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Mosquito Pick-and-Place: Automating a Key Step in PfSPZ-based Malaria Vaccine Production

Henry Phalen, Prasad Vagdargi, Michael Pozin, Sumana Chakravarty, Gregory S. Chirikjian, *Fellow, IEEE*, Iulian Iordachita, *Senior Member, IEEE* and Russell H. Taylor, *Life Fellow, IEEE*

***Abstract*— The treatment of malaria is a global health challenge that stands to benefit from the widespread introduction of a vaccine for the disease. A method has been developed to create a live organism vaccine using the sporozoites (SPZ) of the parasite *Plasmodium falciparum* (Pf), which are concentrated in the salivary glands of infected mosquitoes. Current manual dissection methods to obtain these PfSPZ are not optimally efficient for large-scale vaccine production. We demonstrate the automation of a key step in this production process, the picking and placing of mosquitoes from a staging apparatus into a dissection assembly. This unit test of a robotic mosquito pick-and-place system is performed using a custom-designed micro-gripper attached to a four degree of freedom (4-DOF) robot under the guidance of a computer vision system. Mosquitoes are autonomously grasped from a mesh platform and pulled to a pair of notched dissection blades to remove the head of the mosquito, allowing access to the salivary glands. Placement into these blades is adapted based on output from computer vision to accommodate for the unique anatomy and orientation of each grasped mosquito. In this pilot test of the system on 50 mosquitoes, we demonstrate a 100% grasping accuracy and a 90% accuracy in placing the mosquito with its neck within the blade notches such that the head can be removed. This is a promising result for this difficult and non-standard pick-and-place task. An analysis of the failure cases provides insights for improvements to be implemented as this robotic pick-and-place system is integrated into a larger automated mosquito dissection system under development.**

I. INTRODUCTION

Malaria presents a tremendous public health burden. The World Health Organization estimates 219 million individuals worldwide were infected with the disease in 2017 and ranked it among the top 20 leading causes of death among both adults and infants in 2016 [1], [2]. With increasing drug and insecticide resistance, it has become ever more difficult for current treatments to maintain efficacy in reducing the prevalence of malaria worldwide [3]. Development of malarial vaccines present a promising way forward in the global effort for malaria eradication [3]. Progress has been made in the development of the Sanaria *Plasmodium falciparum* sporozoite-based vaccine (Sanaria® PfSPZ Vaccine), an effective vaccine manufactured from PfSPZ extracted from the salivary glands of female *Anopheles* mosquitoes [4]–[9]. Such a vaccine may reduce the burden of the disease by providing immunity against Pf, the most common malarial parasite, which was estimated to account for greater than 95% of deaths caused by malaria in 2017 [1], [10].

The process of vaccine production requires salivary gland dissection and to date has only been demonstrated with training-intensive manual or semi-automated processes, presenting a major bottleneck in the scalability of this vaccine. In traditional manual methods, technicians are presented with freshly-sacrificed, lab-grown mosquitoes and process them one at a time, removing the mosquito's head with a needle under microscope and squeezing out a volume of exudate that includes the PfSPZ-laden salivary glands. The exudate from mosquitoes is collected and processed for the isolation of PfSPZ.

The automation of salivary gland harvesting from *in vivo* mosquitoes has been attempted in the past [11]–[13]. However, no literature supports the success of any such process at this time. A semi-automated mosquito micro-dissection system (sAMMS) has been developed and investigated within our research group [14], [15]. In the sAMMS process, a technician uses micro-forceps to sort mosquitoes into cartridges such that their necks extend between cutter blades. Then, the blades are actuated to cut off all the heads, and a comb-like squeezing device is used to extrude all the exudate, which is collected via a suction device. Early experience has shown that this device roughly doubles the throughput of purely manual dissection to an average of 470 mosquitoes per hour and reduces training time to reach peak performance from 39 to 1.5 weeks [15].

While a demonstrable improvement over manual methods, the sAMMS device was developed only as a first step towards a fully automated dissection system to enable large-scale production of enough vaccine for world-wide vaccination efforts. One major bottleneck in the transition to a fully automated system is the visual perception and physical precision it takes to recognize a mosquito, analyze it, and best align it so that the head can be removed. We report our work to overcome these challenges through the development of a vision-guided pick-and-place robotic system for loading mosquitoes into the sAAMS device. As our research group works toward development of a fully automated mosquito salivary gland dissection system, the demonstration of a robust pick-and-place apparatus is a key milestone in realization of that goal.

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H. Phalen, P. Vagdargi, M. Pozin, G. S. Chirikjian, I. Iordachita, and R. H. Taylor are with The Laboratory for Computational Sensing and Robotics (LCSR) at the Johns Hopkins University, Baltimore MD, USA. S. Chakravarty is with Sanaria, Inc. Rockville, MD, USA. H. Phalen is the corresponding author, henry.phalen@jhu.edu

II. SYSTEM DESIGN CONCEPT

A. System Overview

The robotic pick-and-place system described in this paper will function as a subsystem within a fully-automated mosquito dissection system. This larger system will ultimately take freshly-sacrificed mosquitoes suspended in liquid media and output a collection of mosquito exudate including PfSPZ-laden salivary glands. A concept of this dissection system is provided in Fig. 1. We briefly describe this system to clarify the context of the robotic pick-and-place subsystem, which will be the middle of three primary system components.

First, a staging apparatus will separate mosquitoes and present them one at a time to the robot. Freshly-sacrificed mosquitoes sit in a basin of media beneath the system. A spinning rotor in the basin creates a vortex that will carry mosquitoes in solution to the top of a separation cone. This cone has channels in one sector down which media will flow onto a ring of orientable mesh-bottomed cups. This ring will rotationally index around the cone so that, by controlling the vortex speed and concentration of mosquitoes in the basin, the cups will on average have one mosquito on them once they pass beyond the sector of the cone with channels. At an index beyond the channel, a camera will image a single cup and a computer vision algorithm will determine if a mosquito is present. If so, at the next indexed position, the cup will be rotated to orient the mosquito so that the mosqutio's proboscis will point radially outward from the ring. Finally, the ring will be rotated to an index that a sits parallel to a linear stage that will comprise the third subsystem, a dissection assembly line. The development of the staging apparatus is described in detail in [16].

The pick-and-place robot will be positioned on the other side of the linear stage and will reach over to the cup, grasp the mosquito by its proboscis and drag it onto a sAMMS-resembling cartridge attached to a linear stage. Similar to how a human technician would use the sAMMS, the robot will drag the mosquito into a slot and place the mosquito's neck into notches cut in two parallel dissection blades. An overhead camera will be used to provide computer vision feedback of this process. The blades will be actuated, cutting the head. After disposing of the mosquito's head, the robot will return to the ring which will have rotated to present a new mosquito. The linear stage will index laterally immediately after the mosquito is cut. As additional mosquito bodies are positioned on the cartridge, the linear stage will translate and expose mosquitoes to several stations at which the exudate can be squeezed out and salivary glands collected. Work on the dissection assembly line is currently ongoing within our research group.

B. Requirements

We focus here on the robotic pick-and-place system, along with its difficult and important task of picking up mosquitoes presented on a mesh surface, and precisely placing them so that only the neck lies within the blades. In order to extract

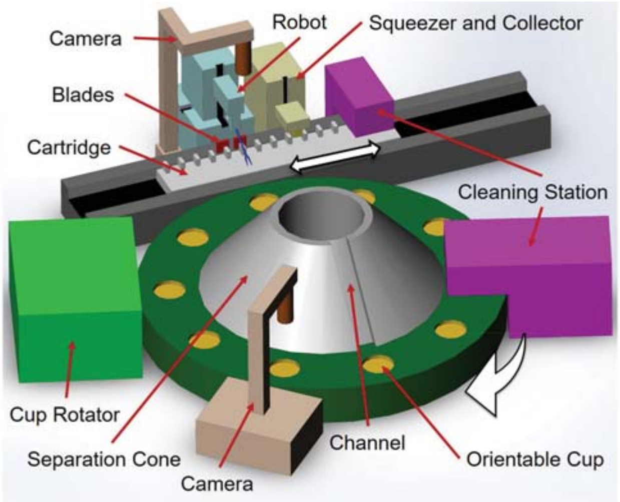

Fig. 1. Concept image for automated mosquito dissection system.

the salivary glands of the mosquito, the dissection point has to be precisely at the intersection of the head and thorax. If the mosquito is not placed far enough into the blade, the cut will occur on the head, leaving no passage for the exudate to be squeezed out, effectively wasting the mosquito and PfSPZ within. Because the salivary glands are located just behind the mosquito's head, placing the mosquito too far into the blade would result in some of the gland being lost in the cut, also decreasing PfSPZ yield. The mosquito neck is approximately 0.3 mm in length, and the blades each have a thickness of 0.002 inches (0.051 mm), leaving only about 200 micrometers for error. Moreover, grasping must occur only on the proboscis to prevent any damage to the body that might ruin the salivary glands or create an alternative opening for exudate to squeeze out of. The proboscis is on average 2.0 mm long, with a diameter of approximately 0.1 mm.

In addition to size challenges, this procedure presents multiple difficulties not typically faced in a standard pick-and-place procedure. One of the main challenges is the mosquito-to-mosquito variation. Some of this is anatomical in origin. Each mosquito varies somewhat in size and is not axis-symmetric, meaning the alignment of the neck relative to the body depends on which side the mosquito body lies on. Further, mosquitoes are very flexible. By grabbing and pulling the mosquito from the proboscis, the body tends to straighten out in time for placement, but first, the mosquito has to be identified and grasped from a variety of twisted, compressed, or otherwise contorted orientations. While upstream processes are expected to align the mosquito's proboscis within 15 degrees of an ideal orientation for the robot to grab it, the mosquito can still be located anywhere on the cup and must be grasped accordingly. Because of its length, the proboscis can still be grabbed even if there is some error in the robot positioning, or computer vision targeting. However, this, combined with the general variability in proboscis lengths, means that the offset between the robot's grasp point and the mosquito's neck is not consistent trial-to-trial. As such, it is not enough to program a sequence of robot movements; these challenges necessitate adaptive automation. Based on visualization of the mosquitos anatomy and its grasping location, the robot must perform customized movements to successfully grasp and place the mosquito.

C. Experimental Setup

The robot used in this procedure is a 4-DOF, linear stage robot by New England Affiliated Technologies, Lawrence, MA (Fig. 2). A dual-axis X-Y table is used as the base for the robot, onto which a Z axis is mounted orthogonally (NEAT: XYR-6060 and NEAT: LM-400 respectively). The robot also has a rotary axis which is not used in this study. Each axis is driven by a 12V DC servo motor, with a leadscrew, has a travel of 100 mm, and is coupled with an incremental encoder. The positioning resolution of these axes was measured with a dial indicator to be approximately 10 micrometers. The entire assembly is mounted to an optical table. Robot motion is driven by a Galil controller (DMC-4143), interfaced to a Linux computer by ethernet connection. Attached to the robot is a custom-designed micro-gripper mechanism visualized in Fig. 3. A cam mechanism attached to a HexTronik HXT900 servo motor drives the rail of a linear guide within its carriage, causing the tooltip to open and close. The tooltip of the micro-gripper is adapted from an Alcon Grieshaber retinal surgical forceps. Movement of the linear guide rail extends or retracts a sleeve over a normally-open gripper jaws. The micro-gripper is controlled by sending position commands to the servo motor via USB serial communication from the computer to an Arduino Uno microprocessor.

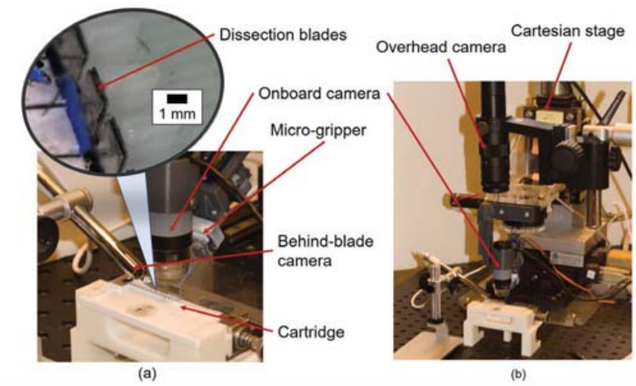

Fig. 2. Experimental Setup. A close-up image of dissection blades and cartridge is inset with length scale for reference.

Mosquitoes are staged for dissection on a modified sAMMS device that is also mounted to an optical table. The sAMMS cartridge is modified to have a hole 23 mm away from the blades in which is placed a 20 mm diameter cup that matches the one used in the upstream staging apparatus. This cup is covered with a nylon 750 micrometer mesh that is used for media drainage in that apparatus. The mosquito

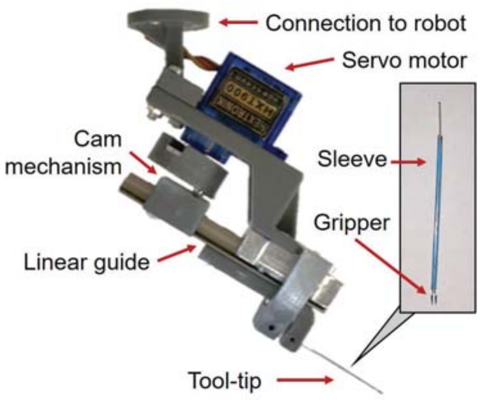

Fig. 3. Custom-designed micro-gripper used to grasp mosquitoes.

is dragged into a slot in the sAMMS cartridge and placed into the sAMMS blades. These are two 0.002 inches (0.051 mm) thick stainless steel blades with 0.5 mm wide by 1.0 mm deep notches cut in them to match the midpoint of the slots. The closest blade to the cartridge is stationary while the further blade can be manually actuated by pressing a button on the side of the device. This action causes the mosquito neck to be caught between the two blades and cut.

The setup also includes three cameras (Fig. 2). An overhead microscopic camera (OptixCam Summit D3K2-5) with an Omano OM-10K zoom lens is used to capture a complete view of the workspace and is used by the computer vision to identify a mosquito's presence and approximate location. A second camera (Plugable USB Microscope Camera), is mounted on the robot and is used to identify the location of the mosquito's body parts for accurate picking and placing. We refer to these as the overhead and onboard cameras respectively. A third camera (Opti-Tekscope USB Microscope Camera) is placed to the side and rear of the setup so that it's visual field is in line with the blades. This camera is not necessary for system operation and is only used to visualize placement so the tester can determine if a trial was successful or not.

The automated procedure uses the overhead and onboard cameras to guide the robot's motion. The procedure consists of three stages. In the first stage, an image of the entire workspace is captured using the overhead camera. This image is converted to HSV space, and the mosquito is segmented out. Next, a bounding box is fit to this region and a weighted centroid is calculated for the mosquito, as shown in Fig. 4(a). The tooltip is moved near the mosquito, to an position where the onboard camera, due to its close proximity, is able to capture am image of the mosquito with more features and details.

In the second stage, a computer vision algorithm identifies the mosquito's proboscis in the detailed onboard camera image shown in Fig. 4(b). The tooltip is moved to a point above the centroid of the proboscis Fig. 4(c), which is used as the grasp location for the mosquito Fig. 4(d). Finally, the robot drags the mosquito to an empty slot on the cartridge near the blades.

In the third stage, the onboard camera captures a final image shown in Fig. 4(e) with the tooltip in view to detect the mosquito head-to-tooltip offset. The robot uses this offset value to position the mosquito with its neck between the dissection blades (Fig. 4(f)). Our group is also investigating the use of a second computer vision processing algorithm [17], which was not used in this study.

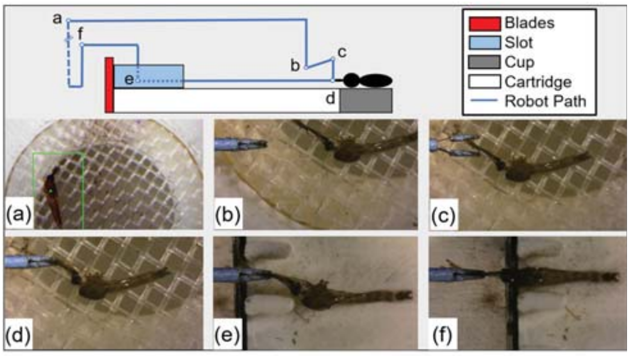

Fig. 4. Side view of robot path and related representative images captured by the vision system. (a) Image captured from overhead camera showing bounding box of detected mosquito. (b) Image captured from onboard camera to determine proboscis centroid. (c) Image captured from onboard camera before grasping. (d) Image captured from onboard camera immediately after grasping. (e) Image of the mosquito taken used to calculate head-to-tooltip offset. (f) Image after aligning the mosquito neck with the blades.

D. Calibration

To relate the robot and camera coordinate systems, we use a two stage calibration process. In the first stage, the tooltip of the micro-gripper is located in the overhead camera frame. The tool is segmented from the background in HSV space using Otsu's binarization [18], and contour identification is used to detect the tool. The lowest point of the tool contour is then used as the tooltip. In the second stage of calibration, the robot is moved through the camera space across a grid of points. The tooltip is detected and recorded at each position. The resulting grid of points from both coordinate systems are used as the inputs for a Bernstein polynomial fitting routine as performed in [19]. This routine fits two fourth degree polynomials, which creates a mapping from the camera coordinates to the robot's encoder coordinates. Such a polynomial fitting method also compensates for radial and aspherical lens distortions.

The tooltip does not move with respect to the overhead camera, so the polynomial fitting method described above could not be used to calibrate this camera. Instead, a pre-calibrated grid of a resolution 5mm x 5mm was placed in the background. The robot was then moved by a known distance along each axis, and images were captured before and after motion. The pixel motion of the grid was calibrated to the corresponding change in robot encoder counts.

The location of the cartridge and blades in robot coordinates were determined using a shim. The robot was slowly advanced until the robot held the shim firmly to the surface of interest and encoder counts at this location were used as a reference.

III. EXPERIMENTAL METHODS

A. Study Design

Testing was performed to investigate the efficacy of the designed system to pick up a mosquito and place that mosquito within blades that can remove the insect's head. The process was performed on 50 non-infected *Anopheles* mosquitoes. Prior to testing, the mosquitoes were kept in an airtight, refrigerated container of phosphate-buffered saline solution (PBS) following sacrifice one day prior. Functioning as a unit test for this subsystem within the eventual automated mosquito dissection system, only the grasping and subsequent positioning of the mosquitoes by the robot were considered for trial success or failure. All actions of the system during the test were performed autonomously with feedback from computer vision, and a manual cut was performed at the end of each trial to facilitate determination of trial success.

B. Pick Procedure

The experimental procedure is demonstrated in Fig. 4. A mosquito is removed from the PBS solution by its proboscis with tweezers and placed anywhere on a circular mesh cup of radius 10 mm with its center placed 23 mm away from the blades as measured on the central axis of the cartridge slot. The mosquito was placed so that the proboscis was positioned forward toward the blades, pointing within 45 degrees this line. One such placement is shown in Fig. 4(a). These conditions were chosen to mimic the worst case expected from the upstream mosquito-staging apparatus that this process will later be integrated with. To further match the expected results of this upstream process, no further attempt at standardization of mosquito starting position were made (e.g. what side the mosquito was lying on, relative straightness of legs). The micro-gripper tooltip begins the trial at a location away from the cup and 3.5 mm above the cartridge surface.

A bounding box around the mosquito is identified by computer vision in an image from the overhead camera, and the robot moves to a point 5.0 mm in front of the centroid of that region (Fig. 4(b)). This brings the mosquito into view of the onboard camera without placing the gripper over top of the mosquito body. By lowering 3.0 mm toward the mesh surface, the mosquito is brought into focus. The centroid of the proboscis region is identified and the robot moves the gripper to a location 2.0 mm above this this point (Fig 4(c)), and then drops down to the mesh surface and the gripper is closed to grab the proboscis (Fig 4(d)).

The robot lifts up 0.8 mm and drags the mosquito to a position 1.5 mm from the blades (Fig. 4(e)). Here, an image from the onboard camera is again analyzed by the computer vision system. This task serves two functions, to confirm successful grasping of the mosquito, and to determine more accurately where on the proboscis the gripper has grabbed. The trial is considered a successful demonstration of grasping if the mosquito is visualized as grasped within the micro-gripper at this point.

C. Place Procedure

The vision system provides the location of the proximal end of the proboscis, where it attaches to the mosquito's head. This location is transformed into robot coordinates and a head-to-tooltip offset is determined by subtracting it from the current encoder values. Only the offset in line with the cartridge grooves (a horizontal offset in Fig. 4(e)) is considered. The robot then executes another set of programmed movements. The robot raises the mosquito head 1.3 mm and moves forward a nominal distance to clear the blades plus the offset, such that the mosquito's neck should be right above the blades (Fig. 4(f)). Then the tooltip moves down 3.0 mm, placing the neck within the notch of the blades if properly aligned. At this point, another subsystem of the automated mosquito dissection system would actuate the blades to cut the head and further process the mosquito. In this unit test, the blade is manually actuated. The test is considered a successful placement if the mosquito's neck is placed into the notch of the dissection blades such that the head could be removed. As a final step of the process, the robot pulls away from the blade, moving the head, if still in its grasp, to a location where it can be cleaned off with a modest jet of air or fluid that does not disturb the tooltip calibration. Video footage from all three cameras is recorded throughout and saved for analysis. The commanded speed of each robot axis was 12.5 mm/s, chosen to achieve rapid movements with negligible overshoot. The speed was decreased to 2.5 mm/s when lowering the mosquito neck into the blades, reducing the inertia of the mosquito and thus the tendency to pivot or flip over the blades rather than settle between them.

IV. RESULTS

Throughout the experiment, there were no issues moving to a mosquito's location, grasping it by the proboscis, or dragging it on the surface of the cartridge. All 50 (100%) of the mosquitoes were observed with the proboscis grasped by the micro-gripper during the second vision check (Fig. 4(e)). Of these 50 mosquitoes, 45 (90%) were placed such that their necks were aligned correctly within the blades. Placement was considered successful if the alignment allowed the blades to cut the neck such that the head could be fully removed. The results were confirmed post-test with close-up video taken of the blades during the placement and cutting steps. An example of a mosquito being accurately placed is provided in Fig. 5(a).

The five mosquitoes that were not accurately placed exhibited similar behavior, flipping over the blades when pulled down by the robot. This action is demonstrated in Fig. 5(b). In these cases the mosquitoes appear to collide with either the slot walls or the blades. That collision point acts as fulcrum, causing the downward motion of the robot to flip them over the blades, rather than pull the neck into the notches. We were unable to correlate this behavior with any other variable including initial mosquito orientation, grasp location of the proboscis, trial number, or a qualitative assessment of the computer vision's head-to-gripper offset estimation.

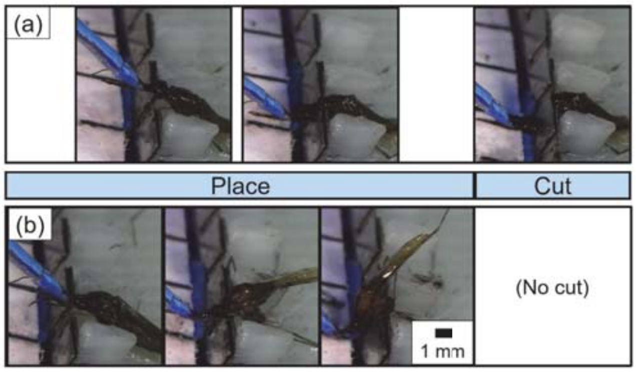

Fig. 5. A demonstration of mosquito placement. (a) A mosquito being accurately placed with neck between the blades. (b) Inaccurate placement of the mosquito, resulting in the body pivoting over the blades when the proboscis is pulled down by the gripper, rather than the neck settling between the blades.

V. DISCUSSION

The robotic pick-and-place subsystem demonstrated highly successful results in this unit test. With no failures in grasping or moving the mosquito, the micro-gripper is shown to be adequately designed for the task. In order to both pick and place any single mosquito, the system was required to navigate to locations that were not explicitly programmed. The ability of the system to achieve these promising results indicates that the computer vision system was effective at providing appropriate adaptations to robot movement.

Although we are still working to improve the system, the 90% success rate from this initial pilot study is very encouraging, especially considering the challenges presented by this non-standard pick-and-place task. It is not surprising that the placing task would prove more difficult than grasping the mosquitoes as it requires more accuracy. In order to move the mosquito, the robot can grasp anywhere on the length of the proboscis. Placing the mosquito's neck between the blades requires more precision and any inconsistencies in grasping, deformation, and anatomy must be accommodated in this step. Using a head-to-tooltip offset determined by the vision system worked well and we will continue to enhance the performance through improvements to the vision algorithm at this step.

In the few cases where adequate placement was not achieved, the mosquitoes were observed to flip over the blades about a contact point with either the blades or the cartridge. This behavior occurred both when the neck appeared to be misaligned with the blade notches as well as in cases when the alignment appeared adequate. When there was neck misalignment, either the head or body of the mosquito, which are wider than the notch within the blades, contacted the top of the blades and caused the mosquito to flip over when the robot pulled the proboscis downward. Our work to better determine the tooltip-to-head offset should improve the accuracy of alignment, and we will also investigate if adjusting the angle from which the images are taken can improve our estimation. As the robot holds the proboscis above the cartridge surface, its length is foreshortened in the top-down view provided by the onboard camera. A better estimation of the offset may be obtained geometrically or from a side-view camera where the proboscis profile should not be distorted. We will also target further improvement through mechanical changes to the blade and cartridge geometries to better guide the mosquito neck into position even in cases of small errors in robot positioning. These modifications should also address the situations in which alignment appeared adequate by video observation, but a flip still occurred.

The robot movements combined took about 7.7 seconds on average in this test. The computer vision steps were running on a CPU at the time of testing and took over four seconds each, but have since been optimized to run on a GPU, bringing processing time down to 0.16 seconds on average per image. Further, the first vision step will happen in parallel with later robot movements, so this time can be excluded from the total count. The pick-and-place procedure would therefore be able to process about 450 mosquitoes per hour. Without hardware changes, we estimate up to 2.5 additional seconds could reasonably be reduced, leading to a rate around 700 mosquitoes per hour, by means of movement optimizations such a less conservative speed for inserting the neck between the blades and movement of the home point of the robot closer to the cup. The final system is expected to use a smaller, lighter robot, which should achieve faster accelerations and may allow for further time improvement through increased nominal speed.

VI. CONCLUSION

We have presented the design and implementation of a robotic system for performing the challenging and non-standard pick-and-place task involved in mosquito dissection. Successful demonstration of this process represents a major milestone in our effort to automate the malaria vaccine production process. The micro-gripper performed with high accuracy and consistency and the system's performance indicates reliable vision and calibration techniques. Our methods proved to adapt well to anatomical and positional differences amongst the tested mosquitoes. These results will help to inform the further design of this subsystem, its companions, and their interfaces, ultimately contributing to an automated mosquito dissection system to facilitate the scalable production of PfSPZ-based malaria vaccines.

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REFERENCES

[1] World Health Organization, "World malaria report 2018," Nov 2018. [Online]. Available: https://www.who.int/malaria/publications/world-malaria-report-2018/report/en/

[2] M. P. Heron, "Deaths: Leading causes for 2016," *Natl Vital Stat Rep*, vol. 67, no. 6, pp. 1–76, 2018.

[3] World Health Organization, *Global technical strategy for malaria 2016-2030*, 2015.

[4] S. L. Hoffman, P. F. Billingsley, E. James, A. Richman, M. Loyevsky, T. Li, S. Chakravarty, A. Gunasekera, R. Chattopadhyay, M. Li, *et al.*, "Development of a metabolically active, non-replicating sporozoite vaccine to prevent plasmodium falciparum malaria," *Human vaccines*, vol. 6, no. 1, pp. 97–106, 2010.

[5] A. S. Ishizuka, K. E. Lyke, A. DeZure, A. A. Berry, T. L. Richie, F. H. Mendoza, M. E. Enama, I. J. Gordon, L.-J. Chang, U. N. Sarwar, *et al.*, "Protection against malaria at 1 year and immune correlates following PfSPZ vaccination," *Nature medicine*, vol. 22, no. 6, p. 614, 2016.

[6] J. E. Epstein, K. M. Paolino, T. L. Richie, M. Sedegah, A. Singer, A. J. Ruben, S. Chakravarty, A. Stafford, R. C. Ruck, A. G. Eappen, *et al.*, "Protection against plasmodium falciparum malaria by PfSPZ vaccine," *JCI insight*, vol. 2, no. 1, 2017.

[7] M. S. Sissoko, S. A. Healy, A. Katile, F. Omaswa, I. Zaidi, E. E. Gabriel, B. Kamate, Y. Samake, M. A. Guindo, A. Dolo, *et al.*, "Safety and efficacy of pfspz vaccine against plasmodium falciparum via direct venous inoculation in healthy malaria-exposed adults in mali: a randomised, double-blind phase 1 trial," *The Lancet infectious diseases*, vol. 17, no. 5, pp. 498–509, 2017.

[8] K. E. Lyke, A. S. Ishizuka, A. A. Berry, S. Chakravarty, A. DeZure, M. E. Enama, E. R. James, P. F. Billingsley, A. Gunasekera, A. Manoj, *et al.*, "Attenuated pfspz vaccine induces strain-transcending t cells and durable protection against heterologous controlled human malaria infection," *Proceedings of the National Academy of Sciences*, vol. 114, no. 10, pp. 2711–2716, 2017.

[9] B. Mordmüller, G. Surat, H. Lagler, S. Chakravarty, A. S. Ishizuka, A. Lalremruata, M. Gmeiner, J. J. Campo, M. Esen, A. J. Ruben, *et al.*, "Sterile protection against human malaria by chemoattenuated pfspz vaccine," *Nature*, vol. 542, no. 7642, p. 445, 2017.

[10] T. Bousema and C. Drakeley, "Epidemiology and infectivity of plasmodium falciparum and plasmodium vivax gametocytes in relation to malaria control and elimination," *Clinical microbiology reviews*, vol. 24, no. 2, pp. 377–410, 2011.

[11] Sanaria Inc. (2014) SporoBot - Build a Robot. Fight Malaria. Save Lives! [Online]. Available: https://www.youtube.com/watch?v=VblazNXcHFg

[12] I. Lapowsky, "The next big thing you missed: This mosquito-dissecting, malaria-killing robot needs your help," Jun 2014. [Online]. Available: https://www.wired.com/2014/06/the-next-big-thing-you-missed-a-crowdfunded-mosquito-dissecting-malaria-killing-robot/

[13] C. Borchers, "Robot may help fight malaria," May 2014. [Online]. Available: https://www.bostonglobe.com/business/2014/05/07/mosquito-harvest/Qxto58qtpGHhRVfliT6aHI/story.html

[14] R. H. Taylor, A. Canezin, M. Schrum, lulian Iordachita, G. Chirikjian, M. Laskowski, S. Chakravarty, and S. Hoffman, "Mosquito salivary gland extraction device and methods of use," U.S. Patent Application US20 170 355 951A1, June 13, 2016.

[15] M. Schrum, A. Canezin, S. Chakravarty, M. Laskowski, S. Comert, Y. Sevimli, G. S. Chirikjian, S. L. Hoffman, and R. H. Taylor, "An efficient production process for extracting salivary glands from mosquitoes," *arXiv:1903.02532*, 2019.

[16] M. Xu, S. Lu, Y. Xu, C. Kocabalkanli, B. K. Chirikjian, J. S. Chirikjian, J. Davis, J. S. Kim, I. Iordachita, R. H. Taylor, and G. S. Chirikjian, "Mosquito staging apparatus for producing PfSPZ malaria vaccines," Accepted to *2019 IEEE 15th International Conference on Automation Science and Engineering (CASE)*. IEEE, 2019.

[17] H. Wu, J. Mu, T. Da, M. Xu, R. H. Taylor, I. Iordachita, and G. S. Chirikjian, "Multi-mosquito object detection and 2D pose estimation for automation of PfSPZ malaria vaccine production," Accepted to *2019 IEEE 15th International Conference on Automation Science and Engineering (CASE)*. IEEE, 2019.

[18] N. Otsu, "A threshold selection method from gray-level histograms," *IEEE Transactions on Systems, Man, and Cybernetics*, vol. 9, no. 1, pp. 62–66, Jan 1979.

[19] L. Feng, P. Wilkening, Y. Sevimli, M. Balicki, K. C. Olds, and R. H. Taylor, "Accuracy assessment and kinematic calibration of the robotic endoscopic microsurgical system," in *2016 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Aug 2016, pp. 5091–5094.