes (Macey, 2007). The flow cytometer can count the cells as they pass through the laser beam. In addition, the amount of forward scatter and side scatter of the laser is detected by the sensors and these values used to identify cell types (Riley and Idowu, 2003). Different fluorescent dyes or fluorescent-labelled antibodies can be added to the cells, and this fluorescence detected as a labelled cell passes through the laser beam. This adds further discriminatory ability to the flow cytometer (Riley and Idowu, 2003; Abcam, 2017).

Several studies have attempted to adapt flow cytometry to avian blood and many diagnostic laboratories can perform a total avian WBC count using flow cytometers such as the Cell-Dyn 3500 (Abbot Diagnostics, Santa Clara, California) in several avian species. However pathological changes and species differences in blood cell morphology preclude flow cytometry from being used universally for all avian species or disease conditions (Lilliehöök et al., 2004; Samour, 2006).

The major difference between mammalian and avian blood which complicates the use of impedance counters and flow cytometry with avian blood is that avian red blood cells (RBCs) and thrombocytes are nucleated (Moritomo et al., 2002). Prior to flow cytometry or impedance counting, mammalian blood is treated to lyse fragile non-nucleated red blood cells and platelets, leaving leucocytes to be counted (McCarthy, 2007). In contrast, the cell membranes of avian RBCs and thrombocytes are resilient and cannot be differentially lysed from white blood cells (WBC) (Seliger et al., 2012). This challenge can be partially overcome by using fluorescent dyes not able to be taken up by red cells (Moritomo, et al., 2002). This allows a flow cytometer to specifically count the WBCs and thrombocytes, but does not allow certain populations to be differentiated from each other. Lymphocytes and thrombocytes have similar forward and side scatter profiles as do heterophils and eosinophils (Moritomo, et al., 2002; Lilliehöök, et al., 2004). One study in chickens used specific anti-chicken fluorescent labelled antibodies to identify the lymphocytes from the thrombocytes (Seliger, et al., 2012) but still could not reliably distinguish the subpopulations of granulocytes. This technique showed promise as a method to almost completely automate the differential count in chickens, however it is unlikely that leucocyte specific fluorescent antibodies will become available for the wide range of avian species seen in avian practice today.

A recent study approached the challenge of automating the avian white cell differential count using a digital slide scanner and computer vision software (Beaufrère et al., 2013). This method showed promise although it did not produce counts in agreement with manual methods. Computer vision is increasingly used in medical diagnosis and has been experimented with to identify white blood cells in human blood smears and to detect malarial parasites in blood (Cuevas et al., 2013; Das et al., 2015). Commercial white cell counters are also now available which employ computer vision to count 100-200 human white cells per smear (MEDICA EasyCell®, Sysmex Corporation, USA). Given these developments, it was decided to revisit this technology in the context of avian blood smear examination.

At the time of undertaking several research projects on avian blood, our laboratory did not have ready access to a slide scanner capable of evaluating blood smears, and was unlikely to obtain one in the near future. The decision was made to therefore try and make a scanner adapting some of the techniques used to make 3D printers and an old microscope. The aim of this study was to first determine if a low-cost slide scanning robot could be made from inexpensive consumer grade electronic components. The second aim was to test whether this robot could be 'taught' how to; 1) find the monolayer region of an avian blood smear; and 2) identify individual blood cells. The preliminary findings of this work are presented here.

Materials and Methods

Robot construction and components

The slide scanning robot was assembled around an old Olympus CH-2 binocular microscope (Olympus, Notting Hill, NSW) which had been taken out of service due to a broken focus control and faulty light circuit. Stage movement and focus controls were connected to three Nema-11 stepper motors via gears and shaft couplers purchased from several 3D printer suppliers and hobby robotic stores online. The motors were controlled via 5V motor controller circuits connected to a Raspberry Pi Model 2B

single board computer (Raspberry Pi Foundation, United Kingdom). A 5-megapixel (MP) Raspberry Pi Camera Module version 1 (Raspberry Pi Foundation, United Kingdom) was aligned over one eye piece and connected to the computer. Micro end stop switches were placed on the microscope and provided feedback to the computer when the stage or the focus had reached either end of its range of movement along each axis. A library of control functions for the microscope was created in Python 2.7 (Python Software Foundation, 2017). A number of significant functions of the microscope relating to image processing such as focus control, monolayer identification and cell detection, were programmed using Python and its OpenCV library (Open Computer Vision, www.opencv.org).

Monolayer and cell finding algorithms

The robot was designed to scan blood smears using the 40x objective. This objective was selected because it allowed visualization of the details of individual cells. However, this meant that the robot could only address a very small section of the slide at a time resulting in a very slow scan of a blood smear if the robot had to assess every possible field and decide if it was a monolayer. To speed up this process, an algorithm to focus on the region of the smear containing the monolayer was designed and tested.

The algorithm had the robot start evaluating a blood smear by first looking at an empty field, determining the average brightness of this field and saving this as a reference of what an empty field should look like. The user would instruct the robot about the orientation of the smear at the start of the analysis, and then the robot would start from the one corner of the slide closer to the tapered end of the smear, and move towards the thick part of the smear taking a small low-resolution image (100x100 pixels) of each field. At this point of the scanning process, the microscope objective may or may not have been in focus. If it found a field that 1) was less bright than an empty field and 2) contained blue and red objects (which could be determined whether the microscope was focused or not), only then did it take the time to focus the image. After focusing, a second low resolution image was captured. The percentage of objects (cells) versus empty background was then calculated. If the objects covered 50-70% of the field, the field was declared a monolayer and a high-resolution image was captured. If cells covered less than 50% of the field, the robot determined the field to be part of the feathered edge of the smear. The microscope would continue to scan towards the thick end of the smear until 3 thick fields had been encountered (fields with objects covering more than 70% of the field). It would then move to the next scanning plane. However, instead of returning to the edge of the slide, the robot returned to where it had previously found the feathered edge fields in the last scanning plane and start looking for the monolayer fields again. With this algorithm, it was anticipated that the microscope would not have to look at every field to find the monolayers and could therefore scan a blood film relatively quickly.

After initial testing, it was found that the process of identifying individual cells in the high-resolution monolayer images was too computationally intensive for the small Raspberry Pi, and therefore this was performed on a laptop with an Intel Core i7 6700HQ processor and 16Gb of RAM. To identify individual cells, the monolayer images were split into their red, green and blue channels. The

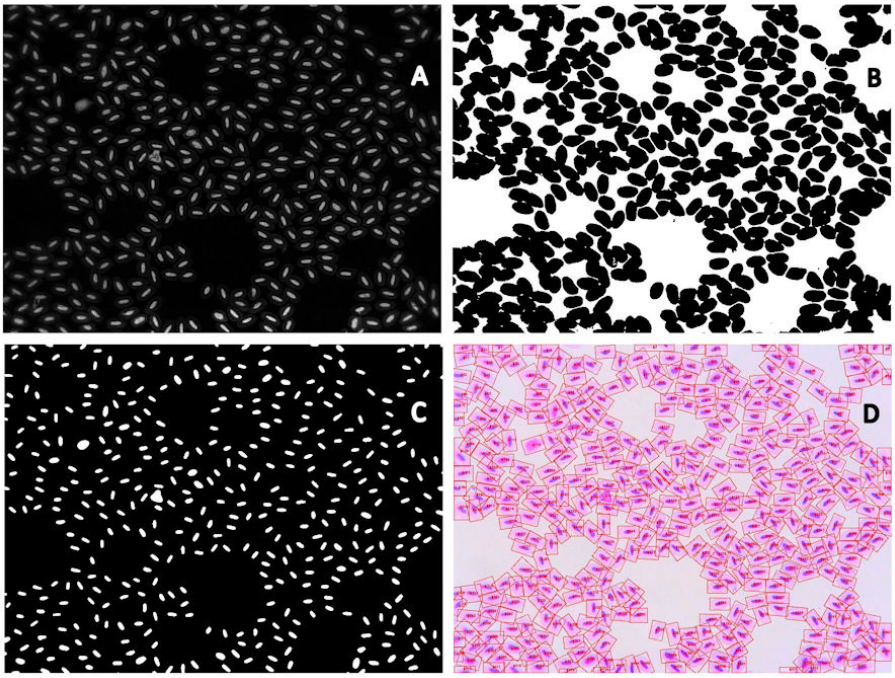

Figure 1. The monolayer image manipulations used to find individual cells. (A) the normalized inverted green channel. (B) the separation of the cells and background using thresholding. (C) the identification of nuclei using thresholding. (D) identification of individual cells. The computer has placed a red box around every object it thinks is a blood cell. Cells that were touching each other were identified as independent objects by using their nuclei as a marker.

contrast between the cells, nuclei and the background was highest in the green channel (Figure 1A) so this channel was first normalized, and the features separated by a process called thresholding (pixels are classified by whether they are above or below a nominated value). One threshold value separated the cells from the background and another separated the nuclei from the background and cytoplasm.

Once the outline of the cells and their nuclei was determined (Figures 1B and C), each cell was identified with a watershed algorithm (Meyer, 1992) using each nucleus as a marker for each cell. This allowed cells that were touching to be detected as separate cells (Figure 1D). Each cell was then saved as an image.

Evaluation of robot's ability to find the monolayer region of a blood smear

Eight smears of adult pigeon blood (Figure 3) were taken from a slide set made during a teaching project at the University of Melbourne, Faculty of Veterinary and Agricultural Sciences (Animal Ethics Permit: 1513461.1). The smears had been stained with Diff-Quik (Fronine, Riverstone, NSW). Five of the smears were of typical thickness but the other three smears were considered too thick. Two of these thick smears had very narrow monolayers and one of these slides had practically no monolayer region. Both good and poor quality smears were used in this initial slide set to understand how the monolayer detection algorithm would function across a wide range of smear qualities. Ultimately, the goal was to see if the robot could be taught how to decide to accept or reject a smear based on its quality.

The slide scanner was asked to map where it had decided that a field was empty, a feathered edge, a monolayer or a field where the blood cells were too thick. These maps were then visually compared to the smears themselves. An operator then evaluated the monolayer field images produced by the robot and decided if they were in fact monolayer fields (where up to 50% of the cells were independent and not touching their neighbours).

Evaluation of the algorithm to identify individual blood cells

The monolayer images of scanned slides were processed on the laptop and the folder containing the images of the individual cells examined by a human to look specifically for artefacts or erroneous cell identifications. This information was then used to decide how to improve the robot and algorithms.

Results

The slide scanning robot could be constructed for less than $500.

The total cost of all the components for this project came to just over $410 (Table 1). The major cost of purchasing a microscope was avoided by salvaging a damaged microscope. Had the microscope needed to be purchased, this would have added $700-1000 to the project. The initial slide scanning robot design (Figure 2) left all image processing to the single board computer, but it was quickly realised that the process of identifying objects from high resolution images was beyond the capacity of this smaller computer, and the images were therefore transferred to a laptop to perform. The smaller computer however was perfectly capable of controlling the movement of the microscope stage and processing low resolution images to determine whether the image was in focus. It was also able to process the low resolution images to identify the percentage of the field covered by cells and therefore could be used to decide if the current field was part of the feathered edge, a monolayer or too thick.

Component	Cost
Computer	$87.95
Camera	$35
Motors	$69
Motor controllers	$20
Miscellaneous (aluminium frame, switches, gears, wiring,etc)	~$200
Microscope	$0 (Salvaged)
Total	$411.95

Table 1. Costs of individual components used in the slide scanning robot build

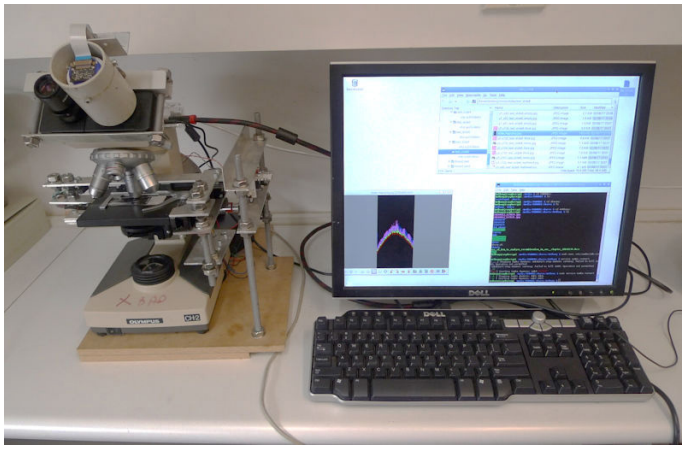

Figure 2. The slide scanning robot prototype

The microscope could find monolayer fields but also classified some feathered edge fields and thick fields as 'monolayers'

The algorithm to find the monolayers of the blood film enabled the robot to look in the correct section of the blood smear for the monolayer fields (Figure 3). However, the algorithm was not stringent enough in filtering

out all fields that were not monolayers as many of these were included alongside field images containing monolayers. The robot erroneously included both feathered edge fields and fields where more than 50% of the cells were touching. In better quality blood smears, the percentage of fields correctly identified as monolayers was 25-33%. In the three poorer quality smears (slides with very narrow monolayer regions), true monolayer fields only comprised 5-10% of the captured fields. The robot was detecting fields that were too thick (more than 50% of cells touching) more so than feathered edge fields, with thick fields comprising 40-90% of the fields declared as monolayers. The proportion of thick fields included as monolayers was not related to the overall thickness of the smear.

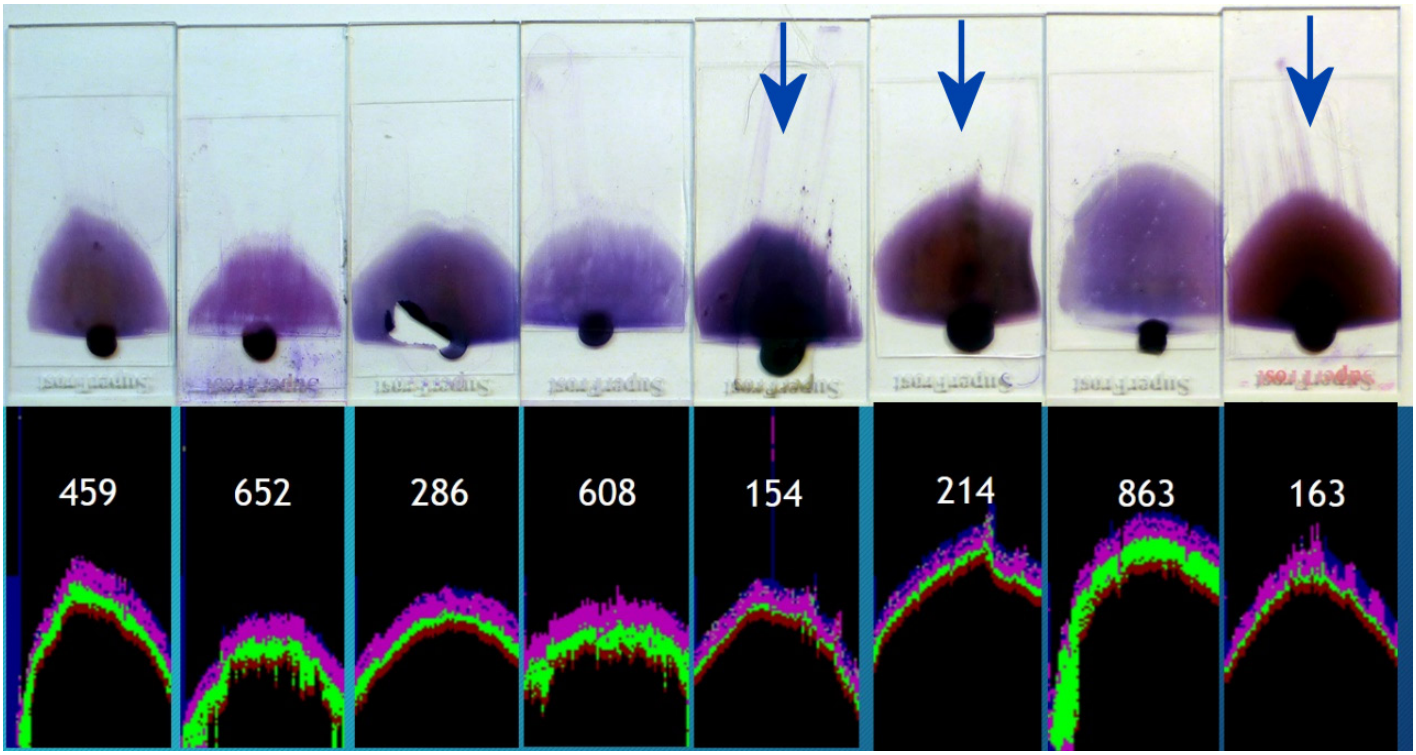

Figure 3. The eight test smears (top) and corresponding smear maps generated by the slide scanner (bottom). Green pixels are fields the robot has detected as monolayers. Pink, blue and red pixels are feathered edge fields, empty fields and thick fields respectively. The numbers indicate the total number of fields identified as monolayer fields per slide. Smears identified with arrows were the thick smears.

Using nuclei as markers for cells allowed the computer to identify individual cells even when cells were touching. However, the best cell pictures were from monolayer fields where most cells were not touching their neighbours.

The robot identified between 100,000 to 350,000 individual cell objects depending on the smear. The quality of the images of the cells was dependent on how well the robot had identified the containing field as a monolayer. If the cells were separate from their neighbours, clear images of cells could be captured (Figure 4). If cells were pressed close to each other, the cell identification algorithm struggled to accurately separate some cells and would detect multiple cells as a single cell (Figure 5,Figure 6), or alternatively incorrectly allocate some of the cytoplasm of a neighbouring cell to the incorrect nucleus (Figure 5).

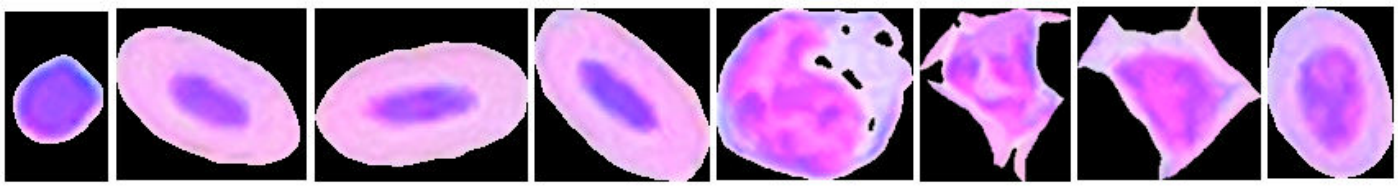

Figure 4. Examples of images of cells where individual cells were not abutting neighbouring cells. The monocyte is showing some artefacts within its cytoplasm from the threshold differentiation of the cells from the background.

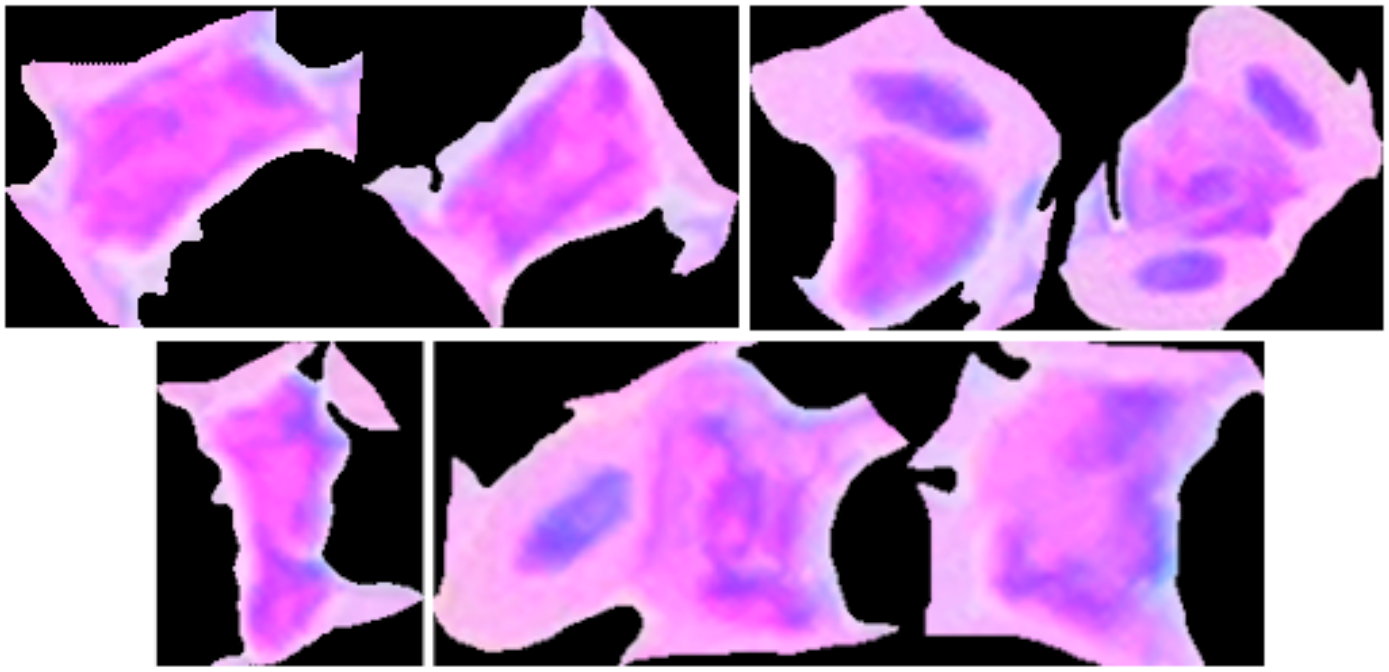

Figure 5. Examples of some cell artefacts when cells were abutting their neighbours. When the margins of nuclei were close together (ie where lymphocytes pressed against neighbouring RBC's) the cell identification system classified two cells as one.

When the cell identification and nuclei identification images were reviewed in the fields where the multiple cells were being identified as a single cell, it was apparent that when two nuclei were pressed close together, or when dark staining cytoplasm was close to a nucleus, the computer was grouping the two adjacent nuclei together (Figure 6A). The error was occurring in the nuclear thresholding algorithm (Figure 6C) and was likely a product of the smoothing occurring during the nucleus detection algorithm and the moderate image resolution (5 megapixels). If the two nuclei were separated by a larger distance in pixels (ie. a higher resolution image), then it is less likely that the smoothing algorithm would have joined these nuclei together as a single object.

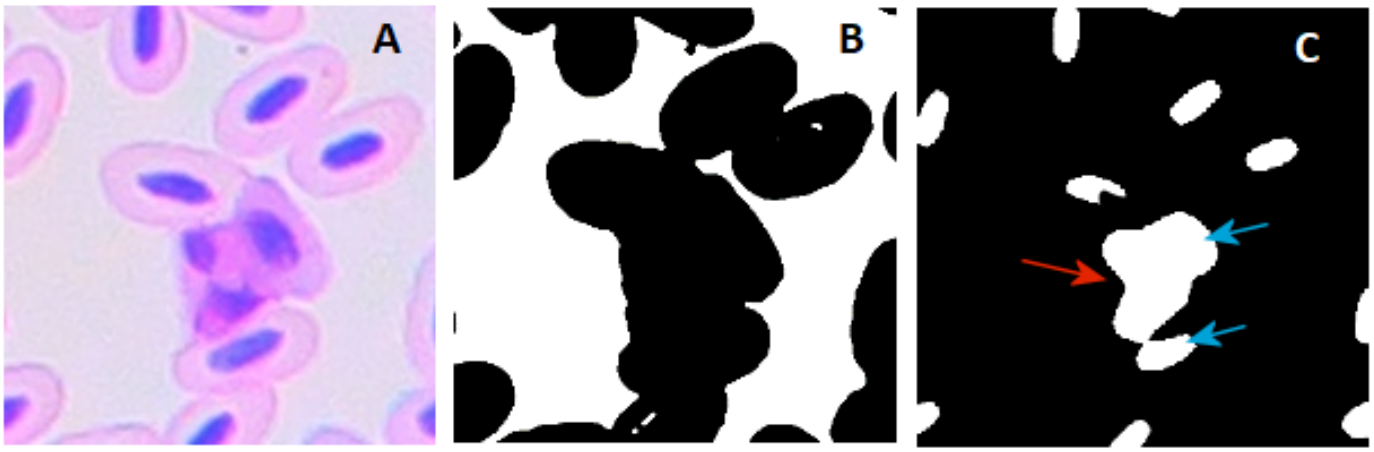

Figure 6. Example of a cell identification error where a heterophil is closely pressed against a RBC. The cell outline detection has grouped the touching cells together (B). Typically, the nucleus identification process (C) separates the cells, but here it has merged together the nuclei of the heterophil (red arrow) and two red blood cells (blue arrows).

Discussion

This study has shown that currently available consumer grade technology can be used to construct a basic slide scanning robot. The $88 single board computer was capable of driving the robot and running the focusing algorithms but was not up to the central processing unit (CPU) intensive task of identifying individual cells from high resolution images. This process however was readily performed on a contemporary high-end laptop. Any future design would most likely connect the camera directly to the laptop and leave only the basic microscope functions to be processed by the smaller computer. This would overcome another limitation of the smaller computer, namely the disk writing speed. During the scanning of a slide, there was a noticeable delay while the small computer wrote the high-resolution field image to its SD card hard drive. On a typical laptop, this would not be noticeable, because typical disk writing speeds are much faster. Additionally, the larger memory on laptops would allow the image to be temporarily stored if the hard drive was busy writing another file at the time of saving the

image.

While the current algorithm guiding how the robot scanned a blood smear did free it from having to evaluate every 40x field of the smear to find the monolayer, it was not stringent enough to prevent the misclassification of some feathered edge and slightly thicker fields as monolayers. This is a crucial step in developing any computer vision system to look at blood smears, as non-monolayer fields mean the computer is more likely to identify multiple cells as one cell or incorrectly assign parts of the cytoplasm to the wrong nucleus. Currently there are two areas of work being pursued to improve this algorithm. The first is allowing the computer to dynamically select threshold values to separate the cells from the background and the nuclei from the cells. Presently, this is a fixed value or a value based on the measurements taken from an empty field at the start of each smear scan. It is possible to give the system the ability to determine these values from each field. This may also help the system better determine the percentage of the field covered by cells if the cells are more consistently identified correctly. It may also help overcome some of the issues associated with staining variability between smears. The second area is narrowing the cut-off threshold percentages on what the computer should accept as a monolayer. The present values were calculated by measuring the percentage of monolayer fields covered by cells on some test slides and the theoretical 78%* percentage of a field that should be covered by elliptical red cells if the cells were perfect arranged and just touching their neighbours with no overlap.

When images of good monolayer fields were captured, the system could produce good images of individual cells. However, even in the best monolayer fields, some cells will be touching their neighbours (e.g. avian lymphocytes and heterophils commonly 'stick' to their neighbours in smears) and therefore the system will have to be able to identify two cells touching each other. The fact that all avian cells are nucleated, which is often a problem in other automated cell counting systems, is beneficial to computer vision as the nuclei are high contrast markers which can be used to separate cells. In the current slide scanning robot, using the nuclei as markers for cells did separate most cells, but the method could benefit from higher resolution images to separate cells when the nuclei get close together. A search of currently available web cameras and eyepiece digital cameras has found an 8 MP eye piece camera for $120 and a 14 MP camera for $225 (as of August 2017). These could be readily substituted into the current system to capture monolayer images and still maintain the relative low cost of the system.

While the current slide scanning robot is a work in progress, it does show that a low cost robotic system to aid in the processing of avian blood smears is feasible. Current work is being done to optimise the accurate recognition of monolayer fields. Work is also underway on testing algorithms to measure and eventually classify individual blood cells. Should a successful prototype be developed it could aid in clinically relevant research involving the evaluation of avian blood smears.

It is possible that one day with the advance of computer vision and robotics that automatic slide scanners become routine tools in diagnostic veterinary laboratories to aid clinical pathologists in their evaluation of avian blood samples. If they are also low cost, then it is possible that such robots become part of in-clinic haematology/biochemistry laboratories by performing preliminary analysis of a smear and sending images to a remote clinical pathologist for expert evaluation. This could potentially remove the need to send slides and blood by courier to the laboratory.

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