Mosquito Staging Apparatus for Producing PfSPZ Malaria Vaccines

Mengdi Xu[1], Shengnan Lyu[1], Yingtian Xu[1], Can Kocabalkanli[1], Brian K. Chirikjian[1], John S. Chirikjian[1], Joshua Davis[1], Jin Seob Kim[1], *Member, IEEE*, Iulian Iordachita[1], *Senior Member, IEEE*, Russell H. Taylor[1], *Life Fellow, IEEE* and Gregory S. Chirikjian[12*], *Fellow, IEEE*

***Abstract*—This paper describes the design of a fully-automated apparatus to dispense mosquitoes into isolate units. This automation system consists of several process units including (1) facilitating the water vortex with a fan-shape rotor to gently transport the mosquitoes to the sorting slides with a conical geometry, (2) exploiting slides to guide mosquitoes to turntables driven by gears one by one, and (3) reorienting the mosquito until its proboscis points outward along the radial direction of the cone, aided by computer vision. This automation system serves as the first processing stage for collecting mosquito salivary glands. The sporozoites contained in the mosquito glands are the source material to produce Sanaria's first generation PfSPZ vaccines. The Mosquito Staging System can dramatically enhance the mass production of Malaria Vaccine which is essential to prevent the propagation of Malaria.**

***Index Terms*—Automation, Design, Medical Robot, Malaria Vaccine**

I. Introduction

The WHO's world malaria report 2018 highlights that no significant progress in reducing global malaria cases was made from 2015 to 2017. 219 million cases and 435,000 related deaths were estimated in 2017 [1]. Malaria is also responsible for the tremendous loss of GDP in high transmission countries. Although existing control measurements result in malaria elimination in some low transmission areas, it is still necessary to develop new tools to achieve a reduction in high transmission areas as well as to protect travelers. Tremendous Work has been done developing whole Plasmodium falciparum sporozoite-based vaccine (PfSPZ) [2]-[7]. Sanaria® PfSPZ Vaccine is based on the only proven approach in humans of inducing robust (100%) and long-lasting (at least 12 months) protective efficiency against Pf malaria by sporozoite (SPZ)-based vaccines. In Sanaria's manufacturing process, the mosquito behaves as a bioreactor, from which parasites are then extracted by microdissection of the insects salivary gland. Sporozoite extraction from mosquitoes is the only method to produce Sanarias first generation PfSPZ vaccines for licensure.

[1]The Laboratory for Computational Sensing and Robotics (LCSR) at the Johns Hopkins University, 3400 N Charles St. Baltimore MD, 21218.

[2]The Dept. of Mechanical Engineering at the National University of Singapore, Singapore.

* Corresponding Author. Email: gchirik1@jhu.edu

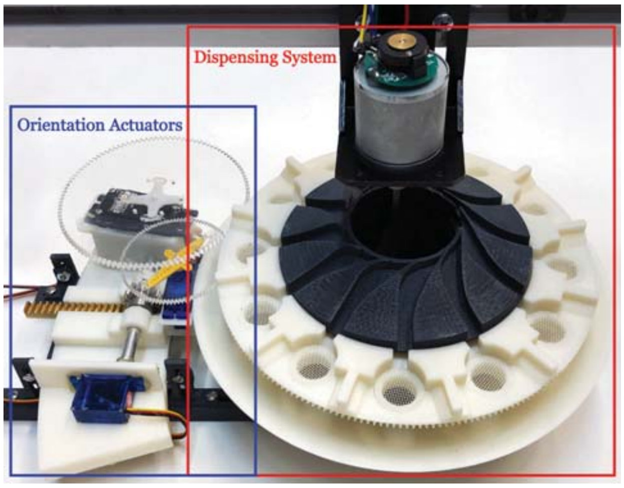


Fig. 1: Mosquito Staging Apparatus Prototype

Mosquito gland extraction using microdissection consists of four steps: (1) mosquito alignment (2) decapitation (3) gland extrusion and (4) gland collection. The manual gland extraction is a simple task considering human dexterity but is a relatively complex one for automation engineering involving mosquito sorting and alignment. The current semi-automated mosquito microdissection system (sAMMS) [8] [9] inspired by pipetting successfully transfers the decapitation and gland extrusion steps from a sequential process to a batch process. The throughput of 470 mosquitoes in one hour per sAMMs unit is triple that of a manual operator's, which is about 150. However, the mosquito dispensing and alignment task is still operated manually which turns out to be the rate-limiting step in the semi-automated system. The bottlenecks that will hinder the success of the automated alignment include the flexibility of the mosquitoes, entanglement of their appendages, aggregation of multiple mosquitoes and the issue of recycling mosquitoes. To achieve the preliminary goal of processing one mosquito per 2 seconds fully-automatically, we explore novel strategies to tackle the design of the apparatus to dispense mosquitoes.

The remainder of this paper is organized as follows to

978-1-7281-0356-3/19/$31.00 ©2019 IEEE

present the first generation of Mosquito Staging Apparatus as shown in Fig. 1. In Section II, we first state the requirements of the Staging Apparatus in terms of efficiency and biosafety. We introduce the detailed design of the dispensing system and orientation actuators in Section III, following with the system integration and realization in Section IV. To give the evaluation of the current staging apparatus, we include the experimental performance in Section V. The conclusion and future work are presented in Section VI.

II. Design Requirements

The main goal is to design a fully automated system to dispense correctly oriented mosquitoes. The whole automation apparatus consists of two subsystems: the dispensing system for isolating tangled mosquitoes and the orientation actuators for positioning the mosquitoes as desired to be processed and dissected. The efficiency requirement is preparing one correctly oriented and isolated mosquito per 2 seconds.

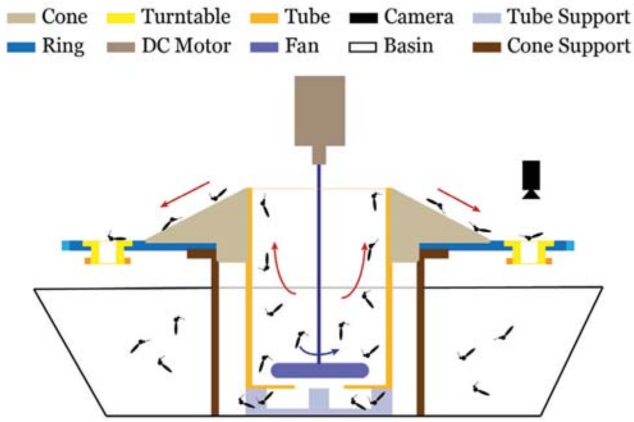
1) Dispensing System: The primary requirement for the dispensing system is to provide sufficient separated mosquitoes so that they can be identified by a computer vision system [10]. The mosquitoes with salivary glands containing live Plasmodium are collected in nutrient solution with high density. After sorting mosquitoes, one robot [11] will conduct the pick and place tasks aided by computer vision to feed mosquitoes to a cartridge for decapitation. Previous work developing the robot can be found in [12]. Therefore, the mosquitoes should be separate enough so that the vision system can recognize qualified mosquitoes.

While getting sufficiently isolated mosquitoes, there should be a decent percentage of mosquitoes that are intact. The fresh mosquitoes stored in Sanaria's nutrient solution are soft and can easily get damaged during the dispensing. For biosafety concerns, water is selected to be the solution to convey mosquitoes. Although the fluid mechanics is heavily involved to generate the water flow gently transporting the mosquitoes, the actuator generating the flow may damage the mosquitoes by the direct collision. Thus the speed-related parameters and the spatial kinematic pair of the dispensing system need to be precisely arranged.

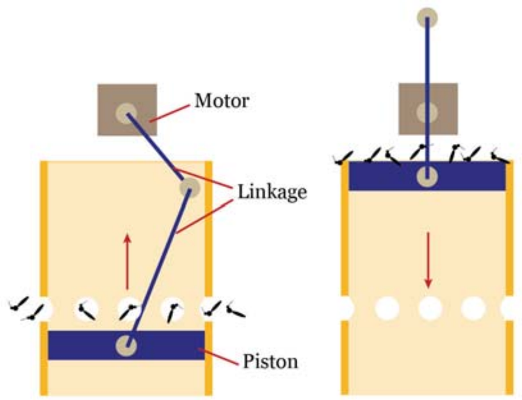
2) Orientation Actuators: After getting the randomly oriented mosquitoes with the dispensing system, the orientation actuators are activated to rotate the turntable until one mosquito's proboscis orientation is in correct orientation for the robot to pick up. Due to the limited workspace of the robot platform, it is required that the relative angles between the proboscis and the home-position of the tweezers are within the ± 5 degree range.

III. Automated Staging Apparatus Design

The automated staging system presented in this paper is the first part of the automated mosquito microdissection system (AMMS). In order to circumvent the aforementioned bottlenecks such as the flexibility of the mosquitoes, and to allow for a large throughput of complete mosquitoes, we propose to use principles of fluid mechanics to facilitate the flow of mosquitoes. To achieve the alignment of the mosquitoes, computer vision aided design will be blended to provide the orientation information of the mosquitoes.



(a) Vortex-actuated Dispensing System



(b) Piston-actuated Design

Fig. 2: Dispensing System

A. Dispensing System

The concept of the dispensing system as shown in Fig.2 is proposed to separate the mosquitoes stored in the basin and deliver isolated mosquitoes to staging areas for further orientation correction. The system can be subdivided to vortex generation, cone module and ring module in terms of functionality and the parts involved.

1) Vortex Generation: Note that while mosquitoes are to be lifted out of the basin to be isolated and are eventually picked up by a robot, extra mosquitoes will be periodically added into the basin with the help of a vision system to keep an approximately fixed amount of mosquitoes.

One way to deliver mosquitoes implies rotary mechanism to generate vortex as shown in Fig.2a. The rotary mechanism device for generating the water flow mainly comprises a basin, a tube, a fan-shape rotor and a motor. The basin is filled with water and assumed to contain a fixed number of mosquitoes. The tube is equipped with a bottom that has a center hole to allow water and mosquitoes to come through. The tube support is a frame structure seating on the ground of the basin. The fan-shaped rotor reaches deep into the water and is concentric with the tube. A DC motor actuates the high-speed spin of the fan-shape rotor. The rotating fan-shaped rotor drives the water inside the tube tangentially to the tube walls. Consequently, the centrifugal force drags the water toward the tube wall and water climbs up to reach the equilibrium point at which the combination of the gravity, water pressure and centrifugal force is 0 along the water profile. When the spinning speed is high enough, the water will overflow the tube top and flow back to the basin after pouring over the cone module and ring module surfaces, which means the water level in the basin remains constant.

Our initial plan for transporting the mosquitoes in water was using the concept of an Archimedes Screw Pump [13]. However, while performing experiments, when the Archimedes Pump is positioned vertically so that is concentric with the tube, it is hard to pump water up especially when the screw and tube walls are not proximate enough. Meanwhile, if the screw and tube walls are too close together, mosquitoes get severely damaged. Hence we finally turned to use a fan-shape rotor to motivate water flow and to move water up like a turbine. One advantage of the current design is that it gives another degree of freedom to control the water volume. For example, by narrowing down the width of the ring structure of the tube bottom, the water volume will decrease due to the reduction of the tube's ability to support water. Therefore, it requires a higher fan speed to lift up the same amount of water.

Another alternative to transfer mosquitoes is using a simple piston to pump mosquitoes up the tube and onto the cone as shown in Fig. 2b. This approach results in mosquitoes moving onto the cone and to the processing stations more discretely rather than through continuous water flow, which might be preferred for easier sequencing and processing of mosquitoes after they are delivered. However, due to the discreteness of delivery, piston-pumping is inherently slower than the vortex, requiring higher concentrations of mosquitoes and additional stirring to guide mosquitoes into the tube for a high rate of transport. Experiments conducted to show that the pumping action does not damage the mosquitoes frequently. The common advantage of the two kinds of pump design compared with the Archimedes Pump is that they can generate near uniform water flow along the cone which guarantees high compliance for future design.

In the final design, we keep the vortex pump considering the requirement of high productivity. Since mosquitoes are flexible, the collision between the fan and mosquitoes will lead to mosquitoes being chopped up especially when the motor speed is relatively high. Note that the required maximum water speed which is proportional to the motor speed increases along enlarging the distance between the water level and the top of the tube. By carefully designing the fan shape, the relative distance between parts and the motor speed, we can keep most mosquitoes coming out of the tube intact.

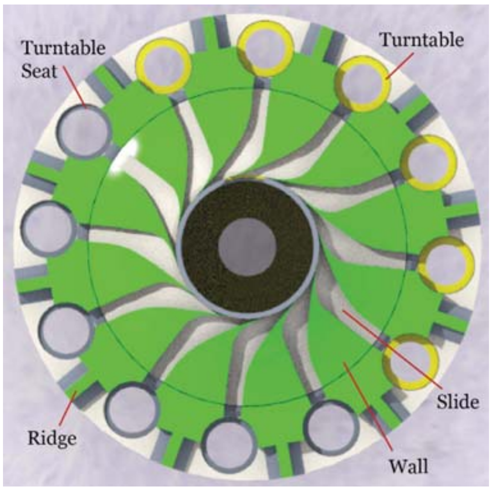
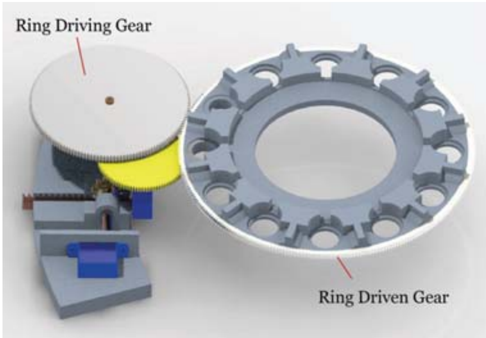


Fig. 3: Cone-Ring Module

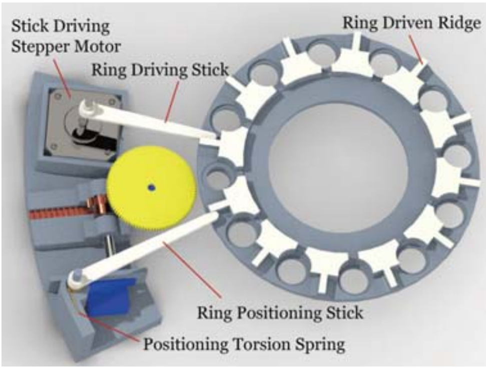
2) Cone Module: After lifting up the mosquito-water vortex, we need to separate the mosquitoes and deliver them to isolate stages. As shown in Fig.2a, the surface of the cone shape is narrow at the top and wide at the bottom. Therefore, water brought to the top of the cone and the mosquitoes in it are expected to flow away from each other as water pours down the cone, dispensing mosquitoes that are separated from each other so that they can be recognized by the computer vision system and be operated on. High, thick walls are placed on the cone to help align mosquitoes effectively as shown in Fig. 3. These walls leave small spiraling slides that grow narrower near the base of the cone for the mosquitoes to follow, guiding them into the small turntables to be oriented.

The curved shape of these slides is designed to be consistent with the free water flow trajectory on a smooth cone without additional structures for the sake of reducing the collision between water flow and slide walls, which may induce turbulence along the flow affecting the motion of mosquitoes. The tangent vector of the slide centerline first points along the tangent of the tube top and then gradually curves back approaching the cone's radial direction. The general slide shape can be modeled by a parabola with a solid physical model. The water on the tube top has high tangent velocity plus near 0 radial velocity, and they decrease and increase respectively due to the work by friction and gravity.

Another method we explored to separate mosquitoes is inspired by the classic lottery machine: we added shallow ridges and protrusions evenly and symmetrically distributed on the cone to constrain and guide the mosquitoes' movement. However, mosquitoes bumping on the raised features fall on stages in a nearly uniform distribution, and the mosquito separation doesn't seem to be enhanced much, partially due to the insufficient height of the protrusions



(a) Gear-based Ring Actuator



(b) Stick-based Ring Actuator

Fig. 4: Ring Rotation Module

and mosquitoes being highly flexible objects contrary to our initial assumption.

In the final design, the slides constructed by high, thick walls are placed evenly on the cone as in Fig. 3. Note that the slide curve orientation matches the situation when the fan-shape rotor spinning clockwise. The number of the slides and the width at the end of each slide is determined by the size of the ring which will be introduced in Section III-A3.

3) Ring Module: A ring is the connection part attached at the bottom of a cone, acting as the support for individual turntables. The key functionality is to guide the water from a slide directly to the center of a turntable. As shown in Fig. 3, the wall on the cone is extended to the ring and forms a half-circle valley around each turntable. It can significantly reduce the probability of mosquitoes being flushed out of the ring or landing out of turntables, increasing the throughput of the system. Considering that the staging system should deliver correctly orientated mosquitoes to the robot platform, we proposed two methods to activate the ring which will be detailed in Section III-B. The structure composing gear actuation consists of an actuator gear and gear teeth attached on the outer face of the ring. For the stick mechanism, the ridges which are parts of the water-guiding mechanism act as the driven structure.

4) Turntable Design: Turntables are parts where the mosquitoes flow into and come to rest in an isolated manner,

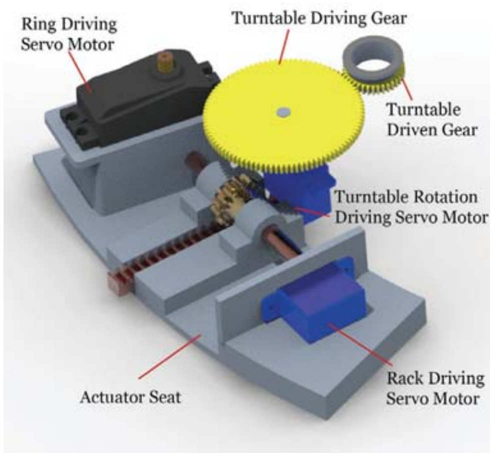


Fig. 5: Turntable Rotation Module

and where they can be staged. Coarse plastic mesh is attached on the top surface for holding mosquitoes and leaking water. There is a large center hole to allow water flowing back to the basin. The middle cylinder of the turntable extrudes beyond the ring thickness for affixing gears. Metal shims are placed between the driven gear and ring to combat friction. The speed of leaking water influences whether mosquitoes can stay on the mesh, and is determined by the water volume from the cone surface slide, the corresponding mesh dimensions on the turntables and the size of the center hole. These parameters are optimized to minimize the number of mosquitoes being washed away back into the basin and maximize the percentage of isolated mosquitoes.

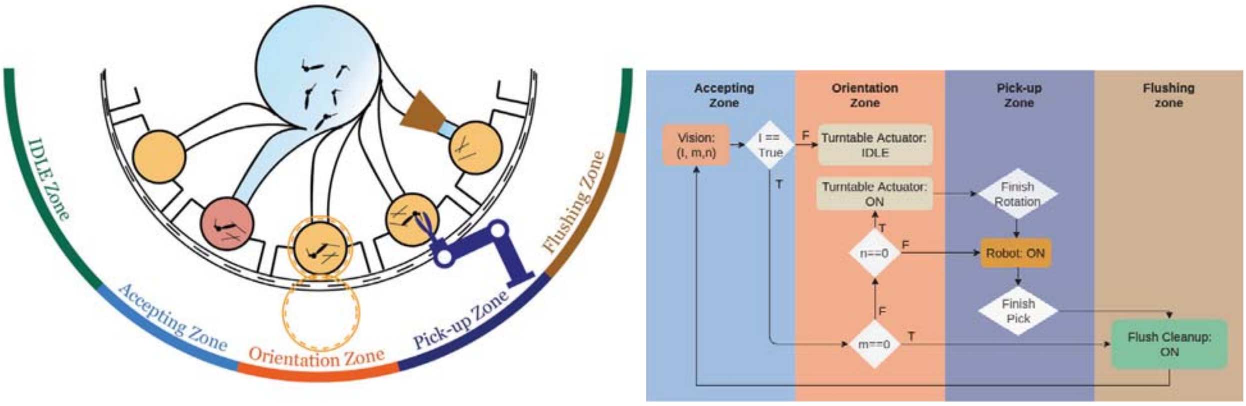
B. Orientation Actuators

The orientation actuators are proposed to first (1) rotate the turntable to make sure the orientation of the mosquito proboscis is within the requirements mentioned in Sec. II-2 and then (2) rotate the ring-cone system to transport turntable for parallel processing, such as delivering the qualified mosquitoes to the robot platform. The orientation actuators consist of two modules: the ring rotation module and the turntable rotation module. In the final design, the two actuator modules are combined into one set as seen in Fig. 4a.

1) Ring rotation module: We proposed two kinds of mechanism to rotate the ring for indexing turntables which are (1) gears and (2) sticks.

Module (1) as shown in Fig. 4a includes a continuous servo motor and its matching ring driving gear meshing with the driven gear on the ring all the time. The advantage of this module is high control flexibility. For instance, when there are multiple mosquitoes or is none mosquito on one turntable, which is rare events though, it is easy to accommodate the overall workflow by changing the rotation magnitude and speed of the driving gear without losing efficiency.

Module (2) consists of a stepper motor and the driving stick mounted on the shaft. In each rotation round, the



(a) Mosquito Staging Apparatus Carousel Concept

(b) System Workflow

Fig. 6: Mosquito Staging Apparatus Workflow. The accepting, orientation, pick-up, flushing and IDLE zones are represented in blue, orange, purple, brown and green, respectively.

stick pushes one ridge on the ring and detach when a new turntable is delivered to the centerline of the slide with water flowing down. The simplicity is the advantage of this module since it uses ridges for guiding water as the driven structure. The disadvantage is that the speed is hard to change dynamically since the ring has to wait for a rotation circle of the stepper motor to be activated. Another advantage is that it requires a positioning mechanism which is the torsion spring constrained stick in Fig. 4b.

2) Turntable rotation module: The turntable rotation module includes two servo motors, one gear, and one rack and pinion set as in Fig. 5. A servo motor with turntable driving gear is mounted on one end of the rack. The rack and pinion set is driven by another servo motor to move the rack forward and backward along the centerline of the ring. When the turntable is transported to the centerline of the rack and it is required to be reoriented before the robot picking, the rack is then driven forward meshing turntable driving gear with the driven gear on the turntable. With the proboscis orientation signal from the Vision system, the turntable is rotated until the proboscis of the target mosquito lies within the pickable range for the robot. The rack is then driven back and remains disengaged until the next turntable is brought into the robot working station.

IV. System Integration and Realization

A. Apparatus Workflow

In the current setup, we assume that there is one robot waiting to drag mosquitoes. By carefully tuning the mosquito flow parameters, we can get 0 or 1 mosquito on the turntable. The workflow for multiple mosquitoes on one turntable or multiple robots is more complicated and will be detailed in future work.

During the whole process, the vision system is activated providing the mosquito information signals (I, m, n) of the target turntable, which are: whether there are mosquitoes on the turntable (I: boolean number), the number of mosquitoes that can be recognized (m) and the number of mosquitoes ready to be picked up (n) respectively. To better demonstrate the workflow, the ring module is divided to 5 sub-zones: (1) Accepting Zone containing the turntable with water flowing on it, (2) Orientation Zone for rotating the turntables via the turntable actuator based on requirements, (3) Pick-up Zone for the robot picking up qualified mosquitoes, (4) Flushing Zone for removing unqualified mosquitoes as well as detached legs and (5) Idle zone. Each turntable will be transported periodically to the 5 zones in each rotation cycle of the ring which forms one process unit as in Fig. 6a. Each turntable will be in the Accepting Zone for around 2 seconds minus the time needed to transport the turntable, hence there will be one mosquito exported for decapitation every 2 seconds in general.

The current workflow for the Mosquito Staging Apparatus is shown in Fig. 6b. For the turntable in the Orientation Zone, if there is a recognizable mosquito that doesn't satisfy the requirements for the robot, the turntable actuator is turned on to correct the orientation aided by the vision system. In the other cases, if there is no mosquito, a mosquito the proboscis of which is undetectable or a mosquito that can be picked, the turntable actuator will be in the IDLE state and then the turntable will be transported to the Pick-up Zone. In the Pick-up Zone, if one mosquito is detected, the robot will be triggered to drag and align it to the decapitation apparatus. Otherwise, the turntable will be directly delivered to the Flushing Zone, where the remaining biomaterials will be flushed off the mesh by strong water flow.

B. Manufacturing and Assembly

The individual parts of the system to be manufactured and assembled consist of a cone, a ring, multiple turntables, a tube, and cone and tube support that rest in the basin to contain mosquitoes and water. These parts are all designed and made to put together through the means of their dimensions as well as certain features like indents and notches, hence can be easily assembled as seen in Fig. 1. In addition to these parts which were designed and rapid-prototyped from scratch, the system features a fan and four actuator motors; one to actuate the fan, one to rotate the ring and two to engage and disengage with and to rotate the individual turntables, as well as a camera to assess mosquito orientation.

The motor and fan are fixed above the cone with the fan dipping down the tube into the mosquito basin. The motor to rotate the ring is placed and supported to be engaged with the ring at all times through gear and the gear teeth fitted around the ring. Although the turntable actuator rotates the turntables similarly by engaging its gear with teeth around the individual turntables, it's default stage is being disengaged to allow for the ring to be rotated independently of the turntables. Cone Module and Ring Module are fixed together in a way that the center of each turntable on the ring coincides with the centerline of one slide on the cone. The blocker and tube are fixed sitting on the basin. To provide the robot and turntable actuator with a relatively dry operation station, there is a cylindrical blocker with one notch placed on the top of the tube as seen in Fig. 3, hence the water can only flow out through the notch as in Fig. 6a. The size of the notch is consistent with the width of the slide near the tube top.

In the current setup, except for the standard parts including the shaft, the shaft coupling, shims and the pinion and rack set, the other novel parts are made by rapid 3D printing while the gears are laser-cut. Toward manufacturing, gears on turntables, the cone and servo motors should be made of stainless steel or aluminum considering that the gear teeth will be meshed and detached frequently. The surface of the ring and cone should be smooth enough, otherwise, mosquitoes can easily get trapped on the slide, which means they can be made of metal or plastic via injection molding. The material used to make the tube and cone support should be stainless for the water-blended working environment.

V. Evaluation

To evaluate the proposed Mosquito Staging Apparatus, rigorous experiments are done to test the effects of key parameters aiming to find the optimal combination generating 0 to 1 mosquito per turntable. Based on the first generation of prototype similar to Fig. 1 but using the stick-based ring actuator as in Fig. 4b, the fan-shape rotor speed is set to about 950rpm under which most mosquitoes are intact when delivered out. The two key parameters that affect the system performance most are the number of mosquitoes in the basin, denoted as "p mosquitoes", and the time duration each turntable spends in the Accepting Zone, represented by the rotation speed of the stepper motor "n rpm" which grows opposite to the time length but is easy to measure.

	Mosq. Number	Ring Speed(rpm)	Total number of tests
1	40	30	10
2	40	20	8
3	60	30	8
4	60	20	7
5	80	30	8

TABLE I: Experiment Conditions

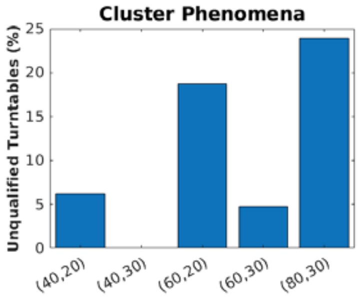
We tested five configurations as in Tab. I. The water volume in the basin is assumed to be the same all the time, hence the number of mosquitoes in the basin is equivalent to mosquito density. In each test, a fixed number of mosquitoes were put into the basin first. The DC motor generating the vortex and the orientation actuators were then turned on at the same time. When there was one mosquito delivered out, the corresponding turntable was annotated as Turntable 1. Before the slide leading to Turntable 1 meeting the notch again, the DC motor was turned off. The numbers of mosquitoes on each turntable were then counted.

Fig. 7a shows that a high mosquito density amplifies the effect of Ring rotation speed. The cluster phenomena as well as the feeding rate as shown in Fig. 7b grows with the increase of mosquito density and the time length. In the configuration pair $(40, 30)$, there are no cups with mosquito clusters. Fig. 7c shows that the configuration pair $(40, 30)$ has the minimal standard deviation considering the average number of mosquitoes in one turntable for each test. From Fig. 7d, we can see that the number of mosquitoes on each cup in one cycle does not seem to deviate much, which means the system is robust to the variation of mosquito number in the basin to some degree. Therefore, the optimal configuration is the one with 40 mosquitoes in the basin leaving each turntable in Accepting Zone for 2 seconds.

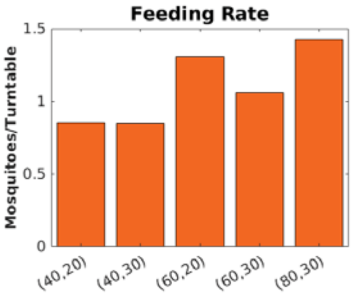
VI. Conclusion and Future Work

This paper proposes a novel fully-automated Mosquito Staging Apparatus serving as the first processing stage for producing PfSPZ Malaria Vaccine. The design is motivated by the dispensing and aligning of mosquitoes being the rate-limiting part of extracting mosquito salivary glands. The main contribution is in 2-folds: the proposed staging apparatus consisting of the dispensing system for separating mosquitoes and the orientation actuators to deliver mosquitoes to various turntables; the fluid mechanism generating vortex activated by a fan-shape rotor. The Mosquito Staging System can dramatically enhance the mass production of Malaria Vaccine which is essential to prevent the propagation of Malaria.

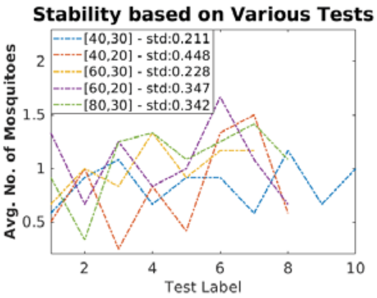
Future work includes further detailed design to enhance the system robustness. Noting that our mechanism relies on fluid mechanics which induces probability that there may be no mosquito on turntables, more experiments should be done to set the best system parameters including the number of mosquitoes in the basin to keep the feeding rate at steady level. Mosquitoes as bioreactor are valuable and expensive to raise. Hence, we have to try our best to avoid the damage



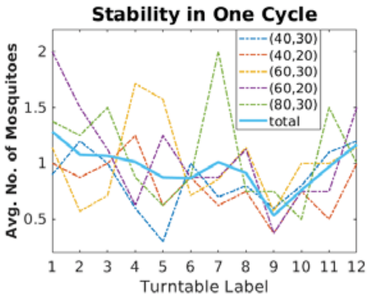
(a)



(b)



(c)



(d)

(e) Experiment Result in the Optimal Configuration

Fig. 7: Evaluation Metrics of Mosquito Staging Apparatus. The label (m, n) represent the number of mosquitoes in the basin and the rotation speed of the ring actuation motor, respectively. In Fig.7a, unqualified turntables are the turntables with clustered mosquitoes that cannot be recognised by vision system. In Fig.7b, the value of each bar indicates the average number of mosquitoes in one turntable in each configuration. Fig.7c shows the temporal stability of the feeding rate by showing the average number of mosquitoes per test. Fig.7c shows the spatial stability by demonstrating the average number of mosquitoes in each turntable in one rotation cycle. Fig.7e shows the result of one test in the optimal configuration $(40, 30)$.

of mosquitoes including modifying the fan shape and its materials. To keep consistent detecting condition for the vision part, we will build the flushing actuator to wash unqualified mosquitoes and detached legs and wings off the turntable. Since the ring and turntable actuator will work near to the basin containing water, a waterproof cover is required to guarantee the dry working space for motors.

ACKNOWLEDGMENT

Thanks go to Ms. Jing Jia, Ms. Xia Wu, Mr. Bolin He and Mr. Mark Shifman for their discussions and help developing the preliminary apparatus. This work is supported in part by NIH SBIR grant 1R44AI134500-01 and in part by Johns Hopkins University internal funds. This work is with the collaboration of Sanaria, Inc. Rockville MD, USA.

REFERENCES

[1] —–, "World Malaria Report", World Health Organization, Geneva2018. https://www.who.int/malaria/publications/world-malaria-report-2018/en/
[2] Hoffman, Stephen L., et al. "Development of a metabolically active, non-replicating sporozoite vaccine to prevent Plasmodium falciparum malaria." Human vaccines 6.1 (2010): 97-106.
[3] Seder, Robert A., et al. "Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine." Science 341.6152 (2013): 1359-1365.
[4] Richie, Thomas L., et al. "Progress with Plasmodium falciparum sporozoite (PfSPZ)-based malaria vaccines." Vaccine 33.52 (2015): 7452-7461.
[5] Ishizuka, Andrew S., et al. "Protection against malaria at 1 year and immune correlates following PfSPZ vaccination." Nature medicine 22.6 (2016): 614.
[6] Epstein, Judith E., et al. "Protection against Plasmodium falciparum malaria by PfSPZ Vaccine." JCI insight 2.1 (2017).
[7] Mordmller, Benjamin, et al. "Sterile protection against human malaria by chemoattenuated PfSPZ vaccine." Nature 542.7642 (2017): 445.
[8] Taylor, Russell H., et al. "Mosquito Salivary Gland Extraction Device and Methods of Use." U.S. Patent Application No. 15/621,875.
[9] Schrum, Mariah, et al. "An Efficient Production Process for Extracting Salivary Glands from Mosquitoes." arXiv preprint arXiv:1903.02532 (2019).
[10] Wu, Hongtao, et al. "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.
[11] Phalen, Henry, et al. "Mosquito Pick-and-Place: Automating a Key Step in PfSPZ-based Malaria Vaccine Production," Accepted to 2019 IEEE 15th International Conference on Automation Science and Engineering (CASE). IEEE 2019.
[12] —–, " SporoBot -Build a Robot. Fight Malaria. Save Lives! (YouTube video)": Sanaria, Inc, 2014, https://www.youtube.com/watch?feature=player_embeddedv=VblazNXcHFg.
[13] Waters S, Aggidis G A. Over 2000 years in review: revival of the archimedes screw from pump to turbine[J]. Renewable and Sustainable Energy Reviews, 2015, 51: 497-505.