Semi-automated Microscope for Canine Semen Evaluation

Proponents:
VELASCO Jessica S., ECE, CAYLAS Jundy M., DE VERA, Edev Paula E., DOMINGO, Flynn Ernest M., GUINTO, John Francis R., RAMOS, Roniel Emerson R.

Abstract— **In this study, a microscope with its mechanisms semi-automated was constructed and tested. This device can easily focus and capture images of the sperm in the semen samples. Image focusing and capturing with the used of buttons in the GUI that is connected to an Arduino microcontroller. This board is equipped with sets of digital and analog input/output pins interfaced to various expansion boards and other circuits. The Arduino is connected to Arduino CNC Shield which has 4 slots in the board for stepper motor drive modules that enable the movement of different knobs in the microscope. This semi-automated microscope aides in the tedious work of breeders in determining if a canine is up for breeding. Also, by using this device, the breeding process can be greatly improved. The average time for the manual process is higher than that of the semi-automated process. All in all, this device offers a low cost but efficient and less-time consuming way of semen analysis.**

Index Terms—**Arduino, Stepper Motor and Computer Aided Semen Analysis (CASA).**

I. INTRODUCTION

According to studies, the infertility of a dog may be caused by three major categories. The first one is the failure to copulate or ejaculate. Next are poor semen quality and lastly the prostatic diseases. Focusing on the poor semen quality, this includes the low sperm count, abnormal motility and abnormal morphology. There are possible causes of the decrease of the semen quality. This includes hormonal imbalance, certain medications, fever from systematic infection and numerous diseases of the testicles of the dogs. In confirmation of the dog's fertility, it will undergo under multiple series of examination to know the cause. The laboratory examination includes complete blood count and biochemistry panel, urinalysis, semen testing, brucellosis titer, ultrasound, hormone testing and other testing depending on the result of other tests. [1]

There had been some updates on the application of CASA on dogs. It stated the capability of CASA systems to detect slight alterations in sperm movement which proved to be very useful for objectively comparing different sperm diluters and for the improvement of cooling and cryopreservation procedures in dogs [2]. Also, CASA systems were used to identify canine sperm cell subpopulations which interestingly offered new insights in the differences in freezability of semen among dogs and which could to a great extent predict the outcome of a cryopreservation procedure of a given semen sample. [3]

For the semi-automate movement of the microscope's mechanism, it moves the slide horizontally and carries the specimen slide into the optical light path of the microscope objective lens for viewing. Once the specimen slide is in the optical light path of the microscope objective lens a signal generated by the microscope viewing electronics will activate the specimen stage assembly Z axis focusing control motor and move the microscope stage in a vertical direction until the specimen is in focus. Other signals generated by the system electronics will activate the X and Y axis positional control motors and accurately move the specimen slide about the horizontal examination plane and place the specimen in any position or series of positions required for examination of the specimen. Focusing of the specimen during these positional changes is automatically corrected by the microscope viewing electronics control signals if required. [4].

A semen analysis evaluates certain characteristics of a male's semen and the sperm contained in the semen. The chance of pregnancy will be reduced, if more than 50 percent of a man's sperm lack movement. Assessing the ability of sperm to move forward through the cervix into the fallopian tubes is a widely used measure of male infertility. [5]

For the collision of the sperms, student from the University of California made an algorithm that can be used in laser optical trapping and sperm motility studies. The algorithm also has a collision-detection feature for real or perceived collision or near-miss cases between two sperms. Same in the instrument made by Leonardo F. Urbano, that has a multi sperm tracking algorithm that has the capability to track simultaneously hundreds of sperm cells and detect dynamic swimming parameters of human sperm cells with fertility and fertilization rates in recorded videos in the microscope. [6]

In relation with the study of Wakimoto et al., the research is all about detecting sperm-immobilizing antibody. The researchers used a Computer-Aided Sperm Analysis (CASA) for detecting sperm-immobilizing antibody and developed a novel method for their study. The study found out that there is a correlation between their study and the traditional method used by the embryologist. The researchers improved and developed the CASA for their study. [7]

In relation with microscopic analysis a Microcontroller-based Automated Microscope for Image Recognition System of Four-class Urine was developed by innovating conventional microscope into analysing urine samples regarding the presence of white blood cells (WBC), red blood cells (RBC), bacteria, and calcium oxalates (CaC2O4) using digital image processing techniques through Artificial Neural Network. Their device examined 100 samples of urine for testing its performance speed and quality of autofocusing. They were able to successfully achieve an automatic image-focusing and stage movement using stepper motors interfaced with PIC18F25J50 Microcontroller. Artificial Neural Network and Digital image processing were created in MATLAB and was used in recognizing the four classes of urine to generate results with an accuracy of 89.5% and average speed of 3.66 minutes. [8]

The objective of this study is to semi-automate the microscope mechanisms to be used for canine semen analysis.

II. MICROSCOPIC ANALYSIS

A. Microscopic Analysis

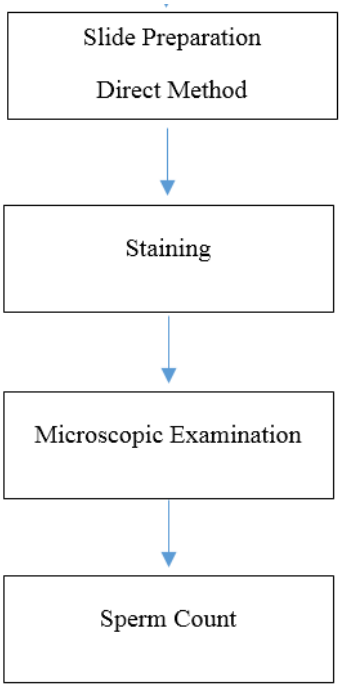

Fig. 1: Steps in Semenalysis for Canine

Figure 1 shows the process of microscopic analysis for semen. After the semen is collected by breeder or veterinarian, it will undergo the preparation process. Then, the specimen will be covered with a cover slip, without staining for motility test and with staining for morphology test. The adjustment knobs of the microscope will be controlled using a Graphical User Interface (GUI) in the connected computer.

B. Semi-automated Microscope Components

1. Microscope

The microscope will be used to magnify the image of the sample. This will make the sample ready for analysis. For viewing Spermatozoa, Red Blood Cells (RBC), and White Blood Cells (WBC), high power field (400x magnification) is needed. The Neubauer with sample on it will be placed under the microscope.

Fig. 2: Harman  Microscope

2. Microscope Camera

The microscopic camera will record a video and capture images of the sample under observation. These will be stored in the database of the system.

Fig. 3: DigiEye Microscope Camera

3. Microcontroller

The Arduino UNO is an open-source microcontroller board that is based on the Microchip ATmega328P microcontroller and developed by Arduino.cc. This board is equipped with sets of digital and analog input/output pins which can be interfaced to various expansion boards and other circuits.

4. Arduino CNC Shield

CNC Shield V3.0 is used as drive expansion board for engraving machine, 3D printer and other devices. There're 4 slots in the board for stepper motor drive modules, can drive 4 stepper motors, and each step stepper motor only need two IO port.

5. Stepper Motor Driver

Allegro's A4988 microstepping bipolar stepper motor driver has adjustable current limiting, over-current and overtemperature protection, and five different microstep resolutions (down to 1/16-step).

6. Stepper Motor

A NEMA 17 stepper motor is a stepper motor with a 1.7 x 1.7 inch (43.2 x 43.2 mm) faceplate. The NEMA 17 is larger and generally heavier than for example a NEMA 14, but this also means it has more room to put a higher torque. However, its size is not an indication of its power.

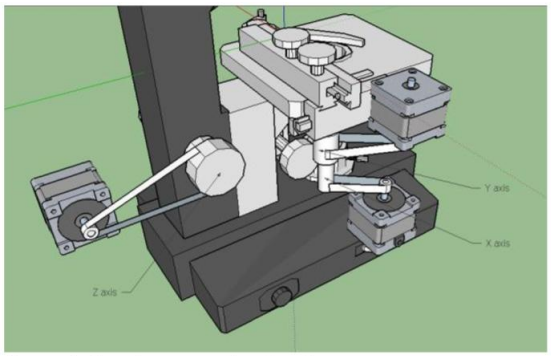

Fig. 4: Semi-automation Design

B. Microscopic Images

Figure 5 shows images taken from the microscope.

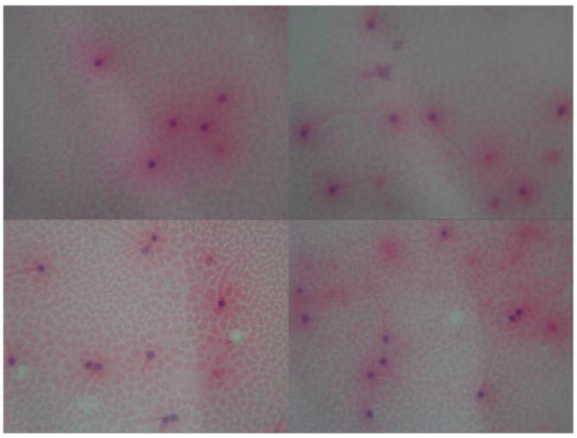

Fig. 5: Datasets Images

V. METHODOLOGY

The block diagram of this study is shown in figure 6 below.

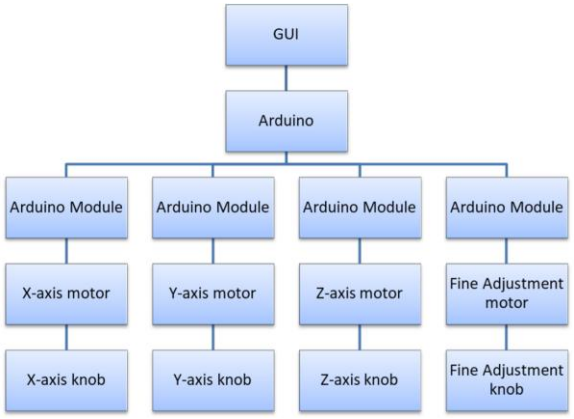

Fig. 6: Block Diagram

A. Block Diagram

The GUI has different tabs with different function for the system which include getting patient information, displaying patient and record information, video recording and image capturing, automatic and manual operation of the microscope, display of results, and PDF generation. With this, the microcontroller will start and wait for the commands. When the commands are already received, it will control the stepper motors connected to the knobs of the microscope to move in accordance with the instruction until all the conditions are satisfied.

B. Computation for the Displacement of the Gear

The proponents used the Neubauer counting chamber as measurement preference. Since at 10X and 40X magnification provides the camera to show a 1mm by 1mm and 0.5mm by 0.5mm respectively, we can determine the necessary displacement for each movement.

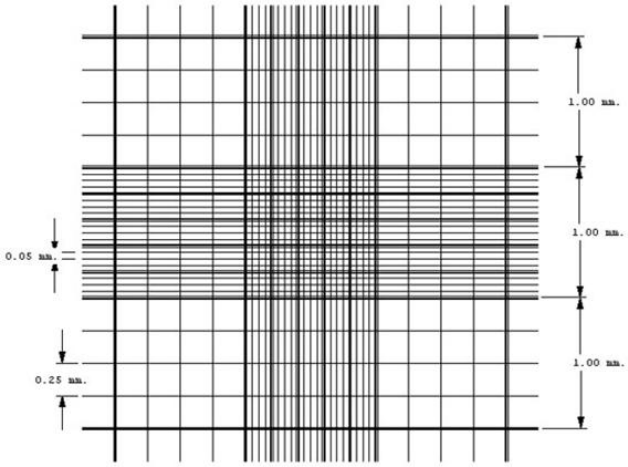

Fig. 7: Neubauer gridlines

Using a 2mm pitch gear attached to the knob and motor, we've determined that one second trigger provided a displacement of 4mm for the x-axis and 2mm on the y-axis. Since we only wanted 2mm displacement for each trigger we've reduced the delay for the x-axis to half a second..

VI. DATA AND RESULTS

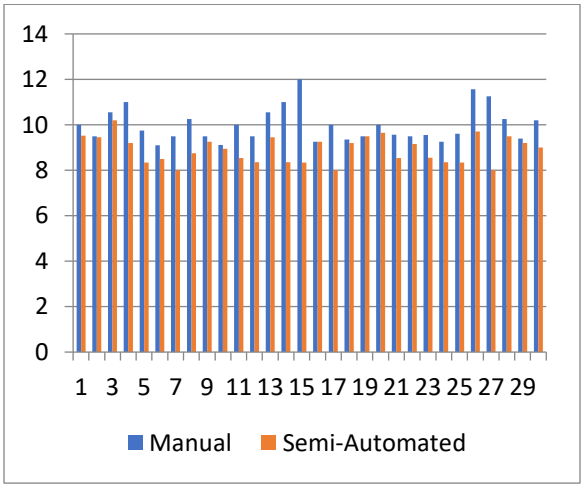

Fig. 8: Graphical comparison of Manual and Semi-automated process

Based on the graph above, it can be determined that the semi-automation process is slightly faster than of the manual process. The average time for manual counting is 9.985while for the semi-automated counting is 8.904.

Table 1. File Size of TLM

TLM	File Size (MB)
MobileNetV1	89.2
Inceptionv2	101.9
ResNet50	134.3
ResNet101	196.5
Inception_ResNetV2	246.6

VII. CONCLUSION

The semi-automation of the mechanisms of the microscope makes the process of semen analysis more efficient and less-time consuming. The GUI was successfully modified by putting buttons in it that makes controlling the knobs of the microscope easier. The program for the microcontroller was successfully achieved using Arduino and through using Arduino CNC Shield which interconnects the microcontroller, stepper motor drivers and stepper motor.

VIII. RECOMMENDATION

The study would like to recommend the full-automation of the system. The authors suggest designing a microscope that will automatically takes microscope specimen slides one at a time for analysis.

ACKNOWLEDGMENT

The authors would like to thank the Technological University of the Philippines-Manila, Mr. Vincent Joharra M. Abo, RMT and Ms. Ira Joy Martin, statistician, who helped in this project untiringly.

REFERENCES

[1] Infertility in Male Dogs. (n.d.). Retrieved from https://vcahospitals.com/know-your-pet/infertility-in-male-dogs

[2] Nizanski, W. (2012, December 24). Computer-Assisted Sperm Analysis in Dogs and Cats: An Update after 20 Years. Retrieved from https://onlinelibrary.wiley.com/doi/full/10.1111/rda.12057

[3] Application of computer-aided sperm analysis (CASA) for... (n.d.). Retrieved from https://www.researchgate.net/publication/322605475_Application_of_co mputer-aided_sperm_analysis_CASA_for_detecting_spermimmobilizing_antibody.

[4] Georges, & P., M. (1981, February 03). Automatic microscope slide - GEORGES; MICHAEL P. Retrieved from http://www.freepatentsonline.com/4248498.html

[5] Semen Analysis: Sperm Count Test Procedure and Results. (n.d.). Retrieved from https://www.healthline.com/health/semen-analysis.

[6] Synthesis of Sperm &Seminal Plasma Sperm Production Maturation &Storage Seminal Plasma Semen Testes: Sertoli Cells Epididymis & Vas Deference Epididymis. - ppt download. (n.d.). Retrieved from http://slideplayer.com/slide/10949189/.

[7] Semen analysis: Find out what it can tell you. (n.d.). Retrieved from https://www.webmd.com/infertility-and-reproduction/guide/what-issemen-analysis#1.

[8] Fernandez et al, (2018). Microcontroller-Based Automated Microscope for Image Recognition System of Four-Class Urine Constituents. Retrieved from Wi-Search archives.